Presenter: Aarreberg, Lauren

Molecular mechanisms of innate immune activation and accelerated liver pathology in HCV/HIV coinfection

Lauren Aarreberg, Amina Negash, Michael Gale, Jr.

University of Washington

Hepatic inflammation is a hallmark feature of pathogenesis during HCV/HIV coinfection, but the processes that propagate this outcome are not understood. Coinfection results more commonly in inflammatory liver disease than monoinfection of either virus, with a quicker progression to end-stage disease. HCV causes significant liver inflammation by triggering potent inflammasome activation and IL-1β release after phagocytic uptake by hepatic macrophages. Also known as "Kupffer cells", these CD4+ cells are targets of HIV infection. Here, we are evaluating differential modulation of innate immune signaling in a hepatocyte/macrophage co-culture model of HCV/HIV coinfection. Furthermore, investigations of the macrophage as a hub of inflammatory signaling have revealed a hereto unappreciated role for IL-1 signaling in the regulation of interferon and antiviral response genes. We therefore hypothesize that the pro-inflammatory signaling molecule IL-1β acts to synergize innate immune activation in response to viral insult in the liver, and that the resulting maladaptive, non-resolving response to chronic infection underlies hepatic injury and accelerated disease in HCV/HIV coinfection.

Presenter: Acharya, Mridu

Alpha(v) integrins engage autophagy components to regulate immune receptor trafficking and signaling

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

Benaroya Research Institute

Integrin-ligand interactions trigger an array of intracellular signaling events and cytoskeletal rearrangements, which include the rapid endocytosis and exocytosis of integrins and other membrane proteins. There is increasing appreciation that these intracellular trafficking events do more than just recycle and deliver integrins to sites of adhesion, but instead play a critical role in signaling. We have uncovered a novel role for alpha(v) integrins in regulating immune cell signaling by modulating trafficking of immune receptors. Specifically, we have found that alpha(v)beta3 integrin regulates Toll-like receptor (TLR) signaling by directing trafficking and maturation of TLR containing endosomes, through a mechanism involving src-family kinases and components of the autophagy pathway. We have defined this mechanism using a combination of biochemical and microscopic techniques in B lymphocytes. In B lymphocytes stimulated with TLR ligands, alpha(v) integrins direct endosomal trafficking of the TLRs from NF?B signaling endosomes to IRF7 signaling endosomes where the TLRs associate with autophagy components LC3 and this leads to down-regulation of TLR signaling. This regulation of TLR trafficking is initiated upon alpha(v) integrin internalization, which results in activation of src and syk kinases and leads to LC3 recruitment to these TLR containing endosomes. When B cells lack either alpha(v) integrins or autophagy components LC3 and atg5 this transition through signaling compartments is disrupted and leads to enhanced TLR signaling and cell proliferation. We have also used av conditional knockout mice to determine the consequences of loss of alpha(v)beta3-mediated immune regulation. Mice lacking B cell alpha(v) show increased antibody responses to antigens with TLR-ligands and develop increased levels of autoantibodies. We therefore propose that alpha(v)-mediated TLR regulation serves to maintain the balance between protective immunity to microbes and potential pathological autoimmune responses. We are currently studying the role of alpha(v) signaling and autophagy components in development of class switched, high affinity antibodies in response to antigens in the context of infection with a pathogen or in response to self-antigens.

Presenter: Agac, Didem

[b]2 adrenergic receptor signaling prevents hyperinflammation through early release of IL-10

Didem Agac, Leonardo D. Estrada, J. David Farrar

University of Texas Southwestern Medical Center

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Presenter: Agarwal, Maria

HPV16 immunity induced by immune responses to mutations in E6 and E7 proteins

Maria Agarwal, Ashley Saint-Fleur, Jie Fu, Hyam Levitsky,Cornelia L. Trimble

Johns Hopkins University

Human papillomavirus (HPV) causes 30% of cancers attributable to infectious pathogens. HPV16 causes most cervical, anal, vaginal, vulvar, and oropharyngeal cancers. Although preventive vaccines targeting HPV16 and 18 are available, rates of vaccination are uneven. The incidence of HPV-associated cancers for which no screening algorithms have been validated continues to increase. Development of squamous cervical cancer and its precursor, cervical intraepithelial neoplasia (CIN) 2/3 is associated with integration of HPV genome into the host genome, and expression of the HPV E6 and E7- gene products, which inactivate p53 and pRb respectively. Since E6 and E7 are constitutively expressed in cervical carcinomas and CIN, they present attractive immunotherapeutic targets. Development of immunotherapies has been limited, in part, by modest immunogenicity of vaccines tested to date. The purpose of this study is to augment the immunogenicity of the E6 and E7 antigens through mutational screening. A subset of CIN2/3 do undergo complete regression. Our lab has carried out a series of prospective clinical studies, including immunotherapeutic interventions, in subjects with CIN2/3. Peripheral blood T cell responses to E6 and E7 are marginal, and do not distinguish regressors in either vaccinated or unvaccinated subjects. However, in vaccinated subjects, the lesion microenvironment is comprised of new tertiary lymphoid structures, evidence of T cell proliferation elicited by cognate antigen, and clonally expanded TCRs. Tissue T cells in vaccinated subjects access dysplastic epithelium, in contrast to T cells in persistent lesions, which were confined to the stromal compartment. These findings suggest that T cells that could mediate regression can be elicited by vaccination. However, strategies to enhance immunogenicity would likely increase therapeutic effect. Here we present an approach designed to determine the effect of mutations in E6 and E7 on antigenicity. Mutations can enhance immune responses to antigens by several mechanisms, including altering antigen processing, trafficking, glycosylation, and affinity to major histocompatibility complexes. Using error prone PCR, we generated libraries containing random mutations in the coding sequence of E6 and E7. Clones from each library are currently being screened in vitro to identify immunogenic mutants, which could be potentially used for multi-epitope vaccines.

Presenter: Agashe, Vrushali

Differential effect of Leukocyte associated immunoglobulin-like receptor 1 (LAIR1) ligation by C1q in a dendritic cell based Th1 response and a monocyte based Th17 response

Vrushali Agashe, Jeremy Sullivan, Ewa Jankowska-Gan, William Burlingham

University of Wisconsin Madison

Background: Leukocyte Associated Immunoglobulin-like Receptor 1 (LAIR1) is a transmembrane receptor expressed by a variety of cells of the immune system. LAIR1 ligands include collagens and collagenous domain containing proteins such as C1q. LAIR1 ligation is though to induce inhibitory signals in Natural Killer cells, T cells and B cells. Recent evidence from our lab demonstrates that LAIR1 ligation through its natural ligand inhibits a Th1 (tetanus) response but augments a Th17 (Collagen type V) based response. Hypothesis: The differential regulation of the Th1 and Th17 response lies in the selective use of APCs: dendritic cells (Th1) vs monocytes (Th17). Results and Conclusions: Pre-incubation of PBMCs with C1q, led to an increase in the number of monocytes expressing IL1(b) and TNF(a), after an overnight stimulation with Collagen Type V, whereas stimulation with tetanus failed to induce either cytokine. Current experiments are underway to study the effect of C1q on DCs. Subsequent experiments will also aim to look at the effect of C1q on Th1 and the Th17 cells.

Presenter: Arroyo, Nicole

Antigen-specific CD4+ T cell differentiation in blood-stage malaria

Nicole Arroyo, Marion Pepper

University of Washington

Malaria is a parasitic disease that kills approximately one million people a year. Although efforts to induce protection from malaria through vaccination have been ongoing for over sixty years, none have produced long-lasting immunity in malaria-endemic areas. This is likely due to a lack of knowledge of the immunological mechanisms required for protection. While it is clear that both CD4+ T cells and B cells are important components of the immune response to the blood stage of infection, little is known about their specific functional contributions. We have used a murine model of Plasmodium infection, P. yoelii, to gain an understanding of the lymphocyte populations that form and are required for protection. Using transgenic parasites and MHC class II tetramers to track CD4+ T cell responses during malaria, we have found that early in the response, the vast majority of CD4+ T cells that form are T follicular helper (Tfh)-like cells, despite the lack of a germinal center at this time point. Interestingly, in the absence of B cells, the CD4+ T cells alternatively develop a significant T effector (Teff) phenotype that appears to be primarily comprised of Th1 cells. Interestingly, while there are stark differences in the type of CD4+ T cells generated in the presence and absence of B cells, parasitemia remains comparable in both WT and uMT mice for approximately 1.5 weeks post-infection, at which point mice deficient in B cells are unable to control the parasite growth. We are currently investigating if CD4+ T cells are responsible for the parasite control at this early stage, how they may be contributing to this control, and which aspects of the Th1 and Tfh responses are important for maintaining low parasitemia. An effective vaccine may need to stimulate both the formation of antigen-specific Teff cells to manage parasite burden until Tfh cells have coordinated effective antibody responses by B cells in order to provide effective immunity against re-challenge.

Presenter: Balakrishnan, Amritha

Pathologic role of dual receptor T cells in chronic graft-versus-host disease.

Amritha Balakrishnan, Burhan Jama, Nicholas Gloude, Gerald Morris

UCSD

Chronic graft versus host disease (cGVHD) is a complication resulting from HLA matched hematopoietic stem cell transplant (HSCT) that manifests as debilitating multi-organ immune pathology. Currently the mechanisms driving disease and the antigens recognized by the activated T cells remain unknown. We have previously shown that T cells naturally expressing two TCRs have disproportionate reactivity to auto and allo antigens and play a role in acute graft versus host disease (aGVHD). Based upon these findings we hypothesized that dual receptor T cells play an important role in the development of cGVHD and that these cells arise due to impaired thymic selection. To study this, we examined by flow cytometry T cells expressing two receptors in patients with cGVHD (n=5) 1.5 - 4 years post-transplant. Dual receptor T cells are increased in number (238.2 + 56.59 /103 T cells, mean+ SEM), and have an activated phenotype as compared to cells from healthy controls (n=5, 79 + 6.4 /103 T cells, P=0.028). To determine the origin of these cells we used a T cell depleted syngeneic HSCT mouse model and found a significant increase in the numbers of dual TCR T cells in post-selection thymocytes and in peripheral T cells of transplant mice. (P

Presenter: Barlow*, Graham

Is there a bias towards tolerance-inducing responses in MAIDS-Susceptible Mice? : IL-10 and Lag-3 Expression following Murine Leukemia Virus Exposure

Graham Barlow, Johnny Le*, Reid Goodman*, Abena Kwaa^ and Sharon Stranford**

Pomona College, Claremont CA* and Mount Holyoke College, South Hadley, MA^

Murine AIDS (MAIDS) is a useful model for studying susceptibility to HIV-induced AIDS and disease progression. In this system, C57BL/6 and BALB/c mice mount differential immune responses upon challenge with the LP-BM5 mixture of Murine Leukemia Virus (MuLV). Whereas C57BL/6 develops severe immunodeficiency after viral infection, BALB/c mice clear the infection within two weeks and establish a protective memory response. Our goal was to probe the differential activation of the adaptive immune response in these two strains of mice. Professional APC activation and antigen presentation is the first and most likely point from which these divergent pathways may evolve. To investigate whether the antigen-presentation microenvironment of these two mouse strains differ we studied two potent immunosuppressive molecules, the cytokine IL-10 and the surface molecule Lag-3, during the early stages of the anti-MuLV response. We find a significant increase in the level of IL-10 in lymph nodes of MAIDS-susceptible mice, which reach a peak at approximately 3 days post infection. Preliminary data suggest that Lag-3 surface expression on T cells may correlate with this enhanced IL-10 expression. Studies are underway to evaluate the levels of soluble Lag-3 in these animals via ELISA and to identify the cellular source of IL-10 expression using flow cytometry. This bias towards a microenvironment of APC tolerance and suppression of adaptive responses may inhibit anti-MuLV responses, promote persistent infection and contribute to virus-induced immune deficiency in C57BL/6 mice.

Presenter: Beaudin, Anna

Identification of a developmentally restricted hematopoietic stem cell that gives rise to innate-like B and T cells

Anna Beaudin, Scott W. Boyer, Gloria E. Hernandez, Jessica Perez-Cunningham, E. Camilla Forsberg

UC Santa Cruz

The generation of distinct hematopoietic cell types, such as tissue-resident immune cells, distinguishes fetal from adult hematopoiesis, but the mechanisms responsible for differential cell production during prenatal hematopoietic development remain to be established. Using an irreversible lineage tracing model, we have identified a novel, definitive hematopoietic stem cell (HSC) that supports long-term multilineage reconstitution upon transplantation into adult recipients, but does not persist into adulthood in situ. Despite their multilineage potential, these novel HSCs display higher lymphoid cell production, lymphoid lineage priming, and greater capacity to generate tissue-resident innate-like B and T lymphocytes as compared to coexisting fetal HSCs and adult HSCs. Our lineage tracing identifies a developmentally restricted HSC that contributes to the formation of a layered immune system and reveals the mechanism underlying developmentally regulated hematopoietic waves. As early lymphoid cells play essential roles in establishing self-recognition and tolerance, defining their origin and generation has critical implications for understanding the development of autoimmune disease, allergy, and tolerance induction upon organ transplantation

Presenter: Brog, R. A.

Breast cancer cells suppress macrophage aggressiveness

R. A. Brog, E. G. Weagel, W. Meng, M. H. Townsend, E. J. Velazquez, R. A. Robison, and K. L. O'Neill

Brigham Young University

There is an estimated 230,000 new breast cancer cases each year. Breast cancer is accompanied by tumors that have managed to evade the immune system to continue development. Surprisingly, it has been shown that macrophages account for up to 50% of the tumor mass. Macrophages are heavily influenced by signals released by cells in the surrounding microenvironment. These signals include cytokines and exosomes, which may change the phenotype of macrophages. Classically-activated macrophages, known for their M1 phenotype, are responsible for activating pro-inflammatory immune responses. Alternatively-activated macrophages, or M2 macrophages, participate in various functions including: secreting anti-inflammatory cytokines, helping in tissue repair, and angiogenesis. A better understanding of the role of macrophages among breast tumors is necessary in order to determine how macrophages can be used to help reduce tumors instead of promoting tumor development. In this study, we tested the effects of MCF-7 and MDA-MB-231 breast cancer cell lines on macrophages. We stimulated U937 cells with phorbol 12-myristate 13-acetate (PMA) for 24 hours. We ensured the activation of the U937 cells by checking for differentiation, morphology changes, and adhesion 24 hours after treatment with PMA. We incubated 500,000 differentiated U937 cells with 500,000 breast cancer cells for 48 hours. Differentiated U937 macrophages were also incubating with 500uL spent media from either MCF-7 or MDA-MB-231 cells for 48 hours. Proceeding the incubation period, fluorescent micro beads were added to the macrophages and incubated for 1 hour to allow for phagocytosis. Macrophages were analyzed using a flow cytometer to quantify microsphere engulfment. We found a 50% decrease in engulfment activity in macrophages exposed to MCF-7 cells, MDA-MB-231 cells, or spent media. When the population of macrophages were analyzed, we found macrophages that engulfed 3+ beads after treatment with MDA-MB-231 cells decreased the rate of engulfment by 57% compared to controls. Macrophages treated with spent media from MDA-MB-231 cells decreased engulfment by 79% compared to controls. For the MCF-7 treatment, the population of macrophages that engulfed 3+ beads was reduced to 54% and 65.4% for media and cells respectively. We also analyzed the gene expression of these macrophages to account for macrophage phenotype (M1 or M2). We found up-regulation on IL-10, however, we also observed down-regulation on TNF-a, and down-regulation on IL-12. These data demonstrate that the macrophages exposed to tumor environments though cancer cells and spent media show an M2-like phenotype. This suggests that the tumor microenvironment may directly affects macrophage function. The microenvironment may be responsible for the decrease in macrophage aggressiveness, and the increased number of M2 macrophages. Further studies are necessary in order to render a more in depth understanding of the relationship between the microenvironment of cancer cells and macrophage function.

Presenter: Brown, Matthew

A novel humanized mouse model incorporating non-fetal tissue

Matthew Brown, Ian G Norman, Ying Zhou, Wanying Lou, Jeremy A Sullivan and William J Burlingham.

University of Wisconsin-Madison

Pre-clinical research into allogeneic tissue transplantation therapies, as well as patient-specific regenerative medicine studies, require animal models that closely mimic the typical patient's immune response. Humanized mouse models offer a tractable in vivo system to experimentally test immunological hypotheses and therapeutic interventions in the context of a human immune system. In the work presented here, we explore the development of a novel humanized mouse model incorporating non-fetal tissue sources: cryopreserved human pediatric thymus tissue and umbilical cord blood hematopoietic stem cells. This model is equivalent to commonly used human fetal tissue models, such as the "BLT" mouse, with regard to frequency of human cell engraftment and distribution and function of various lymphocyte subsets relevant to transplant tolerance and rejection. Further, there are several benefits of this model over fetal tissue-based models. These benefits are associated with the sheer abundance of pediatric tissue available for mouse creation relative to fetal tissue, which allows for in-depth examination of a variety of parameters that are not feasible with limited quantities of fetal specimens. Additionally, and not insignificantly, this non-fetal tissue based model bypasses the ethical barriers associated with conducting fetal tissue research. Finally, the more developmentally mature tissues used in our model may afford a more accurate representation of clinical patient immune responses in terms of T cell gene expression patterns and function.

Presenter: Carden, Sarah

Trojan horses: Infected dendritic cells drive hyperdissemination of African Salmonella Typhimurium isolates

Sarah Carden, Gregory Walker, Jared Honeycutt Renee Tsolis, Juliana Idoyage, Denise MOnack

Stanford University

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Presenter: Carpenter, Susan

Characterization of the regualtory functions of lncRNA in Inflammation

Susan Carpenter, Author Names separated by commas

University of California Santa Cruz

The advent of deep sequencing technology 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. Consortium wide efforts such as the ENCODE project and FANTOM are dedicated to the identification of all the functional elements present in our genomes. The largest group of RNA produced from the genome is Long noncoding RNA (lncRNA). LncRNA are described as transcripts greater than 200 nucleotides in length that do not code for protein. GENCODE represents the gene set of the ENCODE project and its most recent release GENCODE 23 indicates that there nearly 16000 lncRNA present in the human genome. To date there is experimental data available on approximately 1% of known lncRNA. We have developed strategies to enable low to high throughput studies of lncRNA both in vitro and in vivo. In order to study the functions of lncRNA in the inducible inflammatory response we have developed both human and murine NFkB reporter macrophage cell lines. These lines serve as excellent tools for high throughput manipulation of lncRNA, which we are currently testing using whole genome shRNA libraries in addition to Cas9/CrispR editing approaches. We know lincRNA-Cox2 is highly inducible following inflammatory stimulation and we have developed a lincRNA-Cox2 transgenic mouse using site-specific TARGATTTM system and knockout mice are being generated using Cas9/CrispR technology. Such approaches provide an efficient workflow for the characterization of any lncRNA in our model system of inflammation.

Presenter: Cho, Chul-Soo

Increased heparanase expression in keratinocytes promotes dermal fibrosis in scleroderma

Chul-Soo Cho, In-Woon Baek, Ki-Jo Kim, Wan-Uk Kim

Yeouido St. Mary's Hospital, The Catholic University of Korea

Objective: Interactions between keratinocyte and dermal fibroblast via paracrine loop play an important role in wound repair and keloid formation. In this study, we investigated heparanase expression in activated keratinocyte, and tested its effect on the survival of dermal fibroblasts. Methods: Plasma heparanase levels were measured in scleroderma patients, and heparanase expression was evaluated in the skin of bleomycin-induced fibrosis mice and HaCaT keratinocyte (HaCaT). Dermal fibroblasts (DFs) were cocultured with HaCaT separated by transwell insert under serum starvation, and apoptosis was determined using APOPercentage assay. Results: Plasma heparanase levels were significantly higher in 26 scleroderma patients than in 10 healthy subjects, and positively correlated with plasma TGF-β levels. In bleomycin-induced fibrosis mice, increased heparanase expression was observed in keratinocyte layer, but not in dermal layer. Treatment of HaCaT with hypoxia resulted in significant increase in heparanase expression, and this increase was accompanied by concomitant increase of matrix metalloproteinase-9, both of which are known to degrade epidermal basement membrane components. Coculture of DFs and HaCaT in the presence of hypoxia significantly protected the apoptosis of DFs induced by serum starvation, but it was abolished by anti-heparanase antibody or transfection of HaCaT with heparanase siRNA. DFs cocultured with HaCaT exposed to hypoxia exhibited increased Akt phosphorylation, and pretreatment of DFs with LY294002, an inhibitor of phosphatidylinositol 3-kinase, significantly abolished anti-apoptotic effect of heparanase on DFs. Conclusion: These data indicate that hypoxia, caused possibly by microvascular alteration, increases heparanase production in keratinocytes, which promotes fibrosis in scleroderma by inhibiting the apoptosis of DFs.

Presenter: Christian, David

BATF3-dependent dendritic cells are required for the CD4+ T cell response to immunization against parasitic infection

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

University of Pennsylvania

BATF3 has been shown to be a critical transcription factor for the development of a distinct subset of dendritic cells (DCs) characterized by the expression of CD24 and CD103 in the periphery and CD8α in lymphoid tissues. Using mice deficient for BATF3, these DCs have been shown in multiple models to be required for the priming of CD8+ T cells, and are a critical source of the IL-12 needed to generate a protective Th1 T cell response during infection with the protozoan parasite Toxoplasma gondii. Protection from infection by T. gondii is dependent on a T cell response that can be generated by immunization the non-replicating CPS strain of T. gondii. Immunization with a low dose of CPS is capable of generating a CD4+ and CD8+ T cell response that is equivalent in magnitude to the T cell response during active infection. Surpisingly, the CD4+ and CD8+ T cell responses have both been shown to be dependent on BATF3-dependent DCs. The studies presented here aim to determine whether this DC subset is required for the processing and presentation of parasite antigen to CD4+ T cells during CPS immunization, or if these DCs serve as a required source of IL-12 for the differentiation and expansion of parasite-specific T cells.

Presenter: Cozen, Wendy

The effect of environmental exposures on lymphoma-related cytokines, chemokines and soluble receptors.

Wendy Cozen, Yinfei Kong, Otoniel Martinez-Maza, Ann Novak, Megan O'Byrne, Susan Slager, Marta Epedigui, James R. Cerhan

USC visiting prof at ucla

The goal of this study is to determine whether known or suspected environmental risk factors for lymphoma are associated with altered immunological biomarkers that suggest a mechanism of action. Detailed histories for 14 exposure and lifestyle factors associated with lymphoma risk were obtained by questionnaire from 391 Mayo Clinic controls and 23 cytokines, chemokines and soluble receptors important in lymphoma were measured in serum using a multiplex ELISA system (Luminex). We conducted linear (17 continuous biomarker measures) or logistic (4 dichotomous biomarker measures) regression to estimate the association between the exposures and biomarkers. We then used the "best subset selection" method to examine all possible models and selected the best model (combination of biomarkers) that predicted the exposed vs. unexposed subjects using Bayesian information criterion (BIC). For categorical biomarkers, persons exposed to farms (Odds Ratio [OR]farms= 1.39, p= 1.11 x 10-4) and pesticides (ORpesticides= 1.79, p= 2.86 x 10-6) were more likely to have detectable levels of IFNa; but persons exposed to pesticides were less likely to have detectable levels of MIP-1α (OR=0.56, p=3.54 x 10-3), compared to the unexposed. Males reporting statin use were less likely to have detectable IL-1β (OR= 0.34, p= 1.15 x 10-02), while females were more likely to have detectable MCP-1 (OR=2.43, p= 5.89 x 10-3), compared to non-users. For continuous measures IL10, IL-1β, IL1Rα, IP-10, MCP-1 and MIG together explained 18% of total variation by age; EGF, eotaxin, HGF and MCP-1 together explained 10.53% of the total variation by BMI, and eotaxin alone explained 3% of the variation by birth order. Thus, our results suggest that environmental and lifestyle factors are associated with differences in immunological profiles, possibly modified by gender, providing preliminary data for insight into how these exposures may be associated with increased lymphoma risk.

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 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: Delpoux, Arnaud

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

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

University of California, San Diego

Both human cytomegalovirus (HCMV) and murine cytomegalovirus (MCMV) establish persistent infections 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 factors driving memory inflation are still unclear and the molecular pathway for the inflationary memory T-cell development is poorly understood. Mainly, inflationary memory 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 memory precursors for inflationary CD8+ T-cells. To study the role of FOXO1 in CD8+ T-cells during a persistent viral infection, we analyzed mouse bone marrow (BM) 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 (two inflationary dominant epitopes) 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 IFN? and TNFa. 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. A similar loss was found in salivary glands during the persistent phase. 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+). Finally, 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. Collectively, these results demonstrate an intrinsic role for FOXO1 in establishing the inflationary memory program that is essential to forming long-lived effector memory cells.

Presenter: Dolina, Joseph S.

Cytotoxic T lymphocyte dependence on CD4+ T cell help is controlled by pathogen dose during microbial infection

Joseph S. Dolina, Joey Lee, Lara Labarta-Bajo, and Stephen P. Schoenberger

La Jolla Institute for Allergy and Immunology

The in vivo priming, expansion, and survival of CD8+ cytotoxic T lymphocytes (CTL) generally require CD4+ T cell help via ligation of CD40L expressed by activated CD4+ T helper (Th) cells with CD40 on antigen presenting cells (APCs). Whether a CD8+ T cell response is help-dependent or -independent relies on the pathogen, where help-independent CTL responses have been documented for vaccinia virus, lymphocytic choriomeningitis virus, and Listeria monocytogenes (LM). Using LM as a model, we found that the dose, not identity, of a replicating pathogen determines the dependence on CD4+ T cell help. Additional inflammation induced by more bacteria, rather than antigen, underlies this dose response, where removal of CD4+ T cells prevented the provision of help at low dose and boosted the primary CTL response at high dose. Based on immunogen dose, CD4+ T cells can thus be divided into CD4+ Th cells and T regulatory (Treg) cells. The requirement for CD4+ T cells in helping the primary response is restricted at the earliest time points, while their role in regulating it occurs at later time points. The removal of CD4+ Treg cells produces significantly larger primary CTL responses from both low and high doses, which translates into canonical help-dependent immune responses after secondary immunization with non-inflammatory cellular antigen. The paradox in the nature of help-dependence at differing bacterial doses can therefore be explained by heterogeneity seen in the CD4+ T cell response, which is comprised of both Th and Treg cells.

Presenter: Felix, Krysta

A Gut Commensal Bacteria Influences Resistance to Pneumonia.

Krysta Felix, (Kevin) Fei Teng, C. Pierce Bradley, Christina Klinger, Nhan Tran, and H-J. Joyce Wu

University of Arizona

The gut microbiota can have profound effects on an individual's health. The immune system reaction to intestinal colonization mediates a large portion of these effects. Segmented Filamentous Bacteria (SFB) is a commensal bacterium that exerts a powerful pro-inflammatory influence over the immune system, resulting, among other things, in increased antibody production. Immune responses initiated in the intestines can spread to other mucosal sites, such as the respiratory system. We hypothesized that SFB colonization would boost the T-independent II antibody response to Pneumovax-23, an anti-pneumococcal vaccine, to increase resistance to pneumonia induced by Streptococcus pneumoniae. To test this, we used a mouse model of pneumonia in mice colonized by SFB. We monitored the mice for symptoms of illness, bacterial burden, and immune response. In an immunization setting, we found that SFB+ Pneumovax-23 immunized mice are more resistant to pneumococcal pneumonia than are SFB- mice given the same vaccine. Understanding the mechanisms of protection against S. pneumoniae will contribute to devising better ways to prevent infection and design vaccine to this pathogen.

Presenter: Freise, Amanda

Immuno-PET of murine T helper lymphocytes with an anti-CD4 cys-diabody

Amanda Freise, Richard Tavaré, Kirstin Zettlitz, Felix B. Salazar, Anna M. Wu

Crump Institute for Molecular Imaging

OBJECTIVE: Investigating the immune system presents a unique challenge because immune cells traffic between and localize at multiple sites throughout the entire body. Standard diagnostic methods for assessing the dynamic immune system include biopsies, which are site-specific, and blood draws, the results of which can be variable and represent only a small fraction of the total lymphoid population. In contrast, noninvasive imaging of the presence of lymphocytes, specifically CD4+ T cells, throughout the entire body would be an improvement upon current limited methods of analysis. Selective imaging of CD4+ T cells can be accomplished with immuno-positron emission tomography (PET), which utilizes antibody-based probes to detect and quantify cell surface markers. Engineering antibodies to produce fragments allows for customization of pharmacokinetics, clearance route, and conjugation to suit in vivo imaging applications. Based on the GK1.5 rat anti-mouse CD4 antibody, we have engineered a bivalent cys-diabody (cDb) fragment for detecting CD4+ T cells in vivo. The present study aims to characterize the functional properties and imaging capability of GK1.5 cDb. METHODS: GK1.5 cDb is comprised of two single-chain variable fragments connected by a linker. A C-terminal cysteine was introduced to enable site-specific conjugation to maleimide-Alexa488, maleimide-biotin, or maleimide-desferrioxamine (malDFO), a radiometal chelator. The cDb lacks the Fc region of the parental antibody, resulting in increased clearance compared to intact antibodies and removal of Fc-mediated functionality. Flow cytometry was used to confirm GK1.5 cDb binding specificity in vitro and ELISA binding curves were performed to assess affinity. The effect of GK1.5 cDb on surface expression of CD4 on T cells in vivo was investigated by injecting GK1.5 cDb and measuring expression in various lymphoid organs over time. Functional effects of GK1.5 cDb at several doses were assessed using in vivo and in vitro T cell proliferation assays. For imaging studies, GK1.5 cDb-malDFO was radiolabeled with 89Zr and 10-16 μg (25-35 μCi) was injected into untreated, CD4-blocked (bolus co-injection of cold cDb), or CD4-depleted mice. Mice were PET scanned at 20 hours post-injection, followed by biodistribution studies. Additionally, a dose escalation study using 2, 6, 12, or 40 μg GK1.5 cDb was performed to test the effect of protein dose on imaging and biodistribution. RESULTS: Flow cytometry on murine primary lymphocytes demonstrated that GK1.5 cDb retains its specificity for murine CD4. ELISA binding curves showed that the dissociation constant of the cDb (~2.6 nM) was similar to that of the parental antibody (~0.99 nM). Administration of GK1.5 cDb in vivo resulted in temporarily decreased T cell expression of CD4, which recovered within three days. Analysis of CD3+ and CD4+ cell populations showed that GK1.5 cDb does not deplete T cells. Both in vitro and in vivo studies of CD4+ T cell proliferation showed that higher doses of cDb had an inhibitory effect on proliferation, and that lower doses did not alter proliferative capacity. In imaging and biodistribution studies, axillary LNs and spleen showed high uptake of 89Zr-radiolabeled cDb in untreated mice (45, 30% ID/g respectively), in contrast to CD4-depleted mice (3.9, 4.1% ID/g) and CD4-blocked mice (7.1, 6.1% ID/g). The dose escalation study showed that the lowest protein/radiation dose (2 μg/7 μCi) gave better target:background ratios and better targeting of spleen and LN compared to higher doses (6-40 μg/20-136 μCi). CONCLUSION: Characterization at several doses demonstrated that low-dose GK1.5 cDb did not perturb CD4+ T cell numbers or proliferative capacity, and was an effective imaging agent for CD4 expressed in lymphoid organs in wild type mice. GK1.5 cDb is a promising agent for further noninvasive investigation of CD4+ T lymphocytes in vivo.

Presenter: Fujii, Chika

ENGINEERING CD4 EGFP REPORTER MICE WITH DIFFERENT ENHANCER ENVIRONMENTS

Chika Fujii, Melpi Kasapi, Sejiro Littleton, Sophia Sarafova

Davidson College

Present on a variety of immune cells, the glycoprotein cell surface receptor CD4 plays a vital role in the development and maintenance of the immune system. Previously a proximal enhancer, distal enhancer, silencer and promoter have been described, however these regulatory elements fail to describe all of the dynamics of CD4 expression in developing T lymphocytes. Our lab has identified a highly conserved region within the first intron of Cd4 we call NCE. Our previous in vitro experiments indicate that NCE is a developmental stage specific enhancer. To observe directly the dynamic changes in CD4 expression at different stages of development in the presence or absence of NCE we wanted to generate BAC transgenic mice with a destabilized version of EGFP (t1/2=2h) inserted in the Cd4 locus. We modified BACs containing the entire Cd4 locus with or without NCE by inserting EGFP after the Cd4 translation start codon in exon 2, effectively deleting the coding part of this exon, but without affecting its splice donor and acceptor sites. After insertion of the BAC in the mouse genome, we expect EGFP expression to match that of endogenous Cd4 in the presence of NCE, but differ in the absence of NCE.

Presenter: Gerard, Audrey

Integration of IFN[g] and integrin signaling pathways regulates CD8 T cell differentiation

Audrey Gerard, Matthew F Krummel

UCSF

CD8 T cell responses to IFNγ result in diverse and sometimes antagonizing functions. In particular, IFNγ has a dual function in T cell homeostasis, promoting priming, expansion and differentiation of CD8 T cells as well as apoptosis of effector T cells. The cellular and molecular mechanisms underlying the functional dichotomy of IFNγ have not yet been identified. To answer this question, we characterized IFNγ secretion and function over time following Listeria infection. We observed a first wave of IFNγ production early after infection, which was specifically implicated in CD8 T cell differentiation. Whereas both NK cells and early activated CD8 T cells produced IFNγ, only IFNγ produced by CD8 T cells affected CD8 T cell differentiation. IFNγ is shared between recently activated CD8 T cells in the context of secondary T-T synapses, which are required for IFNγ-induced CD8 T cell differentiation. We demonstrated that integrin signaling shapes IFNγ signaling in this context. Recently activated CD8 T cells have impaired Stat1 tyrosine, but not serine, phosphorylation following IFNγ exposure. Integrins can rescue Stat1 tyrosine phosphorylation through Src kinases activation. This suggests that IFNγ leads to differential gene expression patterns according to the microenvironment (i.e whether IFNγ is received in the context of contacts), thereby dictating the functional outcome of recently activated CD8 T cells. Deciphering how IFNγ specific outcomes are established and regulated will be crucial to design better-targeted therapies, allowing us to trigger a specific functional outcome.

Presenter: Ghosh, Debopam

An atypical splenic progenitor population supports antibody production during Plasmodium infection in mice.

Debopam Ghosh, Brian Kennedy, Johnasha Stuart, Jason S. Stumhofer

UAMS

Hematopoietic stem and progenitor cells (HSPCs) function to maintain the immune cell repertoire in the steady state, as well as during emergency situations including infection or stress. Although the bone marrow serves as the primary site of hematopoiesis, extramedullary mobilization and differentiation of HSPCs occurs in the spleen – a critical step in the host immune response during acute Plasmodium infection. Here, we have identified an atypical HSPC population in the spleen of C57BL/6 mice with a Lineage-Sca-1+c-kit- (LSK-) phenotype. These LSK- cells were found to expand in response to acute P. yoelii 17X infection and upon transfer into naïve congenic mice they differentiated predominantly into mature follicular B cells. However, when transferred into infection-matched hosts, LSK- cells matured into B cells capable of responding to Plasmodium by differentiating into germinal center and memory B cells, as well as plasma cells that secreted parasite-specific antibodies. Incubation with parasitized RBC lysate, enhanced the ability of splenic LSK- cells to differentiate into B cells in vitro, suggesting that a component of the parasite directly stimulates expansion and differentiation of LSK- cells. However, differentiation of LSK- cells into B cells after infection was independent of MyD88 signaling. Collectively, we have identified a population of atypical lymphoid progenitor cells in the spleen that is capable of differentiating into B cells in response to Plasmodium infection and ultimately contributes to the overall humoral response by producing antigen-specific antibodies.

Presenter: Glassman, Caleb

The CD4 and CD3de cytosolic juxtamembrane regions are proximal within a compact TCR-CD3-pMHC-CD4 macro-complex

Caleb Glassman, Author Names separated by commas

University of Arizona

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Presenter: Glatigny, Simon

Integrin alpha-4 is required for regulatory B cell control of experimental autoimmune encephalomyelitis

Simon GLATIGNY, Catriona A Wagner and Estelle Bettelli

Benaroya Research Institute

The neutralization of alpha-4 integrin (Itga4) is currently used as treatment in multiple sclerosis. While most studies focused on its function on lymphocyte migration to the central nervous system, we have uncovered the importance of Itga4 expression on B cells for the generation of regulatory B cells in peripheral immune organs and their control of pathogenic T cell response and CNS pathology. Our study underscores the importance of looking at the dual role of B cells in CNS autoimmunity and provides important perspectives regarding the efficacy and side effects associated with Itga4 neutralization and other B cell targeting therapies.

Presenter: Goetz, Anton

Dendritic-T cell interactions in blood stage malaria: the role of reactive oxygen species

Anton Goetz, Maureen Ty, Ana Rodriguez

NYU School of Medicine

Malaria is characterized by high levels of inflammation, and while an early inflammatory response contributes to parasite clearance, excessive and persistent inflammation can lead to severe forms of the disease. At the same time, malaria infections fail to induce durable immunological memory and knowledge of antimalarial immunity is incomplete. Very little is known about the role dendritic cells (DCs) play in the immune response to Plasmodium and how they contribute to activation of CD4+ T cells during blood stage malaria. To address the role of this critical part of the malaria immune response we purified primary human DCs from peripheral blood of healthy naïve donors and co-incubated them with blood stage P. falciparum in vitro. The Although DCs up-regulated surface expression of HLA-DR and co-stimulatory markers, they did not secrete significant amounts of cytokines. Since the lack of cytokine production seems contradictory to the pathogenesis of the disease, especially accompanying up-regulation of activation markers on DCs, we hypothesized that there might be another inflammatory factor involved in vivo. Xanthine oxidase (XO), an enzyme that produces reactive oxygen species, is increased during malaria infection, positively correlates with the severity of the disease, and can activate immune cells. Supplementation of the cultures with XO rescued cytokine secretion by DCs to LPS control levels. We then co-incubated P. falciparum-activated DCs with naïve autologous CD4+ T cells. Although parasite-activated DCs secreted only low levels of cytokines, they were able to activate and polarize naïve CD4+ T cells into Th1 effector cells. A re-stimulation with autologous P. falciparum-activated DCs specifically increased proliferation and cytokine secretion of those primed CD4+ T cells compared to control DCs. Addition of XO to co-cultures increased an initial proliferation response but ultimately lead to low total numbers of responsive T cells, indicating a modulatory effect of XO-produced reactive oxygen species on the immune response to malaria. Our findings might contribute to a better understanding of the mechanisms leading to the absence of sterile immunity in malaria which is relevant for future vaccine designs and therapeutic interventions.

Presenter: Gorman, Jacob

Regulation of human Th17 cell differentiation by the DNA-binding transcription factor TEAD4

Jacob Gorman, Christina Stracener, Bonnie Swerdlow, Geoffrey L. Stephens, Lorraine Clarke, Michael Fung, and Tomas Mustelin

MedImmune

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Presenter: Goulding, John

A role for hypoxia inducible factors in CD4+ T cell differentiation

John Goulding, Laura Shaw, Kyla Omilusik & Ananda Goldrath

University of California, San Diego

Immune cells are unique in their ability to migrate and respond dynamically to tissue specific contextual cues. It is increasingly apparent that established and transient micro-environmental niches that exist during homeostasis and tissue inflammation may influence cell fate decisions and subsequent function. Recent evidence suggests that hypoxia-inducible factors (HIFs), components of the transcriptional response to low oxygen levels, can influence the extent to which CD4+ T cells differentiate into specific T helper subsets. To further investigate this possibility, we utilized CD4+ T cells that lacked components of the HIF / Von Hippel-Lindau (VHL) pathway to dissect the implication of these signals on CD4+ T cell responses. We find that CD4+ T cells lacking HIF-1α and HIF-2α have altered CD4+ T cell subset differentiation as measured by archetypical cytokine production and master regulator transcription factor expression. Collectively these data demonstrate the importance, and growing need, to better understand how local micro-environmental cues can influence the many transcriptional programs and molecular pathways that regulate CD4+ T cell differentiation. This understanding will inform efforts to better develop efficacious vaccines and therapeutic cell therapies.

Presenter: Gray, Elizabeth

The AIM2-like receptors are dispensable for activation of the interferon stimulatory DNA (ISD) pathway.

Elizabeth Gray, Dan Stetson

University of Washington

Detection of intracellular DNA triggers activation of the STING-dependent interferon-stimulatory DNA (ISD) pathway, which is essential for antiviral immune responses; on the other hand, inappropriate immune responses to self DNA result in autoimmunity. Multiple DNA sensors have been proposed to activate the ISD pathway, including cyclic GMP-AMP synthase (cGAS) and a family of DNA binding receptors called the AIM2-like receptors (ALRs). Analysis of cGAS-deficient mice has revealed that cGAS is a key DNA sensor that is required for activation of the ISD pathway; however, whether the ALRs contribute to this pathway remains unclear. Here, we generated mice lacking all 13 mouse ALR genes as a novel tool to explore the function of the ALRs. We show that all ALRs are dispensable for the type I interferon (IFN) response to transfected DNA ligands, DNA virus infection, and lentivirus infection. We also show that the DNA sensor cGAS, but not the ALRs, is required to drive autoimmune disease in the Trex1-deficient mouse model of Aicardi-Goutieres Syndrome. Finally, we used CRISPR to disrupt the human AIM2-like receptor IFI16 in primary human fibroblasts and show that IFI16 is dispensable for the IFN response to transfected DNA ligands as well as human cytomegalovirus (HCMV) infection. Thus, our data reveal that ALRs are dispensable for activation of the ISD pathway and demonstrate that cGAS is the primary DNA sensor that drives the IFN response to DNA.

Presenter: Halec, Gordana

CD40L-carrying HIV-virions stimulate B cell activation in human immune system mice

Gordana Halec, Jonathan Said, Otoniel Martínez-Maza, Marta Epeldegui

Obstetrics & Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, California

Background: HIV infection is known to be associated with chronic B cell hyperactivation. This has significant pathogenic relevance, as B cell activation is associated with enhanced risk for non-Hodgkin lymphoma (NHL), the most common malignancy in AIDS individuals in the post-HAART era. One of the underlying mechanisms of AIDS-NHL is a constant stimulation of B cells by immune stimulatory molecules present in HIV plasma membrane envelope after budding of virions from infected cells. In vitro work from our (Epeldegui et al., 2010) and other laboratories, has demonstrated that one of these crucial molecules is CD40L/CD154. Aim: To confirm the in vitro findings, we aimed to mimic in vivo HIV-induced B cell hyperactivation and early lymphomagenic effects using human immune system (HIS) mouse model which uses NOD/SCIDcG-/- (hNSG) mice implanted with human thymus/liver tissue at 6-8 weeks of age, and subsequently (3 weeks later) injected intravenously with human fetal liver CD34+ hematopoietic stem cells (huBLT). We have injected HIS mice with HIV virions carrying functional human CD40L (CD40L+ HIV), or non-functional CD40L mutant (T147N+ HIV), and compared them with the non-stimulated HIS mice (MOCK group). Results: All mice (total 15 mice with 5 mice per group) were successfully reconstituted and expressed =40% of human CD45+ cells in their blood across the span of 12 weeks as measured by flow cytometry. In addition, in spleens of all mice, we could confirm formation of while pulp into structures corresponding to germinal centers in the human spleen. Immunohistochemical staining of murine spleens revealed a pattern of clustered cells stained positive for cell membrane associated CD10 protein, while positivity for BCL-6 protein was found scattered across the tissue in

Presenter: Harms Pritchard, Gretchen

A role for T-bet in coordinating T cell activation

Gretchen Harms Pritchard, David Christian, Nathan Roy, Janis Burkhardt, Christopher Hunter

University of Pennsylvania

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Presenter: Higdon, Lauren E.

Polyfunctionality of CD8 T Cell Responses to Cytomegalovirus in Transplant Patients

Lauren E. Higdon, Kenneth B. Margulies, Jonathan S. Maltzman

Stanford University, University of Pennsylvania

Polyfunctionality, or production of multiple cytokines and effector molecules, is important in the control of chronic viral infections, such as cytomegalovirus (CMV). CMV infection causes significant morbidity and allograft damage in transplant recipients. Primary CMV infection is rapidly resolved in healthy individuals, and memory T cells control reactivation of latent virus. IFNγ production by CD8 T cells is important for control of CMV, but other CD8 effector functions have not been evaluated in detail in the context of immunosuppression and transplantation. We have analyzed memory T cell responses to two CMV polypeptides, pp65 and IE-1, focusing on the effect of transplant-associated lymphodepletion on T cell specificity and polyfunctionality. In this study, polyfunctionality was defined as co-expression of IFNγ, TNFα, and/or the degranulation marker CD107a. Our analyses were conducted using pre- and up to one year post-transplant PBMC from renal and cardiac transplant patients. All patients received anti-viral prophylaxis and immunosuppression consisting of steroid, calcineurin inhibitor, and cell cycle inhibitor. A subset of transplant patients also received T cell depleting induction therapy. Our results show that pre-transplant and in normal donors, the 11 patients and 4 controls studied thus far can be divided into three groups based on dominant antigen specificity: IE-1, pp65, or equal response to both. The antigen-specific CD8 T cell population contained a similar proportion of cells expressing all three functions (~35%) in patients with IE-1 and pp65 dominance, but a lack of dominance was correlated with a lower proportion of polyfunctional cells (~20%). Cells expressing IFNγ were more likely to be polyfunctional than monofunctional. In addition, the cells expressing two of the three markers were almost uniformly TNFα positive. Some patients had early lymphodepletion-associated decreases in polyfunctional cells, but all returned to a pre-transplant proportion of CD8 T cells by day 180 post-transplant. In addition, the skewing of fine specificity to IE-1 or pp65 was maintained over the first year post-transplant in 8 of 11 patients. In conclusion, pre-transplant TNFα expression is predictive of polyfunctionality both pre- and post-transplant. While long-term maintenance of polyfunctional cells appears to be unaffected by lymphodepletion, further study will be needed to determine whether lymphodepletion has an effect on fine antigen specificity.

Presenter: Hofmann, Christian

Fast Identification and Assembly of HIV-1-Specific T Cell Receptor Gene Therapy Lentiviral Vectors

Christian Hofmann, Christian Hofmann, Christian-Raul Aguilera-Sandoval, Balamurugan Arumugam, Priya K. Patel, Brian Diep, Sangeun Park, Hwee Ng, Otto O. Yang

Division of Infectious Diseases, Department of Medicine, UCLA, Los Angeles

Background: Immune surveillance through HIV-1-specific CD8+ cytotoxic T lymphocytes (CTL) is crucial for the long-term control of HIV-1 replication in vivo. However, most HIV-1-infected persons lack the ability to control the virus with their T cell receptor (TCR) repertoire, due to incomplete coverage of epitope variation. A potential immunotherapeutic strategy that would provide patients with the ability to control the virus without taking medication could be the adoptive transfer of gene-modified T cells, equipped with a combination of TCRs covering all common variants of their targeted epitope. We developed a rapid methodology to identify epitope-specific TCR sequences and insert them into lentiviral vectors without the need for HLA tetramers, cell sorting, or cell cloning. Methods: We focused on five HIV-1 Gag epitopes based on common or protective HLA type and epitope conservation: KRWIILGLNK263-272 (KK10, HLA-B*2705), KAFSPEVIPMF162-172 (KF11, B*5701), GLNKIVRMY269-277 (GY9, B*1501), RQANFLGKI429-437 (RI9, B*1302), and WASRELERF36-44¬ (WF9, B*3501). We identified Gag-specific TCR sequences by expansion after epitope stimulation using a quantitative spectratyping method. Via a one-step reaction we cloned both TCR α and β chains into a lentiviral vector and the functionality of these TCRs was subsequently determined by a Jurkat cell NFAT-dependent GFP reporter assay. Additionally, TCR-transduced primary CD8+ T cells were tested in a chromium release assay against HIV-1-infected T1 cells transduced with the relevant HLA genes. Results: We cloned and functionally tested seventeen TCRs against different HIV-1-specific epioptes (six for KK10, four for KF11, five for GY9, one for RI9, and one for WF9). Several TCRs have been screened for their recognition of epitope variants and functional avidity using synthetic peptides, Conclusions: Our data demonstrate proof-of-concept for rapid TCR cloning into vectors suitable for gene therapy and capacity to screen these TCRs for the ability to recognize epitope variants. This work sets the foundation for combination TCR gene therapy to cover epitope variation.

Presenter: Hsu, Lih-Yun

Destablizing autoinhibitory conformation of ZAP-70 induces thymic upregulation of inhibitory receptors and immuological unresponsivenees

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

University of California, San Francisco

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Presenter: Hudig, Dorothy

Quantitative assay of human antibody-dependent cell-mediated cytotoxicity (ADCC) using peripheral blood mononuclear cells (PBMCs)

Dorothy Hudig, Alexander P. Sung, Jia Jie Jennifer Tang, Michael J. Guglielmo, Doug Redelman, Isabel Barao Silvestre, Julie Smith-Gagen

University of Nevada School of Medicine

Enter your abstract's text here. ADCC can be essential for anti-viral immunity and supports many tumor immunotherapies. It is a challenge to quantify and one wants a fast assay that uses few lymphocytes when patient blood is limited. Three considerations affected our new assay design: 1) normal B cells within PBMCs introduce 'cold target' competition of radiolabeled tumor B cells if anti-B cell antibodies are present in the ADCC assays; 2) type 1 antibodies to CD20 B cell epitopes are internalized and cleared from the cell surface while type 2 anti-CD20 antibodies remain external; and 3) target cells with MHC I initiate KIR-regulation of lysis that will vary among donors. To address these issues, we used Daudi B leukemia cells that lack MHC I and pretreated the Daudi cells with the type 2 anti-CD20 humanized monoclonal antibody. Target cell pretreatment prevented 'cold target' competition. We used 4 hour 51Cr-release for its sensitivity and varied the PBMC to target (E:T) cell ratios to obtain lytic units. Flow cytometric counts of CD16A Fc-receptor positive cells with TrueCountR beads determined the exact number of ADCC effectors within the unfractionated PBMCs. There was low NK activity to Daudi cells, which required subtraction of simultaneous controls without antibody. We plotted ADCC activity as a log function of the E:T ratios and observed that ADCC was dependent upon interactions of multiple CD16A-positive effectors per target cell, which was expressed as lytic units per 106 CD16A cells, that varied 50-fold among donors. In addition, the linear slopes of CD16A cell lytic cooperativity varied 8-fold. In summary, we have established a fast quantitative assay for comparison of human ADCC that uses as few as 8 million PBMCs.

Presenter: Kalekar, Lokesh A.

CD4+ T cell anergy prevents autoimmunity and generates regulatory T cell precursors

Lokesh A. Kalekar, Shirdi E. Schmiel, Na Zhang, Gretta L. Stritesky, Deepali Malhotra, Kristin A. Hogquist, Marc K. Jenkins, Daniel L. Mueller

Center for Immunology, University of Minnesota - Twin Cities

Selective suppression of effector T cell functions in the periphery is required for preventing immune cell-mediated damage to healthy tissues. This is especially true during many health conditions such as pregnancy and organ transplants. Anergy, an acquired state of T cell functional unresponsiveness, is one way in which this suppression can be achieved. However, anergy as a peripheral tolerance mechanism remains poorly understood. In this study, we demonstrate that anergy is selectively induced in fetal antigen-specific maternal CD4+ T cells during pregnancy. A naturally occurring subpopulation of anergic polyclonal CD4+ T cells, enriched in self antigen-specific T cell receptors, is also observed in healthy hosts. Neuropilin-1 expression in anergic conventional CD4+ T cells is associated with thymic regulatory T cell (Treg cell)-related gene hypomethylation, and this correlates with their capacity to differentiate into Foxp3+ Treg cells that suppress immunopathology. Thus, our data suggest that not only is anergy induction important in preventing autoimmunity, but it also generates precursors for peripheral Treg differentiation.

Presenter: Kanemaru, Kazumasa

Tie2 Signaling Enhances Mast Cell Progenitor Adhesion to Vascular Cell Adhesion Molecule-1 (VCAM-1) through [a]4[b]1 Integrin

Kazumasa Kanemaru, Emiko Noguchi, Takahiro Tokunaga, Kei Nagai, Takashi Hiroyama, Yukio Nakamura, Satoko Tahara-Hanaoka, Akira Shibuya

Department of Immunology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan.

Mast cell (MC) activation contributes considerably to immune responses, such as host protection and allergy. Cell surface immunoreceptors expressed on MCs play an important role in MC activation. Although various immunoreceptors on MCs have been identified, the regulatory mechanism of MC activation is not fully understood. To understand the regulatory mechanisms of MC activation, we used gene expression analyses of human and mouse MCs to identify a novel immunoreceptor expressed on MCs. We found that Tek, which encodes Tie2, was preferentially expressed in the MCs of both humans and mice. However, Tie2 was not detected on the cell surface of the mouse MCs of the peritoneal cavity, ear skin, or colon lamina propria. In contrast, it was expressed on mouse bone marrow–derived MCs and bone marrow MC progenitors (BM-MCps). Tie2 is a receptor tyrosine kinase containing two tyrosine kinase domains in the cytoplasmic portion. Upon binding with its ligand Angiopoietin-1 (Ang1), Tie2 mediates an activating signal. Since Ang1 is expressed by peri-endothelial mural cells, we hypothesized that Tie2 would be involved in the transmigration of MCps across the vascular endothelium. Indeed, in vitro assay demonstrated that Tie2 signaling enhanced MCp adhesion to VCAM-1 via α4β1 integrin. These results suggest that Tie2 play an important role in MCp adhesion to VCAM-1 on vascular endothelial cells and transmigration to inflammatory sites.

Presenter: Kovar, Marek

New insight into structure and biology of IL-2/anti-IL-2 mAb complexes

Marek Kovar, Jamie B. Spangler, Jakub Tomala, Vincent C. Luca, Kevin M. Jude, Petra Weberova and K. Christopher Garcia

Laboratory of Tumor Immunology, Institute of Microbiology CAS, v.v.i.; Department of Structural Biology, Stanford University School of Medicine

IL-2 is potent imunostimulatory 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 to either dimeric receptor composed from IL-2Rß (CD122) and common cytokine receptor gamma chain (?c, CD132) or trimeric receptor composed from IL-2Ra, IL-2Rß and ?c. 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 posses 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 found that JES6-1 mAb sterically blocks the interaction of IL-2 with both CD122 and CD132. Moreover, it also allosterically weakens the interaction of IL-2 and CD25 by decreasing affinity of JES6-1 mAb-bound IL-2 to CD25 through a "triggered exchange" mechanism thus favoring CD25high Treg cells in competition for such bound IL-2. On the other hand, S4B6 mAb sterically blocks the interaction of IL-2 with CD25 but it also increases the affinity of S4B6 mAb-bound IL-2 to CD122. This explains why IL-2/S4B6 complexes stimulate all IL-2 responding cells to certain level but much stronger CD122high cells. Further, we found that IL-2/S4B6 complexes possess significant antitumor activity and IL-2/JES6 complexes can be used to extremely expand Treg cells in vivo which in turn protects against DSS-induced experimental colitis (model of autoimmune disease) and enables the growth of tumor cells expressing xenogeneic protein (model of transplantation). We have also shown that IL-2/S4B6 complexes can be produced as fused recombinant protein which exerts in vitro activity comparable to analogical complex and possibly higher activity in vivo. Acknowledgement This work was supported by Czech Science Foundation grant 13-12885S, by the company SOTIO (HS0045) and by Institutional Research Concept RVO 61388971.

Presenter: Krishnamurty, Akshay

Germinal-center derived, somatically hypermutated Plasmodium-specific IgM+ memory B cells are optimally responsive to a secondary blood stage malaria infection

Akshay Krishnamurty, Chris Thouvenel, Gladys Keitany, Brian Hondowicz, Peter D. Crompton, David J. Rawlings, and Marion Pepper

University of Washington

Humoral immunity consists of pre-existing antibodies expressed by long-lived plasma cells (LLPCs) and rapidly reactive memory B cells (MBCs). Classically defined MBCs express class-switched, somatically hypermutated BCRs after undergoing a germinal center (GC) reaction to produce high affinity antibodies within days of infection, making these cells the gold standard target of most vaccine platforms. More recently, this homogenous view of MBCs has been challenged and it is now recognized that diverse MBC subsets exist in both mice and humans. We hypothesized that the development of a system to visualize endogenous populations of distinct MBC subsets that form in response to infection could be used to elucidate the identity and function of various MBC subsets. In this study, we chose to focus on the development of the humoral immune response to Plasmodium, the causative agent of malaria, in which B cells play a critical role in immune protection. To clarify roles for various MBC subsets during malaria infection, novel B cells tetramers were generated containing the Plasmodium-specific protein Merozoite Surface Protein 1 (MSP1) to provide the first direct ex vivo visualization of antigen-specific MBC development and function during blood stage malaria infection in a murine model of malaria, Plasmodium chabaudi. Using these novel reagents we identified distinct long-lived MSP1-specific MBC subsets exist including classically defined, somatically hypermutated, isotype-switched IgG+ MBCs that express CD73 and CD80 and a GC-independent IgMloIgD+ (IgD+) unswitched population that more closely resemble naïve B cells. Interestingly, a third IgM+IgDlo (IgM+) unswitched MBC population also persisted, expressed both CD73 and CD80 and somatically hypermutated BCRs, and was also derived in a GC-dependent manner, more closely resembling IgG+ MBCs. Within three days of a secondary malaria infection, in a T cell dependent manner, MSP1-specific IgM+ MBCs outcompeted clonally related IgG+ MBCs to rapidly form antibody-secreting plasmablasts resulting in an increase in serum IgM antibody levels prior to any IgG+ antibodies. These studies therefore demonstrate that a previously unrecognized population of malaria-specific, somatically hypermutated IgM+, germinal center-derived MBCs can outcompete switched MBC subsets in response to a secondary malaria infection and should an important population targeted by anti-malarial vaccine strategies.

Presenter: Kuan, Emma

Non-tumor derived thymic stromal lymphopoietin regulates breast tumor progression through Ly6Chi monocytes

Emma Kuan, Steven Ziegler

Benaroya Research Institute

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Presenter: Linehan, Jonathan L.

Factors controlling the induction of commensal specific CD8+ T cells in skin

Jonathan L. Linehan, Nicolas Bouladoux, Shruti Naik, Seong-Ji Han, Allyson Byrd, Yasmine Belkaid

NIH-NIAID

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Presenter: Lund, Amanda

Lymphatic vessels are required for efficient viral clearance and adaptive immune induction following epicutaneous vaccinia infection

Amanda Lund, Christopher Loo, Jamie Booth, Ryan Lane, Jeffrey C. Nolz

Oregon Health & Science University

Lymphatic vessels regulate fluid homeostasis and cell trafficking and their aberrant growth is associated with a variety of disease states, however, it remains unclear as to how lymphatic remodeling directly influences pathogen clearance and resulting immune pathology. In this study we used epictuaneous vaccinia (VacV) infection as a model of peripheral challenge. Infection with VacV results in local infection with peak titers by day three and complete clearance of virus from the infected tissue by day 15. Virus-associated lymphatic remodeling was interestingly associated with a decrease in functional lymphatic vessel fluid drainage. Evaluation of lymphatic endothelial cell surface phenotype demonstrated a rapid increase in expression of ICAM-1 and E-selection but not VCAM or P-selectin, which was associated with accumulation of migratory dendritic cells in draining lymph nodes. In the absence of dermal lymphatic vessels (K14 VEGFR3-Ig) immune pathology was enhanced and viral clearance significantly delayed. In the infected tissue microenvironment of these mice there was a notable lack of infiltrating T lymphocytes, thus we evaluated the ability of K14 VEGFR3-Ig mice to initiate an antigen-specific CD8 T cell response following infection. We followed the expansion of antigen-specific CD8 T cells (P14) in K14 VEGFR3-Ig mice or littermate controls. P14 T cells were undetectable at day 7 in draining lymph nodes of K14 VEGFR3-Ig mice and remained undetectable through day 15, while populations in littermates expanded through day 10 and began to contract into day 15. Similarly, no P14 T cells were found in spleens or in infected ears at day 7, however, interestingly were expanded in both these compartments by day 15 suggesting compensatory mechanisms driving T cell priming systemically. In addition we also observed delayed humoral immunity as detected by circulating anti-VacV IgG. Serum antibodies were undetectable at day 10 in K14 VEGFR3-Ig mice but also recovered with time, though their long-term stability appeared impaired. This data demonstrates a requirement for lymphatic vessels in the generation of a potent, antigen-specific adaptive immune response to local, peripheral challenge.

Presenter: Meng, W.

The Effect of Prostate Tumor Microenvironment on Macrophage Aggressiveness

W. Meng, E. G. Weagel, R. A. Brog, M. H. Townsend, E. J. Velazquez, R. A. Robison, K. L. O'Neill

Brigham Young University

Cancer is the second leading cause of death in the United States. The immune system plays an important role in the development and progression of cancer. Infiltration of macrophages to the tumor site has been shown to account for up to 50% of the tumor mass in some solid tumors, suggesting that macrophages have a significant role in tumor progression. Macrophages derive from the myeloid lineage and belong to the innate immune system. Their main function is to phagocytose microbes and clear cellular debris. Two major macrophage phenotypes have been proposed: M1 and M2. M1 macrophages show a pro-inflammatory profile that is characterized by aggressive phagocytosis and anti-microbial properties. M2 macrophages exhibit immunomodulatory, repair, and angiogenesis properties, and have an anti-inflammatory profile. We aimed to explore the effect that prostate cancer cell lines (DU145 and PC3) have on macrophage function by studying engulfment rates and cytokine profiles. In brief, we stimulated a monocytic cell line (U937) with PMA for 24 hours into macrophages, and incubated these macrophages with either prostate cancer cells or their spent media. Macrophages were then collected to be analyzed by the engulfment assay for their aggressiveness. The engulfment rate was measured by incubating macrophages with PE-cojugated beads and using flow cytometry to determine the amount of beads intake by macrophages through phagocytosis. We found that prostate cancer cells exhibit a radical suppression on macrophage aggressiveness. We also found that media had a lower effect on macrophage engulfment overall, indicating cell to cell contact is important to suppress macrophage engulfment. We also studied how these prostate cell lines affected macrophage phenotypes using qPCR to analyze cytokine expression levels, and found that lower engulfment rates correlate with an anti-inflammatory cytokine profiles, suggesting an M2 phenotype. Further studies will help us understand more of the signals that cancer cells give to their environment to down-regulate macrophage engulfment and to help maintain an M2 phenotype.

Presenter: Mercer, Frances

Immune Subversion by the Protozoan Parasite Trichomonas vaginalis

Frances Mercer, Fitz-Gerald Diala, Shek Hang Ng, and Patricia J. Johnson

UCLA

Trichomonas vaginalis is an extracellular protozoan parasite that causes the most common non-viral sexually transmitted infection. While acute symptoms in women may include vaginitis, asymptomatic or untreated infections can persist and are associated with increased HIV susceptibility, infertility, pre-term labor, and higher incidence of cervical cancer. Heightened inflammation is attributed to complications of T. vaginalis infection, however effective cellular immune responses to the parasite have not been characterized. Additionally, re-infection is common; suggesting poor effectiveness of an adaptive immune response. In vivo studies in humans and mice have indicated neutrophil (PMN) recruitment as the major cellular immune response to T. vaginalis infection, but PMN effectiveness at combating the parasite is not known. We established an in vitro co-culture system to assess the interaction between T. vaginalis and primary human leukocytes, using Flow Cytometry and Imaging Flow Cytometry. Cytokine analysis of supernatants from co-culture of T. vaginalis with human monocytes shows a cytokine program dominated almost exclusively by IL-8 secretion, a PMN recruitment chemokine. We also noted some striking differences between common laboratory strains and some clinical isolates of T. vaginalis. Firstly, some clinical isolates are able to lyse T-cells and B-cells, pointing to lymphocyte lysis as a potential adaptive immune subversion strategy. Secondly, PMN are able to efficiently kill laboratory strains of T. vaginalis; however several clinical isolates are resistant. Preliminary data indicate that PMN killing of T. vaginalis proceeds through an engulfment mechanism. Future experiments will determine the molecular players in PMN engulfment and killing of T. vaginalis, and the molecular determinants of PMN subversion by resistant strains.

Presenter: Mittelsteadt, Kristen

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

Kristen Mittelsteadt, Jenna Sullivan, Michael Stolley, Daniel J Campbell

University of Washington, Benaroya Research Institute

Foxp3+ regulatory T cells (Treg) are critical for maintaining immune tolerance and preventing inflammatory disease. Given their potent immunosuppressive capabilities, manipulation of Treg function and/or abundance is a promising therapeutic strategy to either augment or inhibit immune responses in the context of disease. A detailed understanding of the key factors that control Treg development, homeostasis, and function is essential for the successful application of Treg-based therapies. Recent work suggests that inducible T cell costimulator (ICOS) signaling is required for maintenance of effector Treg (eTreg) cells, which migrate to peripheral sites of inflammation. ICOS ligation is most notably a potent activator of phosphatidylinositol 3-kinase (PI3K), however the key signaling pathways downstream of ICOS that support eTreg have not been investigated. In order to study the role of ICOS-dependent PI3K signaling, we obtained mice that carry a knock-in mutation in the Icos gene that alters the cytoplasmic tail motif of the ICOS protein, thereby specifically abolishing ICOS-mediated PI3K activation (IcosY181F mice). The frequency and number of Treg is reduced in these mice, as is the proliferation of ICOShi Treg in the spleen. Early thymic frequencies are altered in IcosY181F thymii, suggesting a developmental bottleneck at the early stages of T cell development in the absence of ICOS-dependent PI3K activation. Furthermore, IcosY181F hematopoietic cells exhibit a selective disadvantage in repopulating peripheral T cell compartments as well as early bulk thymocyte populations compared to WT cells in mixed bone marrow chimeras. To assess function of IcosY181F Treg, we subjected mice to a model of experimental autoimmune encephalomyelitis. IcosY181F mice develop more severe disease earlier than WT controls and have lower eTreg frequencies in the brain and spinal cord. Taken together, preliminary data from our lab suggests an important role for ICOS-mediated PI3K activation in the development, maintenance, and function of Treg.

Presenter: Mueller, Christoph

TREM-1 links dyslipidemia to monocytosis, inflammation and lipid deposition in atherosclerosis

Christoph Mueller, Daniel Zysset, Benjamin Weber, Silvia Rihs, Jennifer Brasseit, Stefan Freigang, Yara Banz, Carsten Riether, Adelheid Cerwenka, Cedric Simillion, P. Marques-Vidal, Leslie Saurer

Institute of Pathology, University of Bern, Switzerland

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a potent amplifier of pro-inflammatory innate immune responses. Its contribution to microbial-induced septic shock is well established, however, the significance of TREM-1 in non-infectious diseases remains less clear. Here, we demonstrate that TREM-1 promotes cardiovascular disease by exacerbating atherosclerotic lesion progression. We find that TREM-1 is expressed in advanced atherosclerotic plaques of human patients and that TREM-1 was highly induced under dyslipidemic conditions on circulating myeloid cells and on lesion-infiltrating monocyte/macrophages in the Apoe-/- mouse model. Furthermore, TREM-1 strongly contributed to the high fat, high cholesterol diet (HFCD)-induced monocytosis in vivo and synergized with HFCD serum-derived factors to promote the pro-inflammatory cytokine responses and foam cell formation of human monocyte/macrophages in vitro. Accordingly, TREM-1-deficient Trem1-/- Apoe-/- mice showed greatly reduced myeloid cell infiltration in their atherosclerotic plaques and were substantially protected from diet-induced atherogenesis. Collectively, our findings illustrate that dyslipidemia induces TREM-1 surface expression on myeloid cells and subsequently synergizes with TREM-1-signaling to enhance monocytosis, pro-atherogenic cytokine production and foam cell formation.

Presenter: Mujal, Adriana

A septin requirement differentiates autonomous and contact-facilitated T cell proliferation

Adriana Mujal, Julia Gilden, Audrey Gerard, Makoto Kinoshita, Matthew Krummel

UCSF

An efficacious immune response requires both rapid antigen-specific expansion of activated T cells, as well as homeostatic turnover and maintenance of naïve and memory T cells. Although these proliferative processes are driven by distinct or synergistic cues of T cell receptor (TCR) signaling or cytokines, it has been thought that equivalent cellular machinery is used to undergo cell division. In particular, the septin cytoskeleton, which consists of a family of GTP-binding proteins, is highly conserved in its role in cytokinesis. One striking exception to the requirement of septins for mammalian cytokinesis, however, has been T cells. Yet, in investigating T cells that lack the septin cytoskeleton, we found that successful cell division has discrete septin –dependent and –independent pathways. Septin-deficient CD8+ T cells undergo robust proliferation when activated by antigen-presenting cells (APCs), but exhibit cytokinetic failure following cytokine-driven division. Surprisingly, APCs facilitate septin-independent cell division through cell-cell contacts and phosphoinositide 3-kinase (PI3K) signaling provided by co-stimulatory and integrin molecules. We could differentiate cytokine- versus antigen-driven proliferation in vivo and thus uncover the potential to selectively target detrimental bystander or homeostatic cytokine-driven proliferation without impacting conventional antigen-specific T cell expansion.

Presenter: Naradikian, Martin

IL-4 and IL-21 reciprocally regulate a unique T- BET driven phenotype in B cells

Martin Naradikian, Susanne Linderman, Lucas Woods, Daniel Beiting, Rosanne Spolski, E. John Wherry, Christopher Hunter, Scott Hensley, Warren J. Leonard, and Michael P. Cancro

University of Pennsylvania

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Presenter: Niizuma, Kouta

Identification and Characterization of CD300H, a New Member of the Human CD300 Immunoreceptor Family

Kouta Niizuma, Satoko Tahara-Hanaoka, Emiko Noguchi, Akira Shibuya

University of Tsukuba, Japan

Recruitment of circulating monocytes and neutrophils to infection sites is essential for host defense against infections. Here, we identified a previously unannotated gene that encodes an immunoglobulin-like receptor, designated CD300H, which is located in the CD300 gene cluster. CD300H has a short cytoplasmic tail and associates with the signaling adaptor proteins, DAP12 and DAP10. CD300H is expressed on CD16+ monocytes and myeloid dendritic cells. Ligation of CD300H on CD16+ monocytes and myeloid dendritic cells with anti-CD300H monoclonal antibody induced the production of neutrophil chemoattractants. Interestingly, CD300H expression varied among healthy subjects, who could be classified into two groups according to "positive" and "negative" expression. Genomic sequence analysis revealed a single-nucleotide substitution (rs905709 (G/A)) at a splice donor site on intron 1 on either one or both alleles. The International HapMap Project database has demonstrated that homozygosity for the A allele of single nucleotide polymorphism (SNP) rs905709 ("negative" expression) is highly frequent in Han Chinese in Beijing, Japanese in Tokyo, and Europeans (A/A genotype frequencies 0.349, 0.167, and 0.138, respectively) but extremely rare in Sub-Saharan African populations. Together, these results suggest that CD300H may play an important role in innate immunity, at least in populations that carry the G/G or G/A genotype of CD300H.

Presenter: Nolz, Jeffrey C.

Local Antigen in Non-Lymphoid Tissue Promotes Resident Memory CD8+ T cell Formation During Viral Infection

Jeffrey C. Nolz, Tahsin N. Khan, Jana L. Mooster, Augustus M. Kilgore, Jossef F. Osborn

Oregon Health and Science University

Tissue-resident memory (TRM) CD8+ T cells are functionally distinct from their circulating counterparts and are potent mediators of host protection against re-infection. One largely accepted paradigm is that activated CD8+ T cells are able to freely migrate to non-lymphoid tissues and differentiate into TRM independent of local antigen recognition. In contrast, using skin infections with Vaccinia virus (VacV) expressing model antigens, we found that local antigen recognition had a profound impact on TRM formation. Activated CD8+ T cells trafficked to VacV-infected skin in an inflammation-dependent, but antigen-independent manner. However, following viral clearance, there was a subsequent ~50-fold increase in CD103+/CD69+ TRM formation when antigen was present in the tissue microenvironment. Secondary antigen stimulation in non-lymphoid tissue caused CD8+ T cells to rapidly express CD69 and be retained at the site of infection. Finally, TRM CD8+ T cells that formed during VacV infection in an antigen-dependent manner became potent stimulators of localized antigen-specific inflammatory responses in the skin. Thus, our studies indicate that the presence of antigen in the non-lymphoid tissue microenvironment plays a critical role in the formation of functional TRM CD8+ T cell populations, a finding with relevance for both vaccine design and prevention of inflammatory disorders.

Presenter: O'Hagan, Kyle

Pak2: An essential regulator for regulatory T cell development and function

Kyle O'Hagan, Hyewon Phee

Northwestern University

Although significant effort has been devoted to understanding the thymic development of Foxp3+ regulatory T cells (Tregs), the precise signaling pathways that govern their lineage commitment remain enigmatic. Our work has shown a novel role for the actin cytoskeletal remodeling protein, p21-activated kinase 2 (Pak2), in Treg development, homeostasis and function. We have reported that Pak2 was necessary for generating the high-affinity TCR- and IL-2–mediated signals that are required by developing Tregs for their lineage commitment. Furthermore, deletion of Pak2 in Tregs specifically resulted in a loss of the characteristic Treg phenotype and the onset of a lethal multi-organ autoimmune phenotype, suggestive of a loss in Treg function. Our findings are the first to link Pak2 as an essential regulator of Treg biology.

Presenter: Omilusik, Kyla

The Role of Ubiquitin Specific Protease 1 in Promoting Id Protein Stability During T Cell Responses

Kyla Omilusik, Kyla D. Omilusik, Marija Nadjsombati and Ananda W. Goldrath

University of California, San Diego

CD8+ T cells are necessary components of immune responses against intracellular pathogens and tumours. The activation of effector T cells and subsequent differentiation into memory populations is a complex process with dramatic changes in gene expression. Of interest, E protein transcription factors and their inhibitors, Id proteins, play key roles in lymphocyte development, differentiation, and maintenance. Specifically, one Id protein, Id2, is upregulated in effector CD8+ T cells and maintained in memory cells presumably to induce effector function. Interestingly, Id2 overexpression is also associated with lymphomas. However, the mechanisms controlling normal Id2 expression and how misregulation leads to transformation is largely unknown. Recently, USP1, a deubiquitinase, was shown to stabilize Id2 in osteosarcomas which resulted in decreased E protein activity. Analogously, we propose a role for USP1 in posttranslational control of Id2 in T cells. Here, we show that USP1 is upregulated in T cells following infection and this expression change is mediated through the T cell receptor. Furthermore, we establish a direct interaction of Id2 and USP1 following T cell activation. By defining a regulatory role for USP1 mediated deubiquitination of Id2 and subsequently E protein activity, we will have a more complete understanding of mechanisms required for controlled survival and expansion of healthy T cells and how failure of this process can lead to tumorigenesis. Ultimately, identifying USP1 as an oncogene in lymphomas will provide a novel target for therapeutic interventions.

Presenter: Parrish, Heather

Functional evidence for TCR-intrinsic specificity for MHCII

Heather Parrish, Neha Deshpande, Jelena Vasic, Michael Kuhns

University of Arizona

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Presenter: Perez, Oriana

CD169+ marginal zone macrophages orchestrate innate immune responses to bacterial infection

Oriana Perez, Zhijuan Qiu, Pablo Romagnoli, Alexandre P. Bénéchet, Leigh Maher, Kamal M. Khanna

University of Connecticut Health Center

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Presenter: Phan, Anthony

Constitutive Glycolytic Metabolism Promotes CD8+ T cell Effector Memory

Anthony Phan, Andrew Doedens, Kitty Cheung, and Ananda Goldrath

University of California San Diego

Memory CD8+ T cells can provide enduring protection against intracellular pathogens and tumors. As such, eliciting memory T cells is a primary objective of vaccination strategies. Extensive metabolic changes accompany T cell activation: amongst these are an increase in glycolytic energy production and biosynthesis. Recent studies suggest that reliance on fatty acid oxidation (FAO) and oxidative phosphorylation as well as generation of increased spare respiratory capacity (SRC) following clearance of pathogen is essential for the formation of memory CD8+ T cells. Strikingly, we find that constitutive glycolytic metabolism and active suppression of oxidative phosphorylation due to HIF transcriptional activity, enhances generation of long-lived memory CD8+ T cells. In spite of HIF-driven glycolysis, CD8+ effector cells generate sufficient levels of ATP as memory precursor cells emerge, upregulate IL-7 receptor and key transcription factors associated with the generation of long-lived memory cells, and show a heightened response to secondary challenge. Importantly, increased glycolysis favors formation of effector memory CD8+ T cells, which can provide rapid protection from reinfection. These data clarify the role of cellular metabolism in the differentiation of CD8+ T cell memory, showing that constitutive reliance on glycolytic metabolism does not necessarily hinder formation of memory CD8+ T cells. This suggests that memory subset heterogeneity may be driven by both transcriptional programs and alterations in cellular metabolism.

Presenter: Pieper, Kathrin

A new mechanism of immunoglobulin diversification generates broadly reactive antibodies to *Plasmodium falciparum*

Joshua Tan[1,2,3,], Kathrin Pieper[1,]*, Luca Piccoli[1,]*, Abdirahman Abdi[2], Mathilde Foglierini Perez[1], Roger Geiger[1,4], Claire Maria Tully[2], David Jarrossay[1], Francis Maina Ndungu[2], Juliana Wambua[2], Philip Bejon[2,3], Chiara Silacci Fregni[1], Blanca Fernandez-Rodriguez[1], Sonia Barbieri[1], Siro Bianchi[5], Kevin Marsh[2,3], Vandana Thathy[2], Davide Corti[5], Federica Sallusto[1], Peter Bull[2,3] and Antonio Lanzavecchia[1,4]*

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*These authors contributed equally to this work

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Presenter: Resop, Rachel S.

The Effect of HIV-1 infection on S1P-R1 expression and function during entry into and egress from the human thymus

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

University of California, Los Angeles

Lack of adequate T cell regeneration in HIV infected individuals despite adherence to antiretroviral therapy is likely due in part to a defect pertaining to entry of hematopoietic stem cells (HSC) into, and egress of mature naïve T cells from, the thymus to the periphery. Limited work has been done to further elucidate these phenomena. We studied the effect of HIV-1 infection on the receptors to Sphingosine-1-phosphate (S1P), a chemotactic sphingolipid mediator, during the processes of entry into and egress from the human thymus. Previously, we have shown that human thymocytes migrate toward S1P and that S1P receptor 1 (S1P-R1) is the main S1P receptor responsible for response to S1P humans as it has been shown to be in mice. During T cell development, S1P-R1 expression is significantly increased at the mRNA and protein level in the most mature CD3hiCD69- thymocyte subset about to exit the thymus as mature naïve T cells. We have demonstrated that thymocytes expressing S1P-R1 respond to S1P exposure in vitro with internalization of the receptor upon binding and downregulation of S1P-R1 mRNA. In our current work, we are examining the dynamics of S1P-R1 expression on both immature hematopoietic stem cells (HSC) and mature CD3hiCD69- cells within the human thymus during HIV infection, which has thus far not been described. We profiled CD34+CD38lo HSC in both fetal and postnatal thymus and found that S1P-R1 is expressed at low to moderate levels on this population. Currently, we are examining S1P-R1 expression within the fetal liver in order to correlate these levels to that observed in the thymus. In two series of NSG mice implanted with human fetal thymus/liver (thy/liv) grafts and infected with CXCR4- or CCR5-tropic HIV-1, we observed that CFSE-labeled CD34+ progenitors developed into mature thymocytes in the human thy/liv implant of infected NSG mice. A minor subset of these cells expressed S1P-R1 and likely migrated to the periphery, indicating that entry into the thymus and development are likely functional during early infection. Morever, we examined the effect of HIV on S1P-R1 expression on CD3hiCD69- cells (as well as additional populations) in the human thy/liv implant. We verified persistent infection and immune activation by demonstrating that MxA and ISG15, two Interferon-alpha secondary genes, were upregulated. Surprisingly, our results show that S1P-R1 as well as its transcriptional regulator, Kruppel-Like Factor 2 (KLF2) were both significantly upregulated in mature thymocytes 5 and 9 weeks post HIV infection. Intriguingly, S1P-R1 was not only upregulated within the CD3hiCD69- population, but was also expressed within the CD3+CD69+ population, which normally does not express S1P-R1 in healthy thymi. Moreover, this effect was observed in both intrathymic and systemic HIV-1 infection. Hence, we investigated S1P-R1 function after HIV infection of the human thy/liv implants both in vitro and ex vivo by directly measuring Akt signaling induced by S1P/ S1P-R1 binding in a flow-based pAkt detection assay. Our findings indicate that S1P-R1 signaling may not be impaired in infected thymocytes, which is an intriguing contrast to data in the literature indicating that S1P-R1 response in HIV infection may be impaired in T cells from peripheral lymphoid tissues. Finally, we investigated the mechanism of the increase in S1P-R1 and KLF2. We found that Tumor Necrosis Factor alpha (TNF-a), Interferon-alpha (IFN-a) and Interferon gamma were elevated in the infected thy/liv implant. S1P-R1 expression levels, as measured by the Mean Fluorescence Intensity, increased to a statistically significant extent on mature thymocytes upon treatment with exogenous TNF-a, but not with IFN-a, indicating that secretion of TNF-a in the thymus may contribute to the increase in S1P-R1 expression. If S1P-R1 remains upregulated and fully functional for prolonged periods post HIV-1 infection, this discovery may offer insight into T cell reconstitution mechanisms during infection as well as provide a potential alternate avenues for immunotherapy.

Presenter: Roberts, Ed

Preeminent Role for CD103+/CD141+ Dendritic Cells bearing CCR7 for Tumor Antigen Trafficking and Priming of T cell Immunity in Melanoma.

Ed Roberts, Miranda Broz, Mikhail Binnewies, Mark Headley, Amanda Nelson, Denise Wolf, Tsuneyasu Kaisho, Matthew Krummel

UCSF

Intratumoral dendritic cells (DC) bearing CD103 in mice or CD141 in humans drive intratumoral CD8+ T cell activation. Using multiple strategies, we identified a preeminent role for these DC in trafficking tumor antigen to lymph nodes (LN), resulting in direct CD8+ T cell stimulation and providing antigen handoff to less-stimulatory myeloid cells. CCR7 loss resulted in defective LN T cell priming and increased tumor outgrowth with the loss of CD103+ DC trafficking, as opposed to that of CD11b+ DC, being the critical determinant of tumor specific CD8+ T cell expansion. CCR7 expression levels in human tumors correlate with signatures of CD141+DC and strongly correlated with intratumoral T cells and better clinical outcomes. This work identifies an ongoing pathway to T cell priming which should be harnessed for tumor therapies.

Presenter: Rudd, Brian

Fetal and adult progenitors give rise to unique populations of CD8+ T cells

Brian Rudd, Jocelyn Wang, Erin Wissink, Norah L. Smith, Andrew Grimson, and Brian D. Rudd

Cornell University

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Presenter: Savage, Hannah

Subsets of Natural IgM-secreting cells differ in their requirement for Blimp-1 expression

Hannah Savage, Vanessa M. Yenson, Jacquelyn Dieter, Marc A. Morgan, Elizabeth K. Bikoff, Nicole Baumgarth

University of California, Davis

The precise cellular origins and the signals that induce and regulate the continuous production of natural IgM, which has both immune regulatory and protective functions against pathogens, are largely unknown. Here we explored the need for terminal differentiation by the transcriptional master regulator of terminal differentiation, Blimp-1, for normal IgM production. Distinct populations of natural IgM antibody-secreting cells (ASC) were identified in spleen and bone marrow: Blimp-1-expressing CD138+ plasma cells (PC) and mostly CD138 negative B-1 cells, phenotypically indistinguishable from non-secreting B-1 cells. Using a neonatal chimera approach we demonstrate that both populations are reconstituted by adoptive transfer of FACS-purified peritoneal cavity B-1 cells. FACS-sorting of the various B-1 cells differing in Blimp-1 expression with help of Blimp-1 YFP reporter mice, followed by ELISPOT analysis, revealed a population of Blimp-1 independent B-1 cell IgM-ASC in bone marrow but not the spleen. This was confirmed with mice lacking Blimp-1 expression specifically in B cells, generated by deletion of exon 1A of prdm-1 (PRDM-1?Ex1A mice). These mice had significant reductions in the frequencies of IgM-ASC in the spleen but not the bone marrow. Consistent with these findings, natural IgM levels were reduced but not absent in PRDM-1?Ex1A mice compared to controls. Thus, our data reveal substantial heterogeneity within the pool of natural IgM-ASC. Of particular interest is the discovery of IgM-ASC among Blimp-1neg B-1 cells in the bone marrow that increase in frequency in the absence of Blimp-1 and are likely responsible for much of the remaining IgM in Blimp-1 deficiency. The data suggest that several distinct mechanisms regulate the pool of natural IgM-secreting cells.

Presenter: Sedy, John

Targeting the HVEM-BTLA-CD160-LIGHT network in Psoriasis

John Sedy, Marisol Veny, Jennifer Nguyen, M. Olivia Balmert, Nichole Niemela, Paula G. Norris, Carl F. Ware

Sanford Burnham Prebys Medical Discovery Institute

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Presenter: Shaw, Laura

Id2 reinforces antiviral Th1 cell differentiation and inhibits Tfh cell differentiation

Laura Shaw, Simon Belanger, Kyla D. Omilusik, James P. Scott-Browne, J. Philip Nance, John Goulding, Anna Lasorella, Shane Crotty and Ananda Goldrath

University of California, San Diego

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Presenter: Shin, Ok Sarah

Insights into the role of immunosenescence during Varicella Zoster (Shingles) Virus infection in aging cell model

Ok Sarah Shin, Ji-Ae Kim, Seul-ki Park, Mukesh Kumar, Chan-hee Lee

Korea University School of Medicine

Varicella zoster virus (VZV) is the etiological agent of shingles, a painful skin rash affecting a significant proportion of the elderly population. In this study, we used two aging cell models, Hutchinson-Gilford progeria syndrome (HGPS) fibroblasts and stress or replicative senescence-induced normal human dermal fibroblasts (NHDF), to investigate age-associated susceptibility to VZV infection. VZV infectivity titers were significantly associated with donor age in HGPS fibroblasts, and senescence induction in NHDF. High throughput RNA-sequencing (RNA-seq) analysis was performed to assess global and dynamic changes in host transcriptome of VZV-infected aging cells. Differentially Expressed genes (DEGs) analysis indicated that VZV infection of aged HGPS fibroblasts resembled that of senescent NHDF, contributing to novel insights into the mechanisms of senescence-associated susceptibility to VZV infection. Additionally, we identified stimulator of interferon genes (STING) as a potential VZV sensing receptor. Knockdown of STING expression resulted in increased viral replication in primary fibroblasts, whereas STING overexpression led to suppression of VZV plaque formation. In conclusion, our findings highlight an important role of immunosenescence following VZV infection, and provide significant insights into the mechanisms by which VZV is sensed and induces immune responses in aged skin cells.

Presenter: Sidler, Daniel

Role of TWEAK/Fn14 in chronic inflammatory disorders of the skin

Daniel Sidler, Rana Herro, Yuko Kawakami, Toshi Kawakami, Linda Burkly, Michael Croft

La Jolla Institute for Allergy and Immunology

Atopic dermatitis and Psoriasis are chronic inflammatory disorders of the skin with significant morbidity and characteristic extra-cutaneous manifestations. Despite particular differences in respect of etiology, pathogenesis and systemic manifestations, these diseases share characteristic histological features such as epidermal hyperplasia, dermal fibrosis and hypervascularization. Discovering molecules that might contribute to the development or maintenance of both diseases could provide new opportunities for therapeutic intervention. It is widely accepted that keratinocytes and dermal fibroblasts play a pivotal role in both diseases by amplifying and maintaining inflammatory and fibrotic responses. TNF superfamily members, including TWEAK (TNFSF12), have been implicated in control of these cell types, but experimental and mechanistic insights are scarce. Employing TWEAK-deficient mice, we demonstrated that the TWEAK/Fn14 axis is indispensable for the development of experimental atopic dermatitis (Der f/SEB model) and psoriasis (Imiquimod model). Furthermore, we found that subcutaneous injection of recombinant TWEAK into naïve mice is sufficient to induce robust cutaneous inflammation with histological and molecular signatures of both atopic dermatitis and psoriasis. Moreover, in vitro experiments revealed that keratinocytes and dermal fibroblasts produced key inflammatory chemokines and cytokines in response to TWEAK that are characteristic of both atopic dermatitis and psoriasis, and these molecules were amplified when TWEAK was combined with the signature cytokines of these diseases, IL-13 and IL-17. Similarly, subcutaneous injection of TWEAK into naïve mice with recombinant IL-13 or IL-17 strongly synergized with respect to infiltration of cells into the skin and intra cutaneous expression of keratinocyte and fibroblast-derived inflammatory cytokines and chemokines. These studies help to understand the complex network of contributing factors active in the pathogenesis of chronic skin inflammatory disorders, and highlight TWEAK as a central regulator of both atopic dermatitis and psoriasis.

Presenter: Slichter, Chloe

Unique signaling requirements facilitate a dichotomous response of human MAIT cells to commensal and pathogenic bacteria

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

Fred Hutchinson Cancer Research Center

Human Mucosal-associated invariant T (MAIT) cells recognize and are activated by bacterial metabolites presented by the non-classical MHC molecule MR1, have a memory T cell phenotype, and are abundant in human blood and mucosal tissues. Given that commensal and pathogenic bacteria share some of the same metabolites known to activate MAIT cells, it raises the question of how MAIT cell effector function is regulated to avoid responses against commensal-derived metabolites, while still responding to pathogen-derived metabolites. To answer this question we examined MAIT cells isolated from blood and mucosal tissues of healthy human donors and found that effector function is controlled in a stringent manner. A TCR plus costimulatory signal is not sufficient to elicit robust effector function, but instead induces a specific monocyte-recruiting chemokine response. Importantly, we demonstrate that inflammatory cytokines are necessary and synergize with the TCR signal to induce robust effector function including interferon-γ and granzyme B expression. To define tissue-based differences of human MAIT cells, we used a single-cell gene expression approach and found that MAIT cells in the mucosal tissue of healthy donors have even more pronounced pro-inflammatory/activating potential than MAIT cells in the blood, but show no direct ex vivo effector function. This further underlines that tight functional control of these effector functions is necessary and occurs in vivo. We propose a novel model in which unique signaling requirements allow MAIT cells to respond to the same TCR signal in a dichotomous and situation-specific manner. These data also have important implications for understanding MAIT cell function following damage to the gastrointestinal luminal integrity such as bone marrow transplant conditioning or HIV infection.

Presenter: Spangler, Jamie

Therapeutic modulation of immune homeostasis through structure-informed design of interleukin-2-targeted antibodies

Jamie Spangler, Jakub Tomala, Vincent C. Luca, Kevin M. Jude, Marek Kovar, & K. Christopher Garcia

Stanford University

Interleukin-2 (IL-2) is a pleiotropic cytokine that regulates immune cell differentiation, growth, and activity by signaling through interleukn-2 receptor-β (IL-2Rβ)/IL-2Rγ heterodimers on both IL-2Rα-high regulatory T (TReg) cells and IL-2Rα-low effector cells (such as memory-phenotype [MP] CD8+ T and natural killer [NK] cells). Its essential role in orchestrating immune homeostasis has made IL-2 an attractive therapeutic target for a wide range of immune-linked disorders such as autoimmune disease, cancer, and chronic infection. Unfortunately, efforts to develop IL-2 as a therapeutic have been limited by its concurrent promotion of both regulatory and effector immune cells, which leads to harmful off-target effects and dose-limiting toxicity. It would therefore be of tremendous therapeutic value to isolate and selectively modulate the immunosuppressive and immunostimulatory effects of IL-2 in order to bias immune cell subset proliferation for particular disease applications. Certain IL-2-directed antibodies have been shown to skew the balance of immune cells to favor either TReg cells (JES6-1) or effector cells (S4B6), but the molecular mechanisms underlying their phenotypic behavior was unknown. Through structural, biophysical, and functional studies, we elucidated the unique and intricate mechanisms through which these antibodies achieve immune cell bias by modulating cytokine-receptor interactions. We found that JES6-1 sterically blocks the IL-2:IL-2Rβ and IL-2:IL-2Rγ interactions, but also allosterically lowers the IL-2:IL-2Rα affinity through a "triggered exchange" mechanism favoring IL-2Rα-high TReg cells. Conversely, S4B6 sterically blocks the IL-2:IL-2Rα interaction, while also conformationally stabilizing the IL-2:IL-2Rβ interaction, thus stimulating all IL-2-responsive immune cells, particularly IL-2Rβ-high effector cells. We harnessed these structural insights to engineer the affinity and species reactivity of JES6-1 to enhance its therapeutic activity and translational significance. Our work provides unprecedented insight into the actions and tunability of anti-cytokine antibodies, presenting a major advance in IL-2 therapeutic development and providing a molecular blueprint for the design of other cytokine-targeted drugs.

Presenter: Steach, Holly R.

Investigating mechanisms that prevent the response of B cells specific for foreign antigens

Holly R. Steach, Justin J. Taylor

Fred Hutchinson Cancer Research Center

In order to prevent antibody-mediated autoimmunity, B cells reactive against self-antigen are subjected to tolerance mechanisms including induction of a state of restricted functionality termed anergy. We hypothesize that anergy also restricts responsiveness of B cells specific for clinically relevant vaccine antigens due to B cell receptors (BCR) cross-reactivity with endogenously expressed molecules. Using a previously published antigen-specific enrichment strategy, we have shown that 60-80% of antigen-specific B cells fail to respond to antigen immunization. While some of this poor responsiveness is likely a result of low affinity for antigen, many B cells binding high levels of antigen are found within the non-responding population. A broadly-inclusive marker for self-reactivity in mice with an intact polyclonal B cell repertoire has been identified using the orphan nuclear receptor Nur77, known to be up-regulated downstream of BCR signaling. Using Nur77-eGFP reporter mice, we have found that populations of B cells specific for non-self antigens display a range of baseline Nur77 expression similar to that of total B cells, perhaps indicating a similar extent of self-antigen recognition. Using adoptive transfer, we have found that antigen-specific naive B cells expressing low levels of Nur77 are more responsive to immunization than their counterparts expressing high levels of Nur77. These data suggest that the response of naive B cells specific for foreign antigens is restricted by cross-reactivity to self-antigens. Our results could lend a unique perspective to the paradigmatic definition of B cell anergy and offer an accession to immunogen design as the principle factor governing protective humoral immune responses following vaccination.

Presenter: Stumhofer, Jason

ICOS regulates host TH1 and TFH cell differentiation in response to Plasmodium chabaudi chabaudi AS infection.

Jason Stumhofer, Daniel J. Wikenheiser, Debopam Ghosh, Brian Kennedy

UAMS

Cell-mediated and antibody-mediated immune responses are required for control of acute and persistent infection with Plasmodium chabaudi chabaudi AS, respectively. The co-stimulatory molecule ICOS can regulate TH1, TH2 and TH17 CD4+ T cell responses, in addition to mediating follicular helper T (TFH) cell induction. TFH cells function to promote B cell survival and facilitate somatic hypermutation and affinity maturation within germinal centers (GC), resulting in production of high-affinity antibodies (Abs). Here, we demonstrate that, in response to P. c. chabaudi AS infection, absence of ICOS resulted in an enhanced TH1 immune response that reduced peak parasitemia. While early TFH cell development was not impaired in the absence of ICOS, by day 21 post-infection Icos-/- mice accumulated fewer TFH cells in the spleen compared to Icos+/+ mice. This resulted in the generation of substantially fewer GC B cells, and a decrease in quality, but not quantity, of parasite-specific isotype-switched Abs. Moreover, treatment of mice with anti-ICOSL Abs revealed a requirement for ICOS in TFH cell differentiation only after day 6 post-infection. Ultimately, over time a decline in quality and quantity of isotype-switched Abs was observed in Icos-/- mice, leading to an impaired control of persistent parasitemia. Collectively, these data suggest that the initial induction of TFH cell differentiation and production of isotype-switched Abs during P. chabaudi AS infection does not require ICOS. However, ICOS is necessary for maintenance of a sustained high-affinity, protective Ab response.

Presenter: Swain, Susan

At a late effector/memory checkpoint, CD4 effectors must again recognize cognate Ag on activated APC to become highly differentiated effectors and to transition to memory cells.

Susan Swain, Bianca Bautista, Priys Deverajan and Allen Ming Vong

University of Massachusetts Medical School

Contrary to several prevailing models, we have found that the fate of effectors generated by viral infection, depends on additional cognate interactions with APC presenting cognate Ag. The CD4 effector cells must interact just as they reach their peak of response with activated APC either from infected mice or after TLR agonist treatment of APC, creating a checkpoint . For transition to memory, the effector cells require costimulation and must make IL-2. If this does not occur 2 logs fewer memory cells are generated. The generation of highly differentiated follicular helper T cells (Tfh) and cytotoxic CD4 T cells (ThCTL) also require cognate interaction with APC, though distinct costimulatory pathways are involved and likely different APC. The fate-determining checkpoint during influenza infection is from 5-8 days post infection, and at later time-points (e.g. day 14) there is no further requirement for Ag. Thus high levels of CD4 memory and highly differentiated Th effector subsets are generated only when Ag persists into the effector phase. Vaccines that lack live pathogens hence lead to low levels of late Ag and costimulation from viral danger signals, so we are testing whether providing Ag stimulation at the memory checkpoint can enhance vaccine-induced protective immunity.

Presenter: Takenaka, Eri

Expression of DNAM-1 (CD226) on inflammatory monocytes

Eri Takenaka, Anh Van Vo, Akira Shibuya, Kazuko Shibuya

University of Tsukuba

Not to be placed on website

Presenter: Thiault, Nicolas

Peripheral Reprogrammed CD4+ Cytotoxic T lymphocytes Efficiently Control Tumor Growth.

Nicolas Thiault, Nicolas Thiault, Mohd. Mushtaq Husain, Hitoshi Iwaya, Hilde Cheroutre

La Jolla Institute for Allergy and Immunology

The initial commitment to either the CTL- or Th-lineage is made during thymic development of T cells, where thymocytes expressing an MHC class II–reactive TCR commit to the CD4+ helper T cell lineage, whereas thymocytes with specificity for MHC class I differentiate into the CD8+ CTL lineage. The molecular regulation is controlled by the action and counteraction of key transcription factors, such as the Th cell transcription factor, ThPOK, which promotes the Th fate and Runx3 which drives the differentiation of MHC class I restricted thymocytes into the CD8+ CTL-lineage. This dichotomy persists in the periphery for mature T cells, where ThPOK continues to suppress the cytotoxic fate of MHC class II–restricted CD4+ Th cells even as they differentiate into effector Th subsets. Recently, our team made a major breakthrough and showed that the thymic lineage commitment of CD4 Th cells is not fixed and that mature peripheral CD4+ T cells have the plasticity to lose ThPOK expression. We demonstrated that the loss of Thpok expression and the Th fate coincides with the re-expression of CD8? and the induction of a typical CTL phenotype, including 2B4 and Granzyme B expression and IFN-? and TNF-? cytokine production. Using an in vitro cytotoxic assay, our lab found that these reprogrammed ThPOK-CD4+ T cells display killer capacities. We also demonstrated that this mechanism is mediated in part by continuous Ag exposure of CD4 T cells that leads to the down-regulation of ThPOK and the induction of the Runx3-controlled cytolytic machinery allowing reprogramming of CD4 Th cells to CTL. Moreover, our data also indicated that peripheral Ag-induced reprogramming of conventional CD4 T cells is not a rare but rather widespread and common phenomenon, which occurs in various conditions such as in response to chronic viral infections (including chronic CMV and LCMV infections). In a new study we now investigate the role of CD4 CTL in anti-tumor immune surveillance. Using the well-described B16-melanoma tumor model, we were able to demonstrate that in vitro differentiated OVA-specific CD4 CTL display anti-tumor capacity and efficiently controlled/killed OVA-expressing B16 tumor cells. Extend studies on CD4 CTL in the cancer field will not only greatly expand our basic understanding of anti-tumor immunity but it will undoubtedly have major implications for translational research to design new cell therapies to prevent and/or treat devastating cancers.

Presenter: Thompson, Thornton W.

Tumor-derived CSF-1 induces the NKG2D ligand RAE-1[d] on tumor-infiltrating myeloid cells

Thornton W. Thompson, Benjamin T. Jackson, Po-Yi J. Li, David H. Raulet

University of California - Berkeley

Expressed by NK cells and some T-cells, the cell-surface receptor NKG2D binds a set of host-encoded ligands typically absent from healthy cells but upregulated upon viral infection and on tumor cells, which targets them for destruction. Unexpectedly, we recently showed that in tumor-bearing mice, persistent interactions between NKG2D and its ligand(s) expressed on non-tumor cells reduce the cytotoxic activity of NK cells against cancer cells and facilitate tumor growth. Using spontaneous and transplanted cancer models, we show here that intra-tumoral macrophages and monocytes are induced to express the NKG2D ligand RAE-1δ. Conditioned media from tumor cells was sufficient to induce RAE-1δ on macrophages ex vivo, so we screened a panel of soluble factors and found that the cytokine CSF-1 was a robust inducer of RAE-1δ. Tumor cells secrete abundant CSF-1, and antibody blockade of the CSF-1 receptor (CD115) prevented macrophage RAE-1δ induction by tumor cell supernatants in vitro. Tumor-bearing mice injected with blocking antibodies against CD115 or CSF-1 rapidly downregulate myeloid RAE-1δ expression in tumors without substantial reduction in macrophage/monocyte numbers. We then used CRISPR/Cas9 to knock out Csf1 from tumor cells, which abrogated the induction of intra-tumoral myeloid RAE-1δ in vivo. Furthermore, we show that induction of RAE-1δ by CSF-1 requires signals from PI3K, and we identify PI3K p110α as the relevant isoform for RAE-1δ induction. Thus, cancer cell secretion of CSF-1 utilizes PI3K to induce the NKG2D ligand RAE-1δ on the cell surface of macrophage/monocyte cells that infiltrate the tumor.

Presenter: Tomala, Jakub

Contrasting effects of wild type and detoxified adenylate cyclase toxin of Bordetella pertussis on proliferation and expansion of CD8+ and CD4+ T lymphocytes

Jakub Tomala, Irena Adkins, Martina Svedova, Radek Spisek, Peter Sebo, Marek Kovar

Institute of Microbiology ASCR, Videnska 1083, Prague 4, Czech Republic

The adenylate cyclase toxin (CyaA) is a key virulence factor of B. pertussis that subverts host defense. CyaA targets CD11b-expressing phagocytes and delivers into their cytosol an adenylyl cyclase enzymatic domain (~400 residues) that subverts cellular signaling by increasing cAMP levels. In parallel, the ~1300 residue-long RTX hemolysin moiety of CyaA forms cation-selective pores and permeabilizes target cell membrane for efflux of cytosolic potassium ions. In this study, we show that CyaA-mediated cAMP signalling of the wild type toxin decreases the capacity of LPS-stimulated dendritic cells (DCs) to induce CD4+ and CD8+ T cell proliferation and expansion in vivo and limits the induction of IFN-? producing CD8+ T cells while enhancing IL-10 and IL-17 production. In contrast, ablation of the adenylate cyclase enzymatic activity of the toxin (CyaA-AC-) converts the molecule into an efficient antigen delivery tool for stimulation of adaptive T cell immune responses. We identified here that CyaA-AC- by its pore-forming activity and potassium efflux enhances the capacity of DCs to stimulate CD8+ and CD4+ T cell expansion in vivo. This revealed a novel self-adjuvanting capacity of the CyaA-AC- toxoid that is exploited as a tool for delivery of immunotherapeutic anti-cancer CD8+ T cell vaccines into DCs. Acknowledgement: This work was supported by Czech Science Foundation grant 13-12885S and Institutional Research Concept RVO 61388971.

Presenter: Tomalova, Barbora

Hemolytic activity of adenylate cyclase toxin is not required for colonization capacity but contributes to virulence of Bordetella pertussis

Barbora Tomalova, Karolina Skopova, Pavel Rossmann, Marek Kovar, Jiri Masin, Ilona Bibova, Nicole Guiso, Radim Osicka and Peter Sebo

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The adenylate cyclase toxin-hemolysin (CyaA, ACT or AC-Hly) plays a crucial role in virulence of the whooping cough agent Bordetella pertussis. The 1706 residue-long bi-functional leukotoxin targets cells expressing the complement receptor 3 (CR3, known as aMß2 integrin, CD11b/CD18, or Mac-1) and delivers into their cytosol an N-terminal adenylyl cyclase (AC) enzyme domain. This ablates bactericidal functions of host phagocytes by unregulated conversion of cytosolic ATP into second messenger molecule cAMP. In parallel, the hemolysin moiety of CyaA forms cation-selective pores that permeabilize target cell membranes and account for the hemolytic (Hly) activity of B. pertussis on blood agar. While importance of the cAMP-elevating activity of CyaA for colonization and immunomodulatory capacities of B. pertussis has previously been established, the role of the pore-forming (hemolytic) activity could not yet be addressed. Here we constructed the first non-hemolytic B. pertussis mutant (AC+Hly-) that produces a CyaA toxin exhibiting an intact capacity to elevate cAMP in host phagocytes. Upon intranasal infection, the AC+Hly- strain colonized mouse lungs to equal levels as the parental AC+Hly+ strain, showing that the pore-forming (hemolytic) activity of CyaA is per se not required for mouse lung colonization by B. pertussis. Compared to AC+Hly+, however, the AC+Hly- strain induced much milder lung pathology, caused lethal infections only at significantly increased bacterial doses and provoked much lower levels of neutrophil, macrophages and dendritic cell infiltration into infected lungs. While infection by the AC+Hly+ strain provoked significant decrease of mean MHC II molecule expression levels on the lung populations of macrophages and dendritic cells, infections by the AC+Hly- strain did not result in reduced MHC II expression levels. Hence, the pore-forming (hemolytic) activity of CyaA synergizes with the cAMP-elevating activity of CyaA in promoting immune evasion of the pathogen. Acknowledgement: This work was supported by Czech Science Foundation grant 13-12885S and Institutional Research Concept RVO 61388971.

Presenter: Townsend, M.H.

Macrophage Polarization in the Colon Cancer Tumor Microenvironment

M.H. Townsend, E.G. Weagel, W. Meng, R.A. Brog, E.J. Valezquez, R.A. Robison, K.L. O'Neill

Brigham Young University

As an integral component of the innate immune response, macrophages are one of the most efficient and skilled killers of foreign pathogens and apoptotic bodies. While extremely effective against external invaders, their role in relation to cancer cells is very different. In certain tumors, tumor associated macrophages (TAMs) make up 50% of the tumor mass, and play an essential role in the survival and propagation of cancer cells. Macrophages in the tumor microenvironment largely switch from an M1 classically activated phenotype towards an M2 phenotype. While M1 macrophages are pro-inflammatory and specialized in phagocytosis, M2 macrophages are characterized by their role in tissue repair. Cellular signals released from the tumor cells influence surrounding macrophages to switch to an M2 profile. These M2 macrophages release anti-inflammatory cytokines, which will stimulate tissue repair and trigger angiogenesis. This will provide the tumor with the necessary nutrients during tumor growth. In this project we evaluated the effects of SW620 and HT29 colon cancer cells on the polarization of macrophages. U937 cell lines were stimulated with phorbol 12-myristate 13-acetate (PMA) over the course of 24 hours. Cells were first evaluated for correct morphology and differentiation changes characteristic of macrophages, then incubated with HT29 or SW620 cells at a 1:1 cellular concentration. Meanwhile, differentiated U937 cells were also incubated in 500µL of spent media from HT29 or SW620's to evaluate signals that may be released from the tumor. Cells were incubated for 48 hours under these experimental conditions. Macrophages were exposed to fluorescent microspheres and incubated for an hour. The U937-derived macrophages were then analyzed through flow cytometry to measure the bead engulfment. We found that there was a 63% decrease in the three bead engulfment and a 47% decrease in the two bead engulfment when exposed to HT29 colon cancer cells. Macrophages exposed to HT29 spent media had reductions of 32% and 18% in the three bead and two bead engulfment population respectively. We discovered that macrophages exposed to HT29 cells were more affected in their bead engulfment than macrophages exposed to SW620 cells. Reductions of 11% in the three bead population and 15% in the two bead population were observed when cells were exposed to SW620 cells. Macrophages incubated in SW620 media decreased by 26% and 35% in the three bead and two bead engulfment populations respectively. These data suggest that the difference in engulfment between the two cancer cell lines may be an indicator of tumor aggressiveness. To further evaluate the polarization of macrophages towards an M2 phenotype in the tumor microenvironment, we evaluated the gene expression of IL-10, IL-1B, and IL-12. While exposed to cancer cells there was a significant increase in the IL-10 production and a decrease in both IL-1B and IL-12 production. This change in gene regulation stimulates the switch from a pro-inflammatory M1 phenotype towards an M2 anti-inflammatory phenotype in the tumor microenvironment. These results provide evidence of a direct relationship between tumor cells and the resulting polarization of macrophages towards a less aggressive, M2 phenotype.

Presenter: Tsau, Jennifer

The role of caspase-8 in regulating dendritic cell activation during both homeostasis and chronic viral infection

Jennifer Tsau, Stephen Hedrick

UCSD

Dendritic cells (DCs) link innate and adaptive immunity by alerting the host to the presence of pathogens. However, DCs must also avoid initiating T cell responses to harmless self-antigens. Caspase-8, required for apoptosis, is also a negative regulator of Rig-I signaling, which is activated upon detection of RNA viruses such as lymphocytic choriomeningitis virus (LCMV). We found that mice with caspase-8-deficient DCs (dcCasp8-/- mice) develop an age-dependent autoimmunity, yet mount an enhanced response to a chronic viral infection. Aged dcCasp8-/- mice develop hyperactivated DCs and T cells, display organ immunopathology, and have CD4 T cells that skew towards a Th1 phenotype when stimulated ex vivo. When we infected young adult dcCasp8-/- mice with the chronic strain of LCMV, we found that these mice had enhanced antigen-specific T cell responses. A month after infection, dcCasp8-/- mice had fewer proportions of exhausted antigen-specific T cells, more LCMV-specific IgG2a antibodies, and lower viral loads. In order to link our observations to a heightened sensitivity of caspase-8-deficient DCs to Rig-I signaling, we transfected DCs lacking caspase-8 with a Rig-I ligand. A larger proportion of CD11b+ Casp8-/- DCs became highly activated upon Rig-I stimulation. Our findings suggest that caspase-8 is important for regulating DC activation, which must be tightly controlled: excessive DC activation aids in viral clearance, but also leads to the development of autoimmunity in the steady state.

Presenter: Ty, Maureen

Malaria Induced Inflammation by Xanthine Oxidase

Maureen Ty, Anton Goetz, Ana Rodriguez

NYU School of Medicine

Malaria blood-stage infection induces high fever and a strong inflammatory response that peaks at the time of rupture of infected erythrocytes. Despite the crucial importance of this inflammatory response in the pathology of malaria, the mechanism of activation by the parasite is not completely understood. An important paradox still unresolved in malaria research, is that in vitro co-cultures of macrophages with infected red blood cells (iRBCs) do not induce strong secretion of inflammatory cytokines. Patients with malaria exhibit elevated levels of oxidative stress markers in the blood, especially of Xanthine Oxidase (XO), an enzyme that converts hypoxanthine to uric acid, releasing reactive oxygen species that cause oxidative stress. When in vitro cultures of macrophages and iRBCs are incubated with XO, a strong secretion of inflammatory cytokines is detected, suggesting that oxidative stress functions as a trigger for the inflammatory activation. The cytokine response observed is comparable to LPS-induced response in magnitude and includes typical inflammatory cytokines that are a signature of malaria: TNF, IL1-beta, IL-10, IL-6 and IL-8. This secretion can be inhibited with the addition of the XO specific inhibitor, Febuxostat. These results suggest that oxidative stress through XO may play a role in the inflammatory response to malaria.

Presenter: Utzschneider, Daniel T

Antigen-driven specialization and differentiation of T cells in chronic infections

Daniel T Utzschneider, Francesca Alfei, Patrick Roelli, David Barras, Stephanie Darbre, Mauro Delorenzi, Daniel D. Pinschewer, and Dietmar Zehn

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Chronic infections induce T cells showing impaired cytokine secretion and up-regulated inhibitory receptor expression such as PD-1. What determines the acquisition of this chronic phenotype and how it impacts T cell function remained vaguely understood. Using newly generated recombinant antigen variant-expressing chronic LCMV strains, we uncovered that T cell differentiation and the acquisition of a chronic "exhausted" phenotype depends critically on the frequency of TCR engagement and less significantly on the strength of TCR stimulation. In fact, we noted that low level antigen exposure promotes the formation of T cells with an acute phenotype in chronic infections. Unexpectedly, we found that T cell populations with an acute or chronic phenotype are maintained equally well in chronic infections and undergo comparable primary and secondary expansion. Thus, our observations contrast the view that T cells with a typical chronic infection phenotype are severely functionally impaired and rapidly transition into a terminal stage of differentiation. Instead, our data unravel that T cells primarily undergo a form of phenotypic und functional differentiation in the early phase of a chronic LCMV infection without inheriting a net survival or expansion deficit and we demonstrate that the acquired chronic phenotype transitions into the memory T cell compartment.

Presenter: Vaden, Kiara

Determining the optimal TCR:pMHC avidity for CD4+ T cell memory generation

Kiara Vaden, K. Scott Weber

Brigham Young University

The development of more effective vaccines requires an understanding of the requirements for strong short and long-term immune responses. Memory cell formation is a hallmark of the adaptive immune system and CD4+ T cells play a central role in the generation of productive recall responses and protective immunity. CD4+ T cells coordinate the adaptive and innate immune responses to infection, but their role as memory cells is not well understood. An unresolved question is what CD4+ TCR:pMHC affinity leads to optimal CD4+ memory cell formation. To address this, we have generated two CD4+ TCR transgenic mouse lines that are specific for the same naturally occurring epitope from Listeria monocytogenes. The TCRs from these mice lines (LLO118 and LLO56) differ by only 15 amino acids and while LLO118 and LLO56 T cells have a similar in vitro response to antigen, their in vivo responses to infection are strikingly different. LLO118 has a stronger primary response whereas LLO56 has a much stronger memory response. Understanding why these TCRs cause such different T cell responses provides a novel system to understand how to improve vaccines and immunotherapies. We have L. monocytogenes mutants with different capacities to stimulate LLO118 and LLO56 T cells to test the role of TCR:pMHC binding affinity in CD4+ primary and memory responses. Our studies are designed to identify the characteristics of CD4+ T cells responsible for differential immune responses and could be highly relevant for improving therapeutic and protective vaccine design and immunotherapies.

Presenter: Velazquez, Edwin J.

Burkitt's lymphoma affects macrophage polarization.

Edwin J. Velazquez, E. G. Weagel, W. Meng, M. H. Townsend, R. E. Brog, R.A. Robison, and K.L. O'Neill

Brigham Young University

The immune system plays an important role in the development and progression of cancer. Macrophages are a heterogeneous population of innate myeloid cells and undergo specific differentiation depending on the local tissue environment. Macrophage activation may exhibit two functional states, which represent the extremes of a continuum of activation states. M1, or classically-activated macrophages, promote inflammation, activate a Th1 immune response, and tumoricidal activities. Whereas M2, or alternatively-activated macrophages, regulate tissue repair, promote a Th2 response, and allow tumor progression. Cells exposed to tumor microenvironments including cancer cells, behave differently. By secreting a number of diverse chemoattractants, primary tumors recruit blood circulating monocytes. Once in the tumor site, these monocytes differentiate to Tumor-Associated Macrophages (TAMs). TAMs have shown to display an M2-like phenotype in most cancers and are thought to be able to maintain their M2-like phenotype by receiving polarization signals from malignant cells. The engulfment activity and cytokine profile expression levels of macrophages are good indicators of macrophage polarization and may help us to understand how tumors are able to evade the immune system. Here, we investigated the influence of cancer cells on the behavior of macrophages. We assessed the engulfment capacity of macrophages using an engulfment assay with fluorescent beads in conjunction with flow cytometry. Using Q-PCR we measured the expression levels of key cytokines in macrophages co-cultured with Burkitt's lymphoma cancer cells. During these experiments, we exposed U937 cells to phorbol 12-myristate 13-acetate (PMA) for 24 hours to allow differentiation. Then we co-cultured these macrophages with Raji cancer cells for 48 hours. Subsequently, we allowed the macrophages to phagocytose fluorescent microbeads for 1 hour and we measured their engulfment using flow cytometry. In parallel, we measured the expression levels of IL-1B, IL-10 and IL-12 in PMA-stimulated U937 cells before and after exposure to a Raji spent media, Raji cells, and normal media. We found a significant decrease of the total engulfment and engulfment capacity of the U937 cells after being exposed to Raji spent media and Raji cells. The U937 cells showed a decrease of almost the half their engulfment capacity. 12% and 19 % decrease of the total engulfment was observed in U937 cells exposed to Raji spent media and Raji cells respectively. We observed a 5 fold increase in the IL-10 expression for those cells exposed to Raji spent media and Raji cells, whereas a down regulation of IL-B and IL-12 was observed for the same treatments. These results show that PMA-stimulated U937 cells undergo macrophage polarization from M1 to M2 phenotype after exposure to cancer cells and their microenvironment through immunomodulatory cytokine signals. This suggests that cancer cells greatly affect the immune system by downregulating macrophage aggressiveness, allowing for further tumor development. Currently, we are performing additional experiments to better understand the complexity of macrophage polarization exposed to various types of cancer microenvironments.

Presenter: von Moltke, Jakob

Tuft cell-derived IL-25 regulates an intestinal ILC2 - epithelial response circuit

Jakob von Moltke, Ming Ji, Hong-Erh Liang, Richard Locksley

UC San Francisco

Parasitic helminths and allergens induce a type 2 immune response leading to profound changes in tissue physiology, including hyperplasia of mucus-secreting goblet cells and smooth muscle hypercontractility. This response, designated "weep and sweep", requires IL-13 production by tissue resident group 2 innate lymphoid cells (ILC2s) and recruited type 2 helper T cells (Th2). Experiments in mice and humans have demonstrated requirements for the epithelial cytokines IL-33, thymic stromal lymphopoietin (TSLP), and IL-25 in the activation of ILC2s, but the sources and regulation of these signals remain poorly defined. In the small intestine, the epithelium consists of at least five distinct cellular lineages, including tuft cells, whose functions are unclear. Here we show that tuft cells constitutively express IL-25 to sustain ILC2 homeostasis in the resting lamina propria. After helminth infection, tuft cell-derived IL-25 further activates ILC2s to secrete IL-13, which acts on epithelial crypt progenitors to promote differentiation of tuft and goblet cells, leading to increased frequencies of both. Tuft cells, ILC2s, and epithelial progenitors therefore comprise a response circuit that mediates epithelial remodeling associated with type 2 immunity in the small intestine, and perhaps at other mucosal barriers populated by these cells.

Presenter: Wang, Haiguang

Localization of iNKT Subsets Determines Their Cytokine Response

Haiguang Wang, You Jeong Lee, Kristin Hogquist

Center for Immunology, University of Minnesota

Invariant natural killer T (iNKT) cells are a specialized subset of aß T cells recognizing lipid antigens presented by CD1d molecule. They can be rapidly activated by a-galactosylceramide (a-GalCer) without priming, a-GalCer and analogues of it are being actively tested for clinical use as immunological adjuvants or therapeutics for cancers and autoimmune diseases. iNKT cells modulate immunity through cytokines production at steady state or during infection to influence the development, activation and recruitment of other immune cells. Instead of being an homogenous population, iNKT cells were comprised of three effector subsets, NKT1, NKT2, and NKT17, which produce distinct cytokines at steady state or after stimulation, but little is known about their distribution and localization. Here, we report the systemic distribution and anatomic localization of these subsets and measured their cytokine production at steady state or after stimulation. NKT1 cells were found to be mainly microvasculature associated while NKT2 cells preferentially localized in tissue parenchyma. Furthermore, intravenous injection of a-GalCer robustly activated microvasculature associated NKT1 cells in spleen and liver, but not lymph node (LN) or thymic iNKT cells, leading to a systemic production of interferon-? and IL-4. In contrast, oral administration of a-GalCer solely activated NKT2 cells in the mesenteric LN, resulting in localized IL-4 effect. These findings indicate that the localization of iNKT subsets determines their cytokine response. Given most iNKT cells are resident rather than circulating, these results demonstrate how the steady-state localization of iNKT subsets governs their systemic or localized cytokine effect, providing critical considerations to the design of iNKT based therapeutic applications.

Presenter: Weagel, E. G.

Prostate cancer exosomes affect macrophage polarization and phagocytosis

E. G. Weagel, W. Meng, R. A. Robison, and K. L. O'Neill

Brigham Young University

Prostate cancer is the second most common cancer in men in the United States after skin cancer and the fifth most common cancer-related death in the world. This study explores the effects of prostate cancer-derived exosomes on macrophages. Exosomes are small vesicles secreted by cells and found in virtually found in all bodily fluids. Exosomes contain parts of the genome of the cell of origin, some protein, and large amounts of RNA, including miRNAs. Exosome secretion is thought to be upregulated in cancer patients, as it is found abundantly in cancer patients' secretions (urine, serum, etc.). One of the functions of exosomes is to mediate cellular communication. This happens as they are secreted from one cell and they distribute their contents into another cell. Some exosomes specifically target immune cells. The miRNA the exosomes carry can silence genes involved in the activation of these immune cells, leading to immune suppression and tumor growth. Macrophages are often found surrounding the tumor microenvironment, as they are recruited by tumor signals to promote angiogenesis and metastasis. These macrophages, which are often called tumor associated macrophages (TAMs), are exposed to various signals deriving from the tumor microenvironment, including exosomes. Our lab has previously generated data that suggests that the co-culture of macrophages with PC3 and DU145 cell lines results in a decrease in macrophage phagocytosis and an M2-like polarization state. For this study, we exposed U937 cells to phorbol 12-myristate 13-acetate (PMA) for 24 hours to allow for differentiation. Then, we co-cultured these macrophages with isolated exosomes from spent media from both PC3 and DU145 cell lines at various time intervals (1, 2, 3, 4, 5, 6, 12, and 24 hours). We then allowed the macrophages to phagocytose fluorescent microbeads for 1 hour. Total bead phagocytosis decreases at 3 hours of exosome co-culture compared to control. Interestingly, total bead phagocytosis resumes to normal after 12 hours exosome co-culture. Gene expression analysis of these macrophages suggests an M2 phenotype from hours 2-24. These results suggest that prostate cancer-derived exosomes can target macrophages and affect their phagocytosis. Further research is required to elucidate the specific genes these exosomes target.

Presenter: Weberova, Petra

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

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IL-2 is important for the proliferation, differentiation, and survival of lymphocytes that display antitumor activity, thus stimulating considerable interest for the use of cytokines in cancer immunotherapy. More than 20 years ago, IL-2 was approved by the FDA for treatment of metastatic renal cell carcinoma and malignant melanoma [1,2]. However, although standard high-dose IL-2 therapy results in a modest clinical response rate of 14% [3], the treatment is limited by substantial toxicities [3,4], vascular leak syndrome (VLS) being the most serious one [5–7]. Alternatively, low-dose IL-2 treatment has shown activity in renal cell cancer (response rates of 18–23%), without the toxicity of high dose IL-2 [8,9]. Yet, IL-2 cancer therapy is of continued interest in part because about one-third of the clinical responses are complete, durable remissions. So far, IL-2 immunotherapy has been shown to be beneficial in a variety of clinical trials [10–14]. For example, studies to identify antigen-nonspecific strategies for enhancing immune reconstitution in individuals with HIV infection include those using IL-2 [15]. In order to increase IL-2 half-life in vivo, we covalently conjugated synthetic semitelechelic polymeric carrier based on N-(2-hydroxypropyl)methacrylamide (HPMA) to IL-2. Thus, we synthesized IL-2-poly(HPMA) conjugate containing 2-3 polymer chains per IL-2 molecule in average. Such conjugate exerts much higher activity than IL-2 in vivo as shown by expansion of memory CD8+ T, NK, NKT, ?dT and Treg cells. IL-2-poly(HPMA) shows also much longer half-time in circulation than IL-2 (~4h versus ~ 5min). Collectively, modification of IL-2 with poly(HPMA) chains dramatically improves its potency and pharmacologic features in vivo, which have implications for immunotherapy.

Presenter: Zhang, Yang

Mechanism of lymph node barrier immunity

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Our ability to mount adaptive immune responses against skin-invading pathogens depends on the delivery of antigens to lymph nodes (LNs) for encounter by naïve lymphocytes. However, activation, clonal expansion and effector lymphocyte differentiation takes several days whereas pathogens can undergo marked replication in a matter of hours. The skin contains immune effector cells that help keep pathogen replication in check, in a process referred to as 'barrier immunity'. Despite this, in many cases, intact pathogens travel within minutes via lymph fluid to draining LNs. Recently there has been evidence indicating the existence of barrier immunity within lymph nodes. Upon infection, the first LN cells exposed to lymph-borne antigens are the CD169+ sinus-associated macrophages. Crosstalk between sinus-assocaited macrophages and IFNg precommited CD8 T cells and NK cells are critical for mounting rapid Th1 like response against acute infection by Pseudomonas aeroguinosa and Toxo gondii. Recently, the Cyster lab and others identified populations of innate (pre-formed effector) lymphocytes that are closely associated with LN CD169+ macrophages and that rapidly produce cytokines (IL17) upon stimulation. In this study, we found those innate lymphocytes are responsible for rapid IL17 production in lymph nodes upon acute bacteria (Corynebacterium Accolens, Staphylococcus Aureus) and fungus (Candida Albicans) infection. In order to find guidance cues for innate lymphocytes positioning, we found CCL20 contributes to position innate lymphocytes in close proximity with CD169+ macrophages. S1P contributes to promote lymphocytes to access subcapsular and lymphatic sinus. LFA1 helps innate lymphocytes get into lymph node parenchyma from lymphatic sinus. We also found evidence for a novel mechanism employed by innate lymphocytes to resist the shear forces of lymph flow while interacting with sinus macrophages.