

Arias, Vanessa J.

Amino acid use due to activation of murine lymphocyte subsets

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Previous research has shown that amino acid availability regulates lymphocyte proliferation and secretion of effector molecules. However, quantitative changes in the requirements of different lymphocyte subsets for amino acids due to activation are not known. Thus, we conducted a series of experiments to identify the changes in protein and amino acid levels in resting and activated lymphocytes. Lymphocyte subsets from adult male C57Bl6 were isolated from spleenocytes using indirect magnetic bead separation. Lymphocyte subsets cultured in RPMI 1640 were stimulated with PMA (20 ng/ml) + Ionomycin (0.225 µg/ml), LPS (5 µg/ml) or no mitogen. After 24-72 hrs, cells were collected and total protein and amino acid levels were determined. BrdU incorporation confirmed proliferation of stimulated cells. Results showed that the amount of protein bound essential amino acids arginine, lysine, threonine, isoleucine, and valine were significantly increased in stimulated CD4 cells compared to non-stimulated cells ($P < 0.05$). Total protein per cell was significantly increased in stimulated CD4 cells ($P < 0.05$). In addition, CD8 and B220 cells showed no significant increase in total protein per cell or protein bound amino acid levels due to stimulation. These data indicate amino acid use changes upon lymphocyte activation and is subset dependent. Supported by USDA Regional Research project 1013.

An in vitro Human Dendritic Cell Platform for Evaluation of Vaccine Formulations

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Dendritic cells are key antigen presenting cells that play a central role in both innate and adaptive immune responses. Uptake and presentation of vaccine antigens by dendritic cells is critical for the induction of a strong adaptive immune response. This process is modulated by adjuvants, such as TLR agonists and other immunostimulatory compounds that induce direct changes in dendritic cell morphology and function. We have established a system for generating human myeloid dendritic cells in vitro for use as a platform for evaluating the immunomodulatory effects of adjuvants and vaccine candidates. To eliminate genetic variability between dendritic cell batches, a stem cell line was used as the starting material. Human CD34 + fetal liver cells were expanded and cultured in serum free medium along with a combination of cytokines including stem cell factor, granulocyte-monocyte colony stimulating factor and IL-4 according to standard protocols to generate immature dendritic cells which express high levels of CD11c and HLA-DR. Immature dendritic cells were then exposed to a panel of TLR agonists and evaluated for maturation, a phenotypic change that affects morphology, expression of co-stimulatory molecules and cytokine secretion. Several individual TLR agonists and their combinations were found to have significant effects that support their use as vaccine adjuvants. This system can be used to evaluate adjuvanted subunit vaccines as well as attenuated viruses and will serve as a tool to support the evaluation of any potential vaccine candidate.

Bajpai, Urmila

Fc Receptor-Like 3 expression on Regulatory T cells in Rheumatoid Arthritis

*Urmila Bajpai, **, Louise A. Swainson*, Jeff E. Mold*, Jonathan D. Graf**, John B. Imboden**, and Joseph M. McCune**

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Fc Receptor-Like 3 expression on Regulatory T cells in Rheumatoid Arthritis Urmila Bajpai**, Louise A. Swainson*, Jeff E. Mold*, Jonathan D. Graf**, John B. Imboden**, and Joseph M. McCune* *Division of Experimental Medicine and **Division of Rheumatology, Department of Medicine, University of California at San Francisco, San Francisco, CA 94110, USA When the immune system is aberrantly activated, as in rheumatoid arthritis, teasing out the key pathogenic abnormalities leading to disease is difficult. Over the years, there has been immense interest in regulatory T cells (Tregs), which are thought to be crucial for controlling development of autoimmune diseases, including rheumatoid arthritis (RA). Recent work from our laboratory has shown that transmembrane Fc receptor-like 3 (FcRL3) is selectively expressed on Tregs. The function of FcRL3 is unknown, but a single nucleotide polymorphism (SNP) at -169 T->C has been associated with an increased risk of RA. We performed a cross-sectional analysis of FcRL3 expression on Tregs from RA patients utilizing flow cytometry. Our preliminary results show that those RA patients with the RA risk-associated FcRL3 SNP have abnormally high expression of FcRL3 and that FcRL3 expression on Tregs correlates with disease activity. We also demonstrate that successful treatment with TNF inhibitors is associated with a decrease in the percentage of FcRL3+ Tregs. In future studies, we hope to explore the effect of FcRL3 expression on T cell signaling to identify a possible mechanistic role of this subset in RA pathogenesis.

Barouch-Bentov1, Rina

A Conserved Salt Bridge in the G-Loop of Multiple Protein Kinases is Essential for Catalysis and for in Vivo Lyn Function

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The glycine-rich G-loop controls ATP binding and phosphate transfer in protein kinases. Here we show that the functions of Src family and Abl protein tyrosine kinases require an electrostatic interaction between oppositely charged amino acids within their G loops that is conserved in multiple other phylogenetically distinct protein kinases from plants to humans. By limiting G-loop flexibility, it controls ATP binding, catalysis and inhibition by ATP-competitive compounds such as Imatinib. In WeeB mice, mutational disruption of the interaction results in expression of a Lyn protein with reduced catalytic activity, and in perturbed B cell receptor signaling. Like Lyn^{-/-} mice, WeeB mice show profound defects in B cell development and function and succumb to autoimmune glomerulonephritis. This demonstrates the physiological importance of the conserved G-loop salt bridge and at the same time distinguishes the in vivo requirement for the Lyn kinase activity from other potential functions of the protein.

Barrigan, Lydia

Prostaglandin E2 Induction Occurs Rapidly After Francisella tularensis Infection

Lydia Barrigan, Matthew Woolard, Tiffany Buckley, Lucinda Hensley, Tom Kawula, and Jeffrey Frelinger

University of North Carolina at Chapel Hill

Prostaglandin E2 Induction Occurs Rapidly After Francisella tularensis Infection Lydia Barrigan, Matthew Woolard, Tiffany Buckley, Lucinda Hensley, Tom Kawula, and Jeffrey Frelinger, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill Francisella tularensis is a facultative intracellular coccobacillus and the causative agent of tularemia. F. tularensis-infected macrophages produce the lipid mediator, prostaglandin E2 (PGE2), which alters the T cell response in vitro and in vivo. We are interested in understanding the mechanism(s) utilized by F. tularensis to induce infected cells to produce PGE2. To identify F. tularensis gene(s) important for the induction of PGE2, we screened a F. tularensis subspecies novicida transposon mutant library. The screen identified twenty genes that are important for the induction of PGE2. To further clarify which genes are important for inducing PGE2, we transcomplemented many of the transposon mutants identified in the screen and examined the transcomplemented mutants' ability to induce PGE2. We next examined whether F. tularensis intracellular location affected the ability to induce PGE2. Using confocal microscopy, we show that the ability to escape the phagolysosome is not required for the induction of PGE2. We then examined whether new protein synthesis in the bacterium is required for the induction of PGE2. Treatment of F. tularensis prior to infection with antibiotics that inhibit protein synthesis prevented PGE2 induction whereas antibiotic treatment one hour post-infection did not block PGE2 induction. In summary, our data indicate that induction of PGE2 by F. tularensis takes place early during infection because mutants unable to escape the phagolysosome are still able to induce PGE2 and induction of PGE2 requires new protein synthesis by F. tularensis.

Baum, Paul

Quantitative measurement of T cell receptor diversity

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Abstract not to be posted.

Bhoopat, Lertlakana

Cell reservoirs of the Epstein-Barr virus in biopsy-proven Lymphocytic Interstitial pneumonitis in HIV-1 sub type E infected children : identification by combined in situ hybridization and immunohistochemistry

Lertlakana Bhoopat, Somrak Rangkulnuwat, Risa Okonogi, Komson Wannasai, Tanin Bhoopat.

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Abstract not to be posted.

Bold, Tyler

Antigen suppression limits the efficacy of the immune response to *Mycobacterium tuberculosis*

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NYU School of Medicine

The adaptive immune response to *Mycobacterium tuberculosis* controls bacterial growth, but it cannot eradicate the infection. We have determined that *M. tuberculosis* antigen-specific CD4⁺ Th1 cells are poorly activated during the chronic phase of infection. We investigated whether this effect limits the success of adaptive immunity and contributes to long-term bacterial persistence in the lungs. Using a mouse model of tuberculosis, we measured the activation of Th1 cells specific for the P25 epitope of *M. tuberculosis* Ag85B, an immunodominant antigen. We adoptively transferred Th1-polarized CD4⁺ P25 TCR transgenic T cells (P25Th1) into infected mice and measured their stimulation in vivo. Transferred P25Th1 cells trafficked to the lungs of infected mice and exhibited antigen dependent production of IFN- γ . During the acute phase of infection 1-2% of P25Th1 cells were stimulated, but this percentage declined as infection progressed. Interestingly, decreasing P25Th1 cell stimulation correlated with bacterial down-regulation of *fbpB* (encoding Ag85B) expression, suggesting that *M. tuberculosis* suppresses antigenic protein expression to evade adaptive immunity during chronic infection. A recombinant strain of *M. tuberculosis* designed to express high levels of *fbpB* during chronic infection stimulated P25Th1 cells at higher frequencies and was attenuated during chronic infection in a CD4⁺ T cell-dependent manner. These results indicate that in addition to defective MHC II antigen presentation, reduced expression of bacterial antigens during chronic infection may prevent complete clearance of *M. tuberculosis* by CD4⁺ T cells.

Bonnevier, Jody L.

A New and Improved Kit for the Isolation of Untouched Human and Mouse NK Cells

Jody L. Bonnevier, Nathan J. Downing, Sherry Lin, Joy Aho, Ernesto Resnik, and Jessie H.T. Ni

R&D Systems, Inc.

We have developed new kits for the isolation of untouched Natural Killer cells from both mouse and human preparations that achieve levels of purity as high as 95%. Undesired populations are negatively depleted using a cocktail of monoclonal antibodies that specifically react with non-NK cell populations. These cell types are then tagged with magnetic beads and separated from the desired NK cells by magnetic force. A typical isolation is achieved in 45 minutes. We have used the most recent and established markers and techniques to extensively characterize the isolated NK cells. We show here that the highly pure NK cell populations (both mouse and human) express NK cell-specific markers (NKp46, Nkp80, NKp30, CD56, NKG2D, KIR3DL1 and NTB-A in human, and NKp46, NKG2D and CD49b (DX5) in mouse samples). We also tested the functionality of the isolated NK cells. Isolated human NK cells were probed in a degranulation assay by measuring expression of CD107a (LAMP-1) on NK cells in a flow cytometric assay after stimulation with myelogenous leukaemia K562 cells. We tested the efficacy of our new MagCelect NK cell kits by comparison to other systems in the market, with typically better or similar results. But unlike other commercially available kits that require a costly set of magnets and supplies, our kits were developed to work with different kind of commercially available magnets and systems, providing more flexibility, simplicity and cost-efficacy.

Brennan, Catherine

Bacterial molecules exported from the phagosome as immune signals: a genetic approach in *Drosophila*

Catherine Brennan,

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As first responders to bacterial infection, phagocytic cells have a dual imperative: to inactivate engulfed pathogens, and to communicate information about the infection to other immune cells. The phagosome, the vesicle that harbors and then destroys engulfed microbes, plays a central role in linking the two functions. This is true both for adaptive immunity, where the phagosomal events that prepare MHC class 2 antigens for presentation are well known, and for innate immunity, where the mechanisms are less well understood. Our finding that degradation of engulfed bacteria by *Drosophila* phagocytes is necessary for the activation of immune gene expression throughout the organism established the fly as an *in vivo* model to investigate the coupling of phagosome function and innate immune signaling. We have recently found that defects in immune gene activation that occur in *psidin* phagosome maturation mutants can be rescued by supplemental direct injection of a purified bacterial cell wall component, peptidoglycan. The ability of peptidoglycan to substitute for the phagocyte immune signal, in conjunction with the capacity of insect phagocytes to export bacterial molecules, suggests that peptidoglycan is the physiological ligand released by phagocytes during infection to activate immune gene expression in other tissues. We have established a tractable genetic system to identify more mutants with phagosome maturation and signaling defects, and have identified sets of genes that positively and negatively regulate this process. Through epistatic analysis in double mutants we are ordering the genes into functional pathways. This is the first reported use of a microbe-derived molecule as an immune signal, and could represent an antecedent of the mammalian system of antigen presentation. Our *in vivo* genetic approach promises to elucidate the cellular mechanisms that execute signaling from the phagosome ; and by extending our studies into mammalian macrophages we will determine the importance of this novel immune signaling pathway in mammals.

Characterization of a Mutant Mouse Strain with Altered Peripheral CD4 T Cell Homeostasis

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Davidson College

The mechanisms regulating peripheral CD4⁺ T cell homeostasis are not completely understood. Previous research at Davidson College has identified a mutant strain of mice with an inverted CD4/CD8 ratio in peripheral lymphoid organs and blood. Furthermore, the mutant strain shows an increased incidence of encephalomyelitis, right-eye blindness, and skin lesions in the absence of infection, indicating a possible autoimmune condition. The purpose of this study is to explore the mechanisms behind the altered T cell ratio, homeostasis, and potential autoimmunity in these mice. Cell counts indicate that the mutant strain exhibits a highly reduced frequency of CD4 T cells while the CD8 T cells remain largely unaffected when compared to control strains. First, we examined whether the CD4 cells in the mutant mouse are more susceptible to apoptosis. AnnexinV/7AAD staining found no differences between mutant and control mice in the frequencies of AnnexinV⁺CD4⁺ or AnnexinV⁺CD8⁺ cells in the presence or absence of TCR stimulation. Then, we examined the role of various factors involved in peripheral homeostasis. We investigated IL-7 signaling in peripheral T cells by culturing lymphocytes overnight in IL-7 deficient medium and analyzing the dynamics of IL-7R expression using flow cytometry. The data show that IL-7R is upregulated normally in the mutant strain when compared to a control of the same genetic background, indicating that IL-7 signaling is not impaired. Finally, we investigated the functionality of mature T cells from the mutant strain. In vitro TCR stimulation of CFSE-labeled T lymphocytes with either alloreactive APCs or anti-TCR-beta antibody demonstrated that CD4⁺ and CD8⁺ cells from the mutant activated and proliferated in numbers comparable to the control strain. In order to evaluate T cell homeostatic proliferation, we performed an in vivo BrdU labeling experiment, followed by flow cytometry. Results indicate that more BrdU incorporation occurred in CD8 cell than CD4 cells in the mutant, but that both classes of T cell proliferated at roughly double the rate of the control strain. This finding could partially explain the skewing of the CD4/CD8 ratio in favor of the CD8 cells. We think that this may also be consistent with the putative autoimmune condition in the mutant strain. If CD4 cells are infiltrating non-lymphoid tissues, more rapid proliferation of both cell types could be a compensatory mechanism occurring to fill the niche vacated by the autoreactive CD4 cells. This would explain both the decreased frequency of CD4 cells and the increased rate of proliferation.

Castelli, Luca

TH17 AND TH1 CELLS TRAFFICKING WITHIN THE CNS

Luca Castelli, Stromnes Ingunn, Goverman Joan

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Abstract not to be posted

Evaluating the selective pressures exerted on *M. tuberculosis* antigen genes by the human adaptive immune response

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New York University School of Medicine

The human adaptive immune response is insufficient to eradicate *Mycobacterium tuberculosis* from the host, leading to persistence of the pathogen in a state of latent infection. However, the mechanisms by which *M. tb* evades immunity still need elucidation. Many pathogens are able to escape immune responses by varying their epitope sequences, and we are interested in whether *M. tuberculosis* makes use of this strategy to avoid elimination by human adaptive immunity. To answer that question, we made use of whole genomes sequences of 22 phylogenetically diverse strains from the *M. tb* complex, and amino acid sequences of 491 experimentally determined *M. tuberculosis* T cell epitopes from 78 antigen genes, as reported in the Immune Epitope Database. We found that the rates of nucleotide sequence variation amongst antigen genes were no greater than those of the remainder of the genome. We then undertook a positive selection analysis to determine the implications of nucleotide variations on protein coding. Within the antigen genes we calculated dN/dS values for those regions defined as T cell epitopes and those defined as non-epitope regions. The epitope containing regions had a significantly lower dN/dS value than the non-epitope regions (0.25 vs. 0.49), and the rest of the genome. Furthermore, 466 (95%) of the epitope sequences had invariant peptide sequences across all 22 strains. Taken together, the latter two results imply that the epitope sequences are not under selective pressure to mutate in response to human immunity. In fact, these sequences are being hyperconserved relative to the rest of the genome over the course of evolution. This suggests that recognition of epitopes by human T cells may in fact be beneficial to *M. tuberculosis* to promote its survival or transmission.

Crabtree, Matthew

Systemic Lupus Erythematosus (SLE) Anti-maternal T Cell Responses Include Elevated TNF α and IFN-gamma that Correlates with Increased Disease Activity

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Systemic Lupus Erythematosus (SLE) Anti-maternal T Cell Responses Include Elevated TNF- α and IFN-gamma that Correlates with Increased Disease Activity **BACKGROUND:** Maternal cells passing into the fetus can persist for decades after birth, creating a state of microchimerism. Loss of T lymphocyte tolerance to maternal antigens could lead to chronic inflammatory diseases, as demonstrated by the mouse model of SLE-like graft-versus-host-disease induced by parental to F1 allograft. Inflammatory cytokines IFN-gamma and TNF- α are implicated in the pathogenesis of both SLE and GVHD. **HYPOTHESIS:** Lupus patients with increased disease activity have pro-inflammatory T cell responses to maternal cells. **METHODS:** Pediatric PBMC (SLE and control) were stimulated by maternal cells in one-way MLRs for 5 days. Multi-color flow cytometry was used to quantify cytokine production among T cell subsets. Maternal microchimerism was measured with Real-Time QPCR for non-inherited maternal alleles using >100,000 genome equivalents of genomic DNA isolated from PBMC. Statistical analysis was performed using STATA SE. **RESULTS:** We found that IFN-gamma production by CD4 $^{+}$ and CD8 $^{+}$ T cells was increased in response to maternal cells in patients with more active disease. We also found a correlation between TNF- α production by CD4 T cells and disease activity. Levels of maternal microchimerism were not increased in SLE patients with active disease. **CONCLUSIONS:** The elevated T cell responses to maternal cells we observed for IFN-gamma and TNF- α are consistent with a model of T cell-mediated immune rejection of maternal cells in the periphery as well as target organs.

De Groot, Annie

T cell epitopes (Tregitopes) contained in IgG Constant Domains activate natural Tregs: Relevance for autoimmune disease, gene therapy, and less immunogenic biologics

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Working with a set of putative natural T regulatory epitopes derived from IgG (□Tregitopes□as described in Blood, 2008; 112: 3303-3311), we have now confirmed that co-administration of Tregitopes with a range of proteins (such as Ovalbumin, allergens, Class I epitopes and Class II epitopes) in vitro and in vivo leads to suppression of T cell and antibody responses to the test antigens. In addition, the development of diabetes is delayed and suppressed in NOD mice treated with the Tregitopes (Scott, Su). In the gene therapy context, CTL effector expansion in the presence of Tregitope resulted in 79% to 89% inhibition of cytotoxic activity against peptide-pulsed and AAV-transduced target cells. Regulatory T cell staining supported these findings, demonstrating a significant increase of this cell population in PBMCs expanded with the AAV-specific CD8⁺ T cell epitope in the presence of Tregitope (62.2% of CD4⁺ cells were CD25⁺FoxP3⁺) compared with PBMCs expanded with the AAV epitope alone (3.63%), or with the irrelevant peptide (1.94%) (Hui, Mingozzi, High). Tregs expanded more from day 0 (4%) to day 5 in Hu PBMC coincubated with alloantigen and Tregitope²⁸⁹ as compared to controls (15% vs. 6%) coincubated with flu peptide (10mcg/ml). In a secondary MLR assay, Tregitope-treated cells proliferated less (CD4⁺CFSE⁺ 55% vs 45%) and produced lower amount of pro-inflammatory cytokines (IFN-g 853±90 vs 2093±388, p<0.01; TNF-a 385±41 vs. 638±7, p<0.03; IL1-b 123±10 vs. 256±160, p<0.02; IL-6 3255±100 vs.5920±20 pg/ml, p<0.002). (D□addio, Najafian and Sayegh). The mechanism of suppression appears to be due to the induction of antigen-specific adaptive tolerance induction (De Groot AS et al. Activation of natural regulatory T cells by IgG Fc-derived Peptide

Dennis, Jr., Glynn

Soluble Clm5-Fc prevents inflammatory arthritis in mice by blocking a distinct pathway of neutrophil-dependent, mast cell-independent vascular permeability and cellular infiltration.

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Background: The Clm5 cell-surface glycoprotein is an activating member of the murine CD300 multi-gene family. Members of the CD300 family are expressed by myeloid lineage cells and are postulated to play an important role in regulation of the immune system. We set out to investigate the immunoregulatory role of soluble Clm5 by testing its efficacy in mouse models of rheumatoid arthritis (RA). Methods: Soluble Clm5 proteins (Clm5-Fc) were constructed by fusing the extracellular Ig-like domain of Clm5 with the Fc portion of mouse IgG2a. Clm5-Fc was tested for efficacy in arthrogen-induced arthritis (AIA), collagen induced arthritis (CIA), and serum transfer arthritis (K/BxN). Mechanism of action studies were performed in normal and genetically modified mice using near-infrared fluorescent imaging (NIRF), 2-photon in vivo microscopy and bioluminescence imaging techniques. Results: Clm5-Fc significantly reduced the clinical and histological signs and symptoms of arthritis in three mouse models of RA. The preventative effect of Clm5-Fc depended on the Clm5 Ig-like domain as well as Fc binding to Fc-gamma receptor 3 (FcGR3), but did not require FcGR2b and did not alter anti-collagen antibody levels, systemic cytokines, leukocyte counts or complement activation. Mechanism of action studies using the K/BxN serum transfer model demonstrated that Clm5-Fc blocked K/BxN induced vascular permeability (VP). This blockade was refractory to further Fc-mediated stimulation for more than 5 days, but was responsive to histamine stimulation within 24 hours. Persistent vascular desensitization was due the fact that Clm5-Fc itself caused VP in the joints of wild-type, mast cell deficient and irradiated wild-type mice, but not in irradiated mast cell deficient mice. Unlike the VP induced by K/BxN, Clm5-Fc induced VP was histamine independent and did not require mast cells. Further in vivo dissection revealed that K/BxN serum induced a mast cell dependent, FcGR3 dependent induction of neutrophil myeloperoxidase (MPO) activity in and around the joints that preceded cellular infiltration. K/BxN serum administration to mice pretreated with Clm5-Fc failed to produce MPO activity and lacked cellular infiltration into the joints. Conclusions: Reduction in RA symptoms by soluble Clm5-Fc resulted from a specific blockade of neutrophil activation, vascular permeability and cellular infiltration. Importantly, this blockade was mast cell-independent and mediated through inhibition of neutrophil activation. These findings suggest that Clm5-Fc acts on an immunoregulatory pathway that lies at the interface between neutrophil activation, vascular permeability and cellular infiltration.

Desvignes, Ludovic

Interferon-gamma-responsive nonhematopoietic cells regulate the immune response to *Mycobacterium tuberculosis*

Ludovic Desvignes, Ernst J.D.

New York University School of Medicine

Immunity to *Mycobacterium tuberculosis* in humans and in mice requires interferon gamma (IFN-gamma). Whereas IFN-gamma has been studied extensively for its effects on macrophages in tuberculosis, we determined that protective immunity to tuberculosis also requires IFN-gamma-responsive nonhematopoietic cells. Bone marrow chimeric mice with IFN-gamma-unresponsive lung epithelial and endothelial cells exhibited earlier mortality and higher bacterial burdens than control mice, under-expressed indoleamine-2,3-dioxygenase (IDO) in lung endothelium and epithelium, and over-expressed interleukin-17 (IL-17) with massive neutrophilic inflammation in the lungs. We also found that the products of IDO catabolism of tryptophan selectively inhibit IL-17 production by Th17 cells, by inhibiting the action of IL-23. These results reveal a previously unsuspected role for IFN-gamma responsiveness in nonhematopoietic cells in regulation of immunity to *M. tuberculosis* and illustrate the role of IDO in the inhibition of Th17 cell responses.

Infection Mediated Induction of Distinct CD4+ T Helper Cell Subsets

Rebecca Elsner, Nicole Baumgarth

University of California Davis, Microbiology Graduate Group, Center for Comparative Medicine

Humoral immune responses rely on CD4+ T helper cells (TH) for induction, affinity maturation, and generation of long-term immunological memory. While the role of TH cells in germinal center responses has been studied extensively, their role in the establishment and maintenance of extrafollicular foci is less well understood. Recently CD4+ ICOS+ CXCR4+ PSGL-1^{low} TH cells were identified in multiple models of autoimmunity. Based on their location in extrafollicular foci it was proposed that they represent "extrafollicular" TH cells. Whether this or any other distinct extrafollicular TH population is induced as part of a pathogen-induced response is currently unknown. To address this question and to begin to elucidate the infection-induced development of TH populations and their function(s), we examined CD4+ T cell responses in two distinct murine models of infection: Acute respiratory tract infection with influenza virus and systemic non-resolving bacterial infection with *Borrelia burgdorferi*, the Lyme Disease agent. Using both models in conjunction with multicolor flow cytometry, a subset of the "classical" ICOS+CXCR5+ follicular TH cells (TFH) that co-expressed CXCR4 was identified in regional lymph nodes. Activated CXCR5+ single- and CXCR4+ CXCR5+ double-expressing TH cells were present by day 6 of influenza virus infection with single expressers peaking earlier than the double expressers: day 10 versus day 14-28 post infection, respectively. Single CXCR4 positive CXCR5 negative TH cells were not found following either influenza or *B. burgdorferi* infection for up to 28 days. Based on these and functional studies to be presented and previous studies demonstrating the presence of the CXCR4 ligand CXCL12 in sub-compartments of germinal centers, our data suggest that ICOS+ CXCR4+ CXCR5+ TH cells represent a subset of germinal center TFH cells. Based on the kinetics of their induction and given that the double-expressers appeared more highly activated than the CXCR5+ single expressing TH cells, the data furthermore indicate that the double-expressers might develop from single-expressers over time. This also suggests that CXCR5 single-positive cells provide help to both extra- and intra-follicular B cell responses.

GLI Transcription Factors Induce TGF- β 1 in Human CD4+ T-cells Following Activation.

Robert Furler, Ruth Getachew, Christel Uittenbogaart

University of California-Los Angeles

BACKGROUND: The depletion of Th17 and increase of Treg in the gut have been implicated in chronic immune activation in HIV-infected patients and NHP models of progressive HIV infection. The balance of human Th17 and Treg cells can be altered by TGF- β 1, a pleiotropic cytokine that has been shown to be induced by HIV-1 Tat and is increased in progressive HIV-1 disease. We have investigated the role of the human GLI proteins, which have previously been reported to interact with Tat and transactivate the LTR of HIV-1, in TGF- β 1 induction. Here we report that GLI proteins regulate TGF- β 1 during CD4+ T-cell activation which may lead to the skewing of human CD4+ T-cells from Th17 cells, that protect mucosal barrier integrity, to iTreg that can suppress HIV-specific immune responses. **METHODS:** Human naïve CD4+ T-cells were negatively selected from PBMC and stimulated with aCD3/aCD28 microbeads for 24 hours prior to RNA extraction. RT-PCR of GLI1 mRNA was done and standardized to 18S rRNA to test the activation state of GLI2 and GLI3 following CD4+ T-cell stimulation. For in silico screening of putative GLI binding sites in the human TGF- β 1 promoter 293T cells were cotransfected with TGF- β 1 promoter-luciferase constructs along with pGLI2 Δ N (activator), pGli3PHS (repressor), or pcDNA3 control. Putative GLI-binding sites were mutated using site-directed mutagenesis. Luciferase activity was read 48h post-transfection. The Wilcoxon Rank Sum test was used for statistical analysis of the data. **RESULTS:** We found that there are six putative GLI-binding sites surrounding the human TGF- β 1 promoter. RT-PCR of GLI1 mRNA, a marker for GLI2 and GLI3 activation, confirmed that stimulation of CD4+ T-cells through CD3/CD28 activates GLI2 and GLI3 which induces GLI1 mRNA >10-fold and TGF- β 1 mRNA 4-fold. A constitutive Gli activator (GLI2 Δ N) significantly induced transcription at the TGF- β 1 promoter by 4-fold over control. Mutagenesis of the putative GLI binding sites abrogated this induction. Subsequently, a constitutive GLI repressor (GLI3-PHS) significantly repressed TGF- β 1 transcription 14-fold over control. **CONCLUSIONS:** The GLI proteins which have previously been shown to transactivate the HIV-1 LTR, are activated following CD4+ T-cell stimulation and induce TGF- β 1 by binding to its promoter. The increased levels of TGF- β 1 induced by the GLI proteins may be one of the underlying factors for the Th17/Treg imbalance seen in HIV-1 disease.

Gravano, David M.

Thymic Epithelial Cell-Specific Expression of the ADAM17 Metalloprotease is Not Required for T Cell Development

David M. Gravano, Leonard K. Vu, Sonia Mathew, Bryce McLelland, Jennifer O. Manilay

University of California Merced

Development of T cells in the thymus is highly dependent on signals provided by the thymic epithelial cells (TECs). The metalloprotease ADAM17 cleaves the extracellular domain of many membrane bound proteins and functions by shedding them from the cell surface or modulating the activity of cell surface receptors such as Notch and EGFR. Previous studies in ADAM17-deficient mice revealed a severe block in early thymocyte development, presumably due to the absence of ADAM17 in thymic stromal cells. However, the specific thymic stromal cell subset responsible for this T cell developmental defect was not identified. In an effort to characterize ADAM17's non-cell autonomous role in T cell development, we generated a TEC-specific ADAM17 knockout (KO) mouse model (Foxn1-Cre/ADAM17-floxed). In this KO mouse model ADAM17 expression in EpCAM⁺ TECs is 3% of control mice, but is expressed normally in thymocytes and other thymic stromal cells. T cell development in the ADAM17 KO was analyzed at 4, 8, and 12 weeks of age. Surprisingly, we did not observe any phenotypic differences in thymocyte development between the ADAM17 KO mice and controls. Analysis of TEC subsets in the ADAM17 KO showed that cortical and medullary TEC (mTEC) subsets are present in ADAM17 KO mice, and preliminary analysis suggests that the frequency of mTECs might be reduced in the KO. Taken together, our data clearly show that ADAM17 in TECs is dispensable for thymocyte development and suggest that non-TECs are the critical ADAM17-expressing cellular component for thymic development. Indeed, ADAM17 and many of its substrates are expressed on non-TECs (including MTS15⁺ fibroblasts) and ADAM17 remains highly expressed on non-TECs in the ADAM17 KO mice. Our findings implicate non-TECs as an important cellular component for thymic development and suggest the role of ADAM17 in thymic development is localized to these cells.

Thymic Stromal Lymphopoietin Expression in Respiratory Syncytial Virus-Infected Airway Epithelial Cells

Lee Hai-Chon, Mark B. Headley, Michael Gale, Jr. & Steven F. Ziegler

Benaroya Research Institute, University of Washington

Respiratory syncytial virus (RSV) is important viral agent of pediatric respiratory diseases that infects airway epithelial cells (AEC), causing a severe acute bronchiolitis and asthma exacerbation. AEC are responsible for the production of cytokines and chemokines that are important mediators of the exacerbated airway inflammation triggered by the host in response to RSV infection. Herein, we report that infection of AEC by RSV, and its murine counterpart Sendai virus (SeV), induced expression of thymic stromal lymphopoietin (TSLP), a cytokine implicated in allergic asthma. We found that RSV and SeV were capable of inducing rapid TSLP production in primary human bronchial airway epithelial cells. The induction of TSLP by SeV was dependent upon RIG-I- and IPS-1-dependent NF- κ B activation, but not IRF-3 and IRF-7 activation. We also demonstrated that siRNA-mediated RIG-I significantly inhibited SeV-induced TSLP gene expression. This study demonstrates for the first time that TSLP is induced by respiratory viral infection through RIG-I pathway in airway epithelial cells.

Han, Hongwei

**Intradermal Administration of Thymic Stromal Lymphopoietin Aggravates
Experimental Asthma**

*Hongwei Han, *, Weihui X. Shi*, Steven F. Ziegler*

Benaroya Research Institute, University of Washington

Approximately half of AD patients will develop asthma, particularly those with severe AD, suggesting that AD is an "entry point" for subsequent allergic disease. We have established a mouse model to study this "atopic march". Here we show that mice exposed to antigen, in the presence of TSLP, in the skin develop severe airway inflammation when challenged later with the same antigen intranasally. Interestingly, local TSLP production in the lung or circulating TSLP are not required to drive an aggravation of the asthma that was generated upon subsequent antigen challenge. TSLP-induced responses were significantly, but not completely reduced in the absence of CD4 T cells. This study, which provides a clean mouse model to study human "atopic march," indicates that keratinocyte-produced TSLP may represent an important factor in the link of atopic dermatitis to asthma.

Haney, Danielle

Sorting Viable Antigen-Specific Polyfunctional CD8+ T cells

Danielle Haney, Tedi Asher, David Ambrozak, David Price, Danny Douek, Michael Betts

University of Pennsylvania

Abstract not to be posted.

Harris, David

Phosphoinositide 3-kinase-C2[a] (PI3K-C2[a]) is Essential for Normal Renal Function in Mice

David Harris, Peter Vogel, Marie Wims, Karen Moberg, Juliane Humphries, Kanchan Jhaver, Chris Da Costa, Melanie Shadoan, Nianhua Xu, Gwenn Hansen, Jan Domin, David Powell, Tamas Oravecz

Lexicon Pharmaceuticals, Inc.

Phosphoinositide 3-kinase-C2A (PI3K-C2A) is Essential for Normal Renal Function in Mice. David P. Harris, Peter Vogel, Marie Wims, Karen Moberg, Juliane Humphries, Kanchan Jhaver, Chris Da Costa, Melanie K. Shadoan, Nianhua Xu, Gwenn Hansen, Jan Domin*, David Powell, Tamas Oravecz. Lexicon Pharmaceuticals, Inc. The Woodlands, TX, 77381, USA. *Renal Section, Imperial College London W12 0NN, UK. PI3K-C2A is a class II PI3K enzyme whose significance for mammalian physiology and pathology is unknown. This enzyme is expressed in many tissues including the kidney, where highest levels are found in glomerular epithelial cells and podocytes. We have generated PI3K-C2A null mice and demonstrate a critical role for the PI3K-C2A enzyme in maintaining normal renal homeostasis. PI3K-C2A-deficient mice displayed a profound failure to thrive, exhibited modest polyuria and proteinuria, had elevated serum BUN and creatinine levels and exhibited impaired creatinine clearance. Histopathology demonstrated that these findings were due to a progressive fatal glomerulonephropathy which manifested a range of lesions within affected glomeruli with diffuse mesangial sclerosis and associated tubulointerstitial disease being the predominant presentation. The progression of renal disease in PI3K-C2A-deficient animals was associated with a concomitant development of leukocytosis, resulting in severe splenomegaly, and an increased production of serum IgA and IgM and immune complexes. Importantly, deletion of PI3K-C2A did not affect innate and adaptive immune responses. Furthermore, bone marrow transplantation demonstrated that the glomerulonephropathy observed in PI3K-C2A-deficient mice was not prevented by the production of wild-type hematopoietic cells. These data indicate that the renal injury in knockout mice is not a consequence of autoimmunity but instead it is due to defects in cells intrinsic to glomeruli. Based on these data we propose that expression of the PI3K-C2A isoform is critically required for maintaining the normal structure and function of the kidney.

Hastey, Christine

Dancing alone in *Borrelia burgdorferi* infection: Strong B cell response independent of CD4 T cell signaling

Christine Hastey, Stefan Tunev, Stephen Barthold, Nicole Baumgarth

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Abstract not to be posted.

Headley*, Mark

Thymic Stromal Lymphopoietin represents a molecular link between respiratory viral infection and asthma

Mark Headley, Hai Chon Lee*, Yueh-Ming Loo, Whitney Shih, Aaron Berlin, Michael Gale, Nick Lukacs, Steven F. Ziegler*

University of Washington, Benaroya Research Institute

Early infection with Respiratory Syncytial Virus (RSV) has long been recognized to predispose certain individuals towards the development of asthma and is additionally a leading cause of asthma exacerbation. Though much is known about the pathology of this disease it is still unclear what the exact link is between RSV infection and asthma pathology. Thymic Stromal Lymphopoietin (TSLP) is a cytokine that has been shown to be both necessary and sufficient for the development of asthma. Mice that express a lung-specific TSLP transgene (SPC-TSLP) develop a spontaneous and progressive asthma-like disease while TSLPRKO mice are highly resistant to disease in an antigen-specific asthma model. In this study we highlight a possible mechanistic connection between TSLP, RSV infection, and asthma. We show for the first time that RSV and its relatives lead to increases in TSLP expression by airway epithelial cells both in vitro and in vivo. Further, intranasal supplementation with exogenous TSLP during RSV infection leads to significant increases in asthma associated parameters, including increased IL-13 production and mucus hypersecretion when compared to animals with RSV alone. Importantly, neutralization of TSLP during RSV infection dramatically reduces disease exacerbation upon subsequent sensitization and challenge with allergen. Together these data suggest that TSLP may be a primary link between RSV infection and asthma.

Hensley, Lucinda

A Targeted Approach to the Identification of T Cell Epitopes in *Francisella tularensis*.

Lucinda Hensley, Michael Valentino, Pamela McPherson, Zachary Maben, Jeffrey Frelinger, and John Frelinger

University of North Carolina at Chapel Hill

Francisella tularensis is a facultative intracellular pathogen that causes the zoonotic disease tularemia. Both the innate and adaptive immune systems are important in the response to and ultimate clearance of this pathogen. We have developed an extensive library of T cell hybridomas from *F. tularensis* infected animals using standard hybridoma technology and used them to define T cell epitopes important in the immune response to infection with *F. tularensis*. We recently recloned the Venter Institute SchuS4 ORF library in the pBAD-DEST49 expression vector and expressed and purified each product and arrayed the recombinant proteins in a high throughput 96 well format. In order to expedite epitope identification we used Western blot analysis along with previously identified immune targets to identify and produce a sub-array of 45 gene products which we dubbed "Greatest Hits". Initial screens using this technology have identified hybridomas that react with the *F. tularensis* proteins IglB, GroEL, and DnaK. The use of the "Greatest Hits" approach can dramatically simplify and speed up screening for T cell epitopes and the same reagents and strategies developed for mapping T cell epitopes may also be useful for the mapping of B cell epitopes.

Hess, Paul R.

Cytotoxic class I tetramers prevent in vivo priming of CD8+ T cell responses against the minor histocompatibility antigen HY

*Paul R. Hess, Sabrina L. Murray, Adam S. Buntzman, Ellen F. Young, Benjamin J. Vincent,
Keith R. Miller, Edward J. Collins, Jeffrey A. Frelinger*

NCSU College of Veterinary Medicine & UNC-Chapel Hill School of Medicine

Abstract not to be posted.

Hill, Jonathan

Visualizing Lymphocyte Attack on Pancreatic Beta Cells

Jonathan Hill, Rainer H. Kohler, Jose-Luiz Figueiredo, Jason Gaglia, Ralph Weissleder, Christophe Benoist, Diane Mathis

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Massachusetts General Hospital

One of the hallmarks of type-1 diabetes in humans and mice is the infiltration of CD4⁺ T cells into the pancreatic islets. These lymphocytes are thought to be major players in coordinating the autoimmune response that leads to the destruction of insulin-producing beta cells. Despite significant advances in understanding this disease process we remain fundamentally ignorant of CD4⁺ T cell dynamics at the site of inflammation. In light of the complexities of studying this process in humans, we have utilized genetically manipulated variants of the non-obese diabetic mouse strain to image the lymphocyte attack in the pancreas of living animals through intravital confocal microscopy. By utilizing autoreactive BDC2.5 T cell receptor transgenic CD4⁺ T cells that stably express the red-shifted mRaspberry fluorescent protein under the control of the β -actin promoter, we have tracked these cells as they enter the pancreas and can spatially orient their location and behavior with respect to GFP-expressing beta cells and microvascular imaging probes. This intravital imaging system was optimized through the use of a custom-designed tissue stabilizer and a self-regulating heating chamber in order to maintain the exteriorized spleen and pancreas under physiological conditions while acquiring stable time-lapse images of lymphocyte dynamics. By using endogenously expressed fluorescent protein, rather than cellular dyes, we can study islet infiltration by CD4⁺ T cells over the course of weeks rather than days. In doing so we have visualized early events of lymphocyte entry into the pancreas which is characterized by sparse T cell seeding in perivascular regions in close proximity to beta cells and late events where T cells form a densely packed insulinitic lesion. Further analysis aims to understand how CD4⁺ T cell movement is governed in the pancreatic tissue compared with the spleen, and how regulatory T cells influence the dynamics of insulinitis.

Ingram, Michael

Equine Antibody Response to the Facultative Intracellular bacteria *Corynebacterium pseudotuberculosis*

Michael Ingram, Dina Abdel-Massih, Luca Valle, Elise Burger, Elizabeth Braker, Sharon Spier, Roberta Pollock

Occidental College Department of Biology

Corynebacterium pseudotuberculosis is a gram-positive facultative intracellular bacterium that causes a disease called pigeon fever or dryland distemper in horses. Most infected horses develop large pus-filled external abscesses on the pectoral region, which resolve with a low mortality rate. Some horses develop internal intramuscular abscesses, with a subsequent mortality rate of about 40%. Our hypothesis is that the TH1 to TH2 balance determines the form of the disease: TH1 dominance may lead to external abscesses whereas TH2 dominance may lead to internal abscesses. The different TH subsets are distinguished by their distinct cytokine producing profiles and also induce different IgG subclasses. We developed an ELISA that measures the antibody response to phospholipase D (PLD), the major antigen and exotoxin of the bacteria, and screened 67 infected and uninfected horses. One diagnostic criterion has been that horses with internal abscesses have higher levels of antibodies to PLD, while horses with external abscesses have lower antibody titers. Our results show that while most horses with internal abscesses show high antibody responses to PLD, many horses with external abscesses and some uninfected horses also have high titers to PLD. The ELISA has also been used to measure the IgG subclasses of anti-PLD antibodies made by infected horses and we found no correlation between the IgG subclass antibody level and the type of abscess observed. Lastly, preliminary immunoblot results of *C. pseudotuberculosis* cell lysate indicate that the anti-PLD antibodies in infected sera have similar concentrations to antibodies specific for other bacterial proteins.

Contribution of Dual T Cell Receptors to a CD8+ T cell-mediated CNS Autoimmunity

Qingyong Ji, Antoine Perchellet and Joan Goverman

University of Washington

Contribution of Dual T Cell Receptors to a CD8+ T cell-mediated CNS Autoimmunity
Qingyong Ji, Antoine Perchellet and Joan Goverman Department of Immunology, University of Washington, Seattle, WA 98195 Abstract Multiple sclerosis (MS) is believed to be an autoimmune disease of the central nervous system. The cause of MS is not known but viral infections have been implicated. Experimental autoimmune encephalomyelitis (EAE) is commonly used as an animal model for MS. CD8+ T cells have recently been shown to mediate the pathogenesis of EAE, but how CD8+ T cells become activated is still unclear. In this study, using a CD8+ T cell receptor (TCR) transgenic mouse model we investigated the roles of viral infection in breaking tolerance of autoreactive T cells and triggering EAE. Myelin basic protein (MBP) 79-87-specific TCR transgenic 8.8 (VA8+VB8+) mice maintain tolerance and do not develop EAE spontaneously. Peptide immunization failed to break 8.8 T cell tolerance. Surprisingly, infection with wild-type vaccinia virus caused a weight loss and inflammation in brain and spinal cord (atypical EAE) as efficiently as recombinant vaccinia virus expressing MBP. CD8+ T cells up-regulated activation markers and trafficked to the CNS in vaccinia-infected mice. T cell activation was neither due to TCR cross-reactivity against vaccinia virus, nor due to bystander activation following viral infection. Furthermore, T cell activation did not require the presence of MBP since CD8+ T cells in MBP-deficient mice also displayed activation markers and acquired cytolytic effector function. However, 8.8 RAG-2^{-/-} mice lacking endogenous TCRs did not develop disease following vaccinia infection. Finally, T cells co-expressing the transgenic TCR chains and VB6 were specifically enriched following vaccinia infection of 8.8 mice. In conclusion, the results suggest that viral infections may trigger autoimmune diseases by activating autoreactive T cells through a second TCR on the same T cells.

A role of TSLPR on Th2 cells

Masayuki Kitajima, Hai-Chon Lee

Benaroya Research Institute at Virginia Mason

The cytokine thymic stromal lymphopoietin (TSLP) has been implicated in the development and progression of allergic inflammation in both humans and mice. Although the underlying mechanism is not known, TSLP-stimulated dendritic cells have been shown to prime human CD4 T cells into Th2 cytokine-producing cells. In addition, TSLP can directly enhance expansion of CD4 T cells upon TCR engagement, and can directly drive Th2-mediated responses via the induction of IL-4 production in naïve CD4 T cells. However, its direct effect on effector Th cells has not been extensively investigated. In this study, we show that the level of TSLPR expression on effector Th2 cells is higher than on Th1 and Th17 cells. TSLPR expression in Th2 differentiation was up-regulated in an IL-4 dose dependent manner. Functionally, TSLP treatment to Th2 cells, but not Th1 or Th17, induced proliferation. And, TSLP was also induced the phosphorylation of Signal Transducer and Activator of Transcription (Stat) 5, and expression of anti-apoptotic factor Bcl-2, in Th2 cells. But, these effects were not induced in TSLPR^{-/-} Th2 cells. The TSLP-mediated proliferation was reduced in resting Th2 cells, which were cultured with medium for 1 day following differentiation. However, the ability of rested Th2 cells to respond to TSLP was restored by IL-4 or IL-7 treatment. Taken together, these results indicate that TSLP is directly activated effector Th2 cells, and the response is correlated with the level of TSLPR expression. Thus, the mechanism suggests that TSLP is involved in exacerbation of Th2-mediated allergic inflammation by activating Th2 cells directly.

Ubiquitin specific protease 8 (USP8) is essential for normal T-cell development and immune homeostasis

Klaus-Peter Knobeloch, Agnes Kisser, Almut Dufner, Sandra Niendorf, Christoph Loddenkemper, Marco Prinz, Stefanie Manthey, Caroline Westendorf, Marco Prinz and Klaus-Peter Knobeloch

University Freiburg, Dept. Neuropathology

Ubiquitin modification represents a versatile mechanism to control cellular functions and is counteracted by the activity of ubiquitin deconjugating enzymes (DUBs). More than 90 different DUBs are encoded in the human genome and around 50 DUBs belong to the family of ubiquitin specific proteases (USPs). Despite the high number and diversity the physiological function and biological relevance of individual DUBs especially in discrete cell types is poorly defined. Using cre-loxP mediated gene targeting we have generated mice with T-cell specific deletion of USP8 (deltaT-USP8) in order to analyze the function of this ubiquitin isopeptidase in T-cells, where a pleiotropy of key signalling molecules are controlled by ubiquitin mediated mechanisms. Surprisingly lack of USP8 in T-cells caused severe autoimmune inflammatory bowel disease leading to premature death of the animals. CD4+ cells in the periphery are highly activated demonstrating an essential function of UBPY in the prevention of aberrant T-cell responses in vivo. In addition numbers of CD4+ and CD8+ positive T-cells in spleen, lymphnodes and thymus of deltaT-USP8 mice are strongly reduced caused by impaired transition from the CD4+CD8+ to the CD8+ and CD4+ stage of thymocyte development. In contrast to hepatocytes and fibroblasts where USP8 is essential for the stability of the HRS STAM complex, no reduction in protein levels of these proteins were detected in USP8 deficient T-cells pointing to specific functions of USP8 in different celltypes. As we detected enhanced Erk phosphorylation upon T-cell receptor stimulation in UBPY deficient thymocytes, we propose that USP8 mediated ubiquitin deconjugation is necessary to ensure proper T-cell signalling, a prerequisite for normal thymocyte development and the maintenance of immune homeostasis.

Ku, Chia-Chi

The use of ENU-mutagenesis mouse model to study the role for IL-15 alternative splice variant in the establishment of antigen specific CD8 T cells against Herpes Simplex Virus-1 infection

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IL-15 is a pleiotropic cytokine which mainly supports NK and memory CD8 cells. An ENU-mutagenized mouse, pedigree 191 (P191), generated by the Mouse Mutagenesis Program Core Facility (MMPCF) in Taiwan was characterized by the predominant expression of an alternatively spliced IL-15 mRNA isoform (IL-15^{ΔE7}). Infection of P191 mutant mice via flank skin with HSV-1 showed a much more severely disrupted lesional skin in P191 than in B6 wild type mice as a result of elevated expressions of proinflammatory cytokines such as IL-1B and IL-6 by cDNA microarray and quantitative PCR analysis. Using MHC Class I tetramer expressing HSV-1 gB498-505 epitope to track antigen specific CD8 T cells in HSV-1 infected mice, we found that generation of the tetramer+ CD8 T cells in P191 was delayed by 2 days as were found in B6 mice. Moreover, the number of tetramer+ CD8 T cells was markedly reduced in P191 at 3 weeks and to the lowest level at 8 weeks possibly due to the suppressed expression of CD122 by activated CD8 T cells suggesting the failure to establish memory CD8 T cell pool in P191 mice. When IL-15^{ΔE7} cDNA were expressed in COS-7 cells, confocal immunofluorescence revealed that IL-15^{ΔE7} protein was expressed and largely accumulated in the ER. Culture supernatant from IL-15^{ΔE7} transfected cells not only failed to support HT-2 proliferation but also inhibited the bioactivity of IL-15 prototypic protein in HT-2 assay. Results from our experiments have shed lights on the regulatory roles for IL-15 alternatively spliced protein in mediating skin specific immunity and the establishment of memory CD8 T cell response in response to HSV-1 infection.

Kwon, Myung-Ja

PKC[q] up-regulates Stat3 critical for Th17 differentiation

Myung-Ja Kwon, Ruqing Wang, Young Cho, Zuoming Sun

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Abstract not to be posted.

Laing, Rachel

Expression of the proton-sensing receptor GPR65 by cytotoxic T cells is important for optimal anti-tumor immunity

Rachel Laing, Amanda L. Armijo, Andrew Q. Tran, Caius G. Radu

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Expression of the proton-sensing receptor GPR65 by cytotoxic T cells is important for optimal anti-tumor immunity. Rachel E. Laing (1, 2), Amanda L. Armijo (1, 2), Andrew Q. Tran (1, 2), Caius G. Radu (1, 2) 1. Department of Molecular and Medical Pharmacology, 2. Crump Institute for Molecular Imaging, Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California at Los Angeles (UCLA) G-protein coupled receptors (GPCRs) play a major role in immunity by coordinating signaling events involved in immune cell activation, proliferation, apoptosis and chemotaxis. GPR65 is a proton sensing GPCR predominantly expressed on lymphocytes, especially T cells. We hypothesized that GPR65 may modulate T cell function when these cells are exposed to acidic environments, such as those associated with rapidly growing glycolytic tumors. To test this hypothesis, we used a model of adoptive cell transfer cancer immunotherapy. Melanoma-bearing mice were treated with GPR65 wild type (WT) and knockout (KO) CD8⁺ T cells specific for an epitope derived from the gp100 melanocytic antigen. The ability of WT and KO GPR65 T cells to reject melanoma tumors was determined using caliper measurements. B16 melanoma bearing mice treated with GPR65 WT T cells exhibited drastic tumor regression compared to untreated controls. In contrast, mice treated with GPR65 KO T cells showed a significant delay in tumor regression. While BLI imaging with firefly luciferase transduced T cells showed substantial GPR65 WT T cell accumulation in melanoma tumor, GPR65 KO T cells displayed reduced accumulation and/or expansion at the tumor site. We conclude that GPR65 expression is important for optimal T cell-mediated rejection of highly glycolytic and acidic tumors, such as B16 melanoma. Elucidating the mechanism by which pH-sensing via GPR65 augments the anti-tumor activity of cytotoxic T cells may be important to increase the efficacy of cell based immunotherapies in melanoma and, potentially, other cancers.

TSLP Drives Th2 Responses and Inhibits Th1 Responses in the Skin by Modulating DC Phenotype and Function

Ryan Larson, Masanori Iseki, Miyuki Omori, and Steven F. Ziegler

Department of Immunology University of Washington and Benaroya Research Institute

Thymic stromal lymphopoietin (TSLP) is an IL-7 related cytokine produced by epithelial cells in response to tissue damage, inflammation, or TLR ligation. The presence of TSLP in the lesional skin of atopic dermatitis patients and asthmatic lungs suggests a role in the etiology of these allergic Th2 diseases. Overexpression of TSLP in mouse skin or lung leads to the development of atopic dermatitis and asthma, respectively; suggesting that TSLP is sufficient for the development of these diseases, however, these models do not elucidate the function(s) of TSLP. Furthermore, it has been hypothesized that TSLP serves to dampen aberrant Th1 responses at barrier surfaces, specifically in the intestine. We have utilized a contact hypersensitivity (CHS) model to determine what role TSLP plays in Th2 and Th1 responses, as well as to identify potential cellular targets of TSLP. Initially, we have observed a requirement for TSLP-TSLPR interactions for a Th2-type CHS response to the allergen fluorescein isothiocyanate (FITC). Specifically, TSLP receptor deficient (TSLPR^{-/-}) mice lack the hallmark features of a Th2 response to FITC, with reduced IL-4 expression and eosinophilia in allergen challenged skin, as well as reduced serum IgE compared to FITC sensitized and challenged WT mice. The defective response in TSLPR^{-/-} mice appears to be due to a significant reduction in frequency and number of FITC+CD11c⁺ DCs in the skin draining lymph nodes of FITC-sensitized TSLPR^{-/-} mice. Furthermore, TSLPR^{-/-}-FITC+DCs express reduced CD86 and are reduced in their ability to drive proliferation of naïve CD4 T cells. In addition to defective DC function in TSLPR^{-/-}, we have also observed reduced proliferation of CD4 T cells in TSLPR^{-/-} mice, however, this is not a T cell intrinsic defect, as both WT and TSLPR^{-/-} CD4 T cells proliferate less in TSLPR^{-/-} hosts compared to WT hosts. Furthermore, in a mixed bone marrow chimera setting, TSLPR^{-/-} CD4 T cells are equally capable of proliferating and entering inflamed tissue as their WT counterparts after FITC sensitization and challenge. Moreover, CD4 T cells do not require direct TSLP signals to mediate the FITC CHS response, as TCRB^{-/-} mice that have received either WT or TSLPR^{-/-} donor CD4 T cells experience equivalent FITC CHS responses. These data suggest a cell type other than CD4 T cells, such as DCs, are required to respond to TSLP to mediate the CHS response to FITC. In addition to playing a role in driving Th2 allergic responses, TSLP also regulates Th1 responses in the skin. Specifically, TSLPR^{-/-} mice experience an enhanced response to the Th1-inducing hapten DNFB, as shown by increased ear swelling and increased frequency of Th1 cells compared to DNFB-treated WT mice. Taken together, these results suggest that TSLP plays a role in driving Th2 responses and inhibiting Th1 responses in the skin. We are currently determining the mechanism by which TSLP is able to modulate these responses, focusing on which cell types respond to TSLP and how this affects the phenotype and function of the responding cells.

Migration differences between MHC I-restricted and MHC II-restricted thymocytes during positive selection

Ou Li, Ena Ladi, Ivan Dzhagalov, Paul Herzmark, Yingfang Liao, Ellen Robey

UC berkeley

Positive selection is required for thymocyte maturation. During positive selection, thymocytes that recognize MHC I become CD8 T cells, and the thymocytes that recognize MHC II become CD4 T cells. We used two-photon laser-scanning microscopy, to analyze the migration patterns of MHC I-restricted (F5) and MHC II-restricted (OT-2) thymocytes during positive selection in intact thymic lobes. We found that, whereas F5 thymocytes show fast and random migration pattern, OT-2 thymocytes migrate significantly slower and show hints of confined migration. Our data suggest that there are significant differences in the migration patterns between F5 and OT-2 thymocytes during positive selection that may reflect different modes of TCR signaling during positive selection via MHC-I versus MHC-II.

Requirement for Deoxycytidine Kinase in Lymphocyte Development and Function

Hsiang-I Liao, Gerald Toy, Wayne R. Austin, Hsiang-I Liao, Rachel Laing Donghui Cheng, Arun Singh, Dean O. Campbell, Tomo-o Ishikawa, Lynn W. Lehmann, Nagichettiar Satyamurthy, Michael E. Phelps, Harvey R. Herschman, Johannes Czernin, Owen N. Witte, and Caius G. Radu

University of California-Los Angeles

Deoxycytidine kinase (dCK) is a rate-limiting enzyme in deoxyribonucleoside salvage, a metabolic pathway that recycles products of DNA degradation. dCK also phosphorylates and therefore activates nucleoside analog pro-drugs that are frequently used in cancer, autoimmunity and viral infections. In contrast to its well-established therapeutic relevance, the biological function of dCK remains enigmatic. Highest levels of dCK expression are found in thymus and bone marrow, indicating a possible role in lymphopoiesis. To test this hypothesis we generated and analyzed dCK knockout (KO) mice. dCK inactivation selectively and profoundly affected T and B cell development. A 90-fold decrease in thymic cellularity was observed in the dCK KO mice relative to wild type littermates. Lymphocyte numbers in the dCK KO mice were 5 to 13- fold below physiological values. Secondary lymphoid organs from these mice had structural abnormalities. Furthermore, mature dCK KO T cells failed to proliferate in response to TCR cross-linking. The severe impact of dCK inactivation on lymphocyte development and function was unexpected given that nucleoside salvage has been thought to play a limited, "fine tuning" role in regulating deoxyribonucleotide triphosphate (dNTP) pools produced by the de novo pathway. The dCK KO phenotype challenges this view and indicates that, in contrast to the great majority of other somatic cells, normal lymphocyte development and function critically require the deoxyribonucleoside salvage pathway. These findings provide the rationale for conducting high throughput screens to identify small molecule dCK inhibitors; such compounds may find utility in the treatment of autoimmune disorders, transplant rejection, and potentially, lymphoid malignancies.

Differences that matter: the obscure pMHC-I repertoire of ERAAP-deficient mice

Kristin Camfield Lind, Nilabh Shastri

UC Berkeley

MHC I molecules display peptides (pMHC I) derived from intracellular proteins on the cell surface for recognition by CD8⁺ T cells. The enzyme ERAAP (ER-aminopeptidase associated with antigen processing) is a key component of the MHC I antigen presentation pathway. ERAAP trims the N-terminal ends of antigenic peptides to the optimal length for pMHC I presentation. Because even subtle changes in a given pMHC I complex can elicit potent CD8 T cell responses, we tested whether ERAAP-deficient (ERAAP KO) mice of various MHC haplotypes could present an unedited and immunogenic pMHC I repertoire to wild-type (WT) mice, and have found that ERAAP KO cells are immunogenic to wild-type hosts. To characterize the molecular nature of these ERAAP KO pMHC I differences, we derived T cell hybridomas specific for ERAAP-deficient cells. Interestingly, these hybridomas recognize peptides presented by both classical MHC I and nonclassical MHC Ib molecules. In addition, we seek to understand the extent of the differences between ERAAP KO and WT cells. Although ERAAP-deficient cells do not cause proliferation of wild-type cells in an in vitro mixed lymphocyte reaction, naive wild-type mice nevertheless reject ERAAP-KO cells in vivo.

Linderman, Jessica

Delayed Reconstitution of Donor-restricted T follicular helper cells after Fully MHC-Disparate Hematopoietic Stem Cell Transplantation

Jessica Linderman, Judith A Shizuru

Stanford University

Delayed Reconstitution of Donor-restricted T follicular helper cells after Fully MHC-Disparate Hematopoietic Stem Cell Transplantation

Jessica Ashley Linderman and Judith A. Shizuru

Stanford University School of Medicine, Blood and Marrow Transplant Division

MHC-disparate hematopoietic stem cell transplantation has great promise as a strategy for tolerance induction, a potential treatment for autoimmune disease, and a treatment of hematological malignancy. However, MHC-disparity between donor and host presents a unique set of challenges for immune reconstitution. Impaired interaction of immune cells expressing different MHC molecules and expression of only host MHC on radio-resistant cells of the thymus are believed to affect immune function post-transplant.

Here we use full and mixed MHC-disparate bone marrow chimeras challenged with various antigens to determine T helper cell function post-transplant. In accordance with our previous work, we show that proliferative T cell responses in MHC-disparate chimeras can be seen to donor-restricted peptides as early as 6 weeks after transplantation. However, antibody production to a T-dependent antigen is limited to host B cells in fully MHC-disparate chimeras until 12 weeks post-transplant. Donor and host-derived B cells produce antibody to a T-independent antigen at six weeks in all chimeras studied and to a T-dependant antigen in haploidentical chimeras where donor and host share an MHC allele. Donor-derived T follicular helper cells are present in both fully MHC-disparate and haploidentical animals at 6 weeks post-transplant and do migrate to germinal centers, but are either incapable of providing B cell help or are restricted to only host MHC.

These data suggest that reconstitution of donor-restricted T follicular helper cell function in fully MHC-disparate chimeras is delayed. We hypothesize that there are more stringent developmental requirements for T cell receptor-MHC interaction for T follicular helper cells than other T helper subsets. We believe that further examination of donor-restricted T follicular helper cells in this context could provide important insights into the function and development of T follicular helper cells, as well as for the management of patients receiving MHC-disparate transplants.

Lodoen, Melissa

Toxoplasma gondii differentially affects the expression of MHC class II and costimulatory molecules

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Abstract not to be posted.

The Role of indoleamine 2, 3 dioxygenase in regulating host immunity to Leishmania infection

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Leishmania major (*L. major*) creates immune privilege that attenuates local T cell responses. Cutaneous *L. major* infection induced expression of the immuno-regulatory enzyme indoleamine 2,3-dioxygenase (IDO) in lymph nodes affected by *L. major* infection. Mice were infected with cutaneous *L. major* to target inguinal lymph nodes (LNs). Cells expressing IDO were detected in inguinal LNs of *L. major* infected mice. These IDO⁺ cells potently suppressed T cell responses. Additionally, *L. major* inhibited CD8⁺ T and CD4⁺ T cell clonal expansion to cognate peptide, and IDO inhibitor treatment enhanced T cell clonal expansion. Thus, *L. major*-induced local T cells suppression in vivo when IDO was active. However, when IDO was ablated *L. major*-induced infection induced IL-6-dependent T cell conversion into TH17-like cells. Consistent with these findings, parasite burdens were reduced significantly when IDO was ablated. Moreover, host inflammatory responses to *L. major* were reduced substantially when IDO was ablated, suggesting that IDO attenuated host T cell immunity to *L. major*. We hypothesize that IDO-mediated suppression by DC in dLNs creates a local environment that is suppressive of host anti-*L. major* T cell responses. Grant acknowledgement: (MCG-STP/GIP STP00001, NIH-AI-063402)

Martin, Christopher

Regulation of Gut Antigen Presentation by a Non-Treg CD4 T Cell Subset

Christopher Martin, Joon Youb Lee, David Kim, Charlie Surh

The Scripps Research Institute

Abstract not to be posted.

Miazgowicz, Mike

Investigating the Function of Epithelially Expressed TSLPR in a Murine Model of Asthma

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University of Washington, Benaroya Research Institute

Epithelial cells of the airway and lung have been shown to participate actively in the response to both pathogenic and allergenic environmental stimuli. In particular, the airway epithelium is a potent source of thymic stromal lymphopoietin (TSLP), a cytokine that is strongly associated with asthma and other atopic pathogenesis. In the lung, TSLP acting in concert with an antigenic stimulus is capable of inducing hallmarks of the asthmatic response: eosinophilia and inflammatory cell infiltrates, sub-epithelial fibrosis, goblet cell hyperplasia and mucus production, airway hyperresponsiveness, and Th2 cytokine and chemokine production. The receptor for TSLP, a heterodimer composed of TSLPR and IL7RA, is known to be expressed on cells of the hematopoietic system, most notably cells of the myeloid lineage such as dendritic cells. We present data here that the TSLPR receptor complex is also expressed on several non-hematopoietic cells, including lung epithelial cells. Surprisingly, we have observed that TSLPR expression on radio-sensitive cells is not required for the establishment of disease in a murine model of asthma. Taken together, these results imply a relevant functional role for TSLPR expression outside of its established role in myeloid cell function. Interestingly, non-myeloid cells appear to express a heavily glycosylated form of TSLPR. We are currently investigating what functional consequences this differential glycosylation has on TSLPR signaling in the various cell types in order to better understand how epithelially expressed TSLPR contributes to the response to inflammatory stimuli in the lungs.

Identification of a neuropilin 1 expressing iNKT cell subset in naïve mice: insights into iNKT peripheral autoreactivity

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Invariant natural killer T (iNKT) cells are T lymphocytes with a restricted T cell receptor (TCR) composed of a unique VA14JA18 chain paired with a limited set of VB chains (VB7, VB8.2 and VB2). Unlike conventional T cells, iNKT cells recognize glycolipid antigens presented by the MHC-like molecule CD1d. Upon activation, iNKT cells rapidly release large amounts of cytokines like IL-4 and IFN- γ that modulate a wide variety of immune situations and disorders. Understanding how iNKT cell development, activation and turnover are regulated is therefore of crucial importance. Development of iNKT cells in the thymus is dependent on positive selection by double positive (DP) cortical thymocytes expressing CD1d. Although exclusive CD1d expression on DP thymocytes is sufficient for the development of iNKT cells, there is growing evidence that autoreactivity of iNKT cells with CD1d-expressing cells in the periphery contribute to iNKT cell maturation, homeostasis and function. Neuropilin 1 (Nrp-1) is a transmembrane receptor for neural guidance cues (class 3 semaphorins) and angiogenic factors (vascular endothelial growth factors) which has functions in a wide range of tissues. In the immune system, Nrp-1 is notably expressed on mouse Foxp3⁺ regulatory T cells where it mediates long term interactions with immature dendritic cells. We therefore analyzed whether the autoreactive status of iNKT cells was associated with Nrp-1 expression. We report that Nrp-1 is expressed on a subset of iNKT cells in naïve mice. The relative abundance of Nrp-1⁺ iNKT cells depends on the organ, ranging from less than 5% of total liver iNKT cells to an average of 5%, 15% and 50% of thymus, spleen and lymph node iNKT cells, respectively. In all organs including the thymus, Nrp-1⁺ iNKT cells have a phenotype reminiscent of immature or activated iNKT cells: they do not express NK1.1 and have low levels of CD69, are larger in size and highly express the proliferation-associated marker Ki67. The VB repertoire of splenic Nrp-1⁺ iNKT cells is slightly biased toward VB2 and VB7 usage. Nrp-1 is induced on iNKT cells after in vitro TCR engagement and more than 80% of spleen and liver iNKT cells are Nrp-1⁺ 3 days after intraperitoneal injection of AGalCer. After in vitro activation, sorted Nrp-1⁺ iNKT cells produce 10 times less IFN- γ , twice as much IL-4 and proliferate 4 times more than their Nrp-1⁻ counterparts. Interestingly, Nrp-1⁺ iNKT cells are also the main producers of IL-17 and IL-5 in the splenic iNKT cell population. Altogether, our data characterize a Nrp-1⁺ subset of iNKT cells in naïve mice. The phenotype of Nrp-1⁺ iNKT cells and the induction of Nrp-1 on iNKT cells after activation are consistent with Nrp-1⁺ iNKT cells being autoreactive in vivo. Heterogeneous distribution of Nrp-1⁺ iNKT cells in the immune system, cytokine secretion profile and proliferative behavior after in vitro and in vivo activation of these cells provide insights into the importance of autoreactivity for the diversity of iNKT cell-mediated responses. We are currently investigating the precise mechanisms by which Nrp-1⁺ iNKT cells regulate various iNKT cell-dependent immune responses and whether Nrp-1 is involved in these processes.

Mingueneau, Michaël

The LAT (Linker for Activation of T cells) adaptor molecule is more than just an activator of the T cell signaling cassette.

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Harvard Medical School, Boston, US

The LAT (Linker for Activation of T cells) adaptor molecule is more than just an activator of the T cell signaling cassette. M. Mingueneau 1, R. Roncagalli 2, C. Grégoire 2, A. Kissenpfennig 3, M. Malissen 2, B. Malissen 2 et al. 1 Harvard Medical School, US ; 2 Centre d'Immunologie de Marseille-Luminy, France ; 3 Center for Infection and Immunity, UK Background : The Linker for Activation of T cells (LAT) adaptor molecule has been shown to be a crucial positive regulator of the T cell receptor (TCR) signaling cassette by coordinating the assembly of a multiprotein complex in T cells □ the LAT signalosome □ that links the T-cell specific and the ubiquitous components of TCR signaling pathways. Murine models : Surprisingly, mutations of several key tyrosine residues of this transmembrane adaptor (among which the mutation of the tyrosine 136 responsible for PLC γ -1 recruitment) in knock-in mice led to lymphoproliferative disorders characterized by strong Th2 polarization and massive titers of IgG1 and IgE, among which auto-antibodies. Based on these findings, one mechanistic model postulated that thymic negative selection defects caused by crippled LAT adaptor molecules lead to the emergence of auto-reactive T cells that, once in the periphery, trigger B cell activation and auto-antibodies production, through their imbalanced TCR-LAT signaling axis. Question : What is the relative importance of central versus peripheral defects in the etiology of the disorders associated with LAT-crippled adaptor molecules ? Experimental strategy : By using a conditional expression system under the control of the CRE recombinase, we induced the expression of LAT-crippled molecules or deleted the whole pool of LAT molecules in peripheral T cells that were selected in a LAT-proficient thymic environment. Conclusions : This approach revealed that the unfolding of these LAT-associated lymphoproliferative disorders does not require a prior defect in central tolerance. More interestingly, it showed that LAT constitutes more than just an activator and plays a negative regulatory role in that it contributes to terminate antigen-driven T cell responses by exerting a repressive function on segments of the TCR signaling cassette that lie upstream and/or parallel to the LAT signalosome. In the absence of such LAT-operated negative regulatory loop intrinsic to conventional CD4 $^{+}$ T cells and of no lesser importance than the extrinsic regulatory mechanisms mediated by regulatory T cells, physiologic, antigen-specific CD4 $^{+}$ T cell responses evolve into chronic pro-inflammatory responses that perpetuate themselves in a manner that does not depend on engagement of the T cell receptor. These data underscore that a novel immunopathology proper to defective LAT signalosomes and that we are proposing to call □LAT signaling pathology□ is likely taking shape.

Memory CD4 T cell responses are compromised in the absence of B cells

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Following infection, CD4 T cells become activated and differentiate into effector cells. After the infection is controlled, the majority of these effector CD4 T cells die via apoptosis, however, a subset of the cells remain and constitute the memory pool. Memory CD4 T cells provide protection upon secondary exposure to a pathogen, therefore, it is critical to understand the factors that contribute to the development of a CD4 T cell memory response, as well as maintenance of the memory cells. B cells help to shape the CD4 T cell response through antibody production, antigen presentation and co-stimulation. It is currently unclear what contribution B cells have on the formation of the memory pool. We tested the role of B cells in the establishment of CD4 T cell memory in the context of an intracellular bacterial pathogen, *Listeria monocytogenes* (LM), where antibody production plays a minimal role in pathogen clearance. Following LM infection we observed a diminished CD4 T cell memory response in the absence of B cells. We also examined the requirement for B cells in the maintenance of memory CD4 T cells as well as their ability to participate in a recall response. We found that in the absence of B cells there is reduced maintenance of memory CD4 T cells, and these CD4 T cells do not undergo as robust an expansion upon secondary infection. In order to understand which functions of B cells are critical to CD4 T cell memory formation, we evaluated the impact antigen presentation by B cells has on effector and memory CD4 T cell responses. We found that the ability of B cells to present antigen to CD4 T cells is crucial for the development of optimal effector and memory CD4 T cell responses. Taken together, these studies demonstrate that B cells impact CD4 T cell memory at multiple phases, highlighting the complex nature of B:T cell interactions during protective immunity.

Mouchess, Maria

Proteolysis of Toll-like receptor 9 is required for tolerance to self nucleic acid

Maria Mouchess, Barton, G.M.

U.C. Berkeley

Abstract not to be posted.

Myers, Riley C.

The collaborative interactions between B cells and Follicular Dendritic Cells

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Follicular dendritic cells act as accessory cells to trap Ag and promote B cell responses, and once activated, FDC upregulate FcR and adhesion molecules. FDC activation fails in CD19-deficient mice after Ag challenge. However, adoptive transfer of wt B cells into CD19^{-/-} mice rescued FDC activation. Potential mechanisms for the rescue of FDC activation include the normalized activation of wt follicular B cells or the rescue of Ag trafficking by wt marginal zone B cells. To test whether the loss of Ag trafficking in CD19-deficient mice is responsible for the failure of FDC activation, mice were injected with anti-OVA, followed 1 day later by OVA, allowing passive immune complex (IC) formation. By day 3 after OVA IC injection, FDC in wt and CD19^{-/-} were loaded equivalently with OVA IC. However, GC and FDC activation were observed only in wt mice at day 10, despite IC deposition in wt and CD19^{-/-} mice. Therefore, FDC activation is independent of IC binding, and in this system, FDC only express VCAM-1 and FcR with the appearance of GC B cells. LTB is upregulated on activated B cells and LTBR is expressed on FDC. To test the hypothesis that B cells activate FDC through LTB-LTBR interactions, LTB-deficient B cells were adoptively transferred into CD19-deficient mice. LTB⁺ B cells were able to rescue FDC activation, but despite forming GC, LTB-deficient B cells were not. These results indicate that lymphotoxin B is necessary for FDC activation. Supported by NIH R01AI042265.

Ou, PhD., Jing-Ni

Defective PD-L1 expression on lupus monocytes can be corrected by soluble factors

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Seattle Children's Hospital Research Institute

Background Antigen presenting cells (APC) function as inducers of T-cell mediated adaptive immune responses, and also as regulators to maintain tolerance. Monocytes (Mo) and myeloid dendritic cells (mDC) are APC involved in pathogenesis of SLE and other autoimmune diseases. We found that programmed cell death ligand-1 (PD-L1), a critical regulatory protein for T-cells, is significantly down-regulated in cultured Mo and mDC in SLE patients with active disease compared to healthy controls and patients in remission. This defect in PD-L1 expression in SLE is related to cytokines produced by APC, or T-lymphocytes. Objective Elucidate molecular mechanisms which lead to down-regulation of PD-L1 in active SLE. Methods Peripheral blood mononuclear cells (PBMC) were isolated from juvenile SLE patients and age-matched healthy controls. PD-L1 expression was measured by flow cytometry and real-time RT-PCR. Supernatants of PBMC cultures from patients and healthy donors were assayed for cytokines by Luminex. Results PD-L1 mRNA expression was decreased in SLE cells compared to healthy controls. Surface protein expression of PD-L1 on SLE monocytes was significantly induced by PBMC from healthy subjects, and also by supernatant from cultures of healthy PBMC. The level of TNF- α and IL-10 cytokines detected in SLE PBMC culture supernatants correlated with PD-L1 protein expression. Conclusion PD-L1 expression can be controlled by cytokines, and regulation may occur early at the mRNA level. Therefore, loss of peripheral T cell tolerance in SLE may be mediated by a feedback loop involving PD-L1 expression on monocytes controlled by T-cell factors. Our findings suggest that down-regulation of PD-L1 by cytokines is a potential mechanism leading to loss of peripheral tolerance to autoantigens in SLE.

The miR-29 family in the human neonatal Th1 response

Amy Palin, Shivkumar Venkatasubrahmanyam, Atul Butte, David B. Lewis

Stanford Medical School

Neonates are highly susceptible to severe infection by intracellular pathogens, including herpes viruses and intracellular bacteria, for example *M. tuberculosis* and *Listeria*. A large body of evidence suggests that a limitation in T helper 1 (Th1) responses accounts for much of the neonatal susceptibility to these pathogens. Our lab has previously defined a number of limitations in naïve CD4⁺ T cells of the neonate obtained from umbilical cord blood that restrict Th1 generation in vitro even when optimal signals are supplied by activating antigen presenting cells. These include decreased expression by T cells of the costimulatory molecule CD40 ligand and IFN- γ . Though human neonates are not lymphopenic, neonatal T cells proliferate at a much higher rate than adult T cells. Because of multiple differences in neonatal naïve CD4⁺ T cell function, we hypothesized that miRNAs, upstream regulators of the expression of multiple gene products, account for the impaired generation of Th1 immunity. The miR-29 family (miR-29a, b, and c) of miRNAs is expressed at higher levels in adult naïve CD4⁺ T cells than in neonatal naïve CD4⁺ T cells. Their predicted mRNA targets are expressed at higher levels in neonatal naïve CD4⁺ T cells, as would be expected if miR-29 were repressing these targets. miR-29 downregulates the anti-apoptotic protein Mcl-1, and targets repressors of p53. Through its regulation of the cell cycle and apoptosis, miR-29 may contribute to the proliferative state of neonatal naïve CD4⁺ T cells, which in turn may inhibit the Th1 response.

Pan, Hao

CEACAM1 regulates granulopoiesis through inhibition of G-CSFR-Stat3 signaling in an ITIM-SHP-1 dependent manner

Hao Pan, Hao Pan, John E. Shively

City of Hope Irell & Manella Graduate School of Biological Sciences

CEACAM1 is involved in many biological processes, including regulation of lymphocyte activation. Although granulocytes highly express CEACAM1, the role of CEACAM1 in granulopoiesis and granulocyte dependent host immune responses is not well understood. We showed here that CEACAM1^{-/-} mice had severe neutrophilia associated with hyperproliferation of granulocyte progenitors. Reverse bone marrow reconstitution and bone marrow competition assays revealed that CEACAM1^{-/-} bone marrow myeloid progenitor cells (BMMPCs) acted autonomously and the regulation of CEACAM1 on progenitor cells was granulocytic lineage specific. In vitro treatment of BMMPCs with G-CSF in CEACAM1^{+/+} mice resulted in association of CEACAM1 with G-CSFR, phosphorylation of CEACAM1 on its ITIMs and subsequent recruitment of SHP-1, leading to the down-regulation of Stat3 activity, an essential step in G-CSFR mediated granulopoiesis. Moreover, CEACAM1^{-/-} mice were hypersensitive to *Listeria monocytogenes* infection with accelerated mortality due to enhanced neutrophil liver infiltration and augmented cytokine levels, and in vivo modulation of CEACAM1 isoforms or Stat3 level using retroviral vectors manipulated granulopoiesis and host sensitivity to *Listeria* infection. Thus, we identify CEACAM1 as a co-inhibitory receptor for G-CSFR regulating granulopoiesis and affecting the host innate immune response to a critical pathogen.

Molecular Mechanisms of disease severity in Juvenile Idiopathic Arthritis

Anne Pesenacker, Tessa Crompton, Lucy Wedderburn

UCL - Institute of Child Health

Molecular Mechanisms of disease severity in Juvenile Idiopathic Arthritis Anne Pesenacker, Tessa Crompton*, Lucy Wedderburn, Rheumatology and *Immunobiology Units, Institute of Child Health UCL The molecular mechanisms of inflammation and its severity are not currently well characterized. Several pathways, including the nuclear factor-kappa B (NF-KB) pathway, may induce a pro-inflammatory environment and thus give rise to inflammation. Oligoarticular Juvenile Idiopathic Arthritis (JIA) is the most common rheumatic disease in children. Oligoarticular JIA can persist as a fairly mild disease (persistent) or go on to a more severe form (extended). Thus, JIA provides a good example of different states of inflammation within the same disease. A gene expression profiling project of synovial fluid mononuclear cells (SFMC) from children with persistent and extended oligoarticular JIA implicated several pathways and genes of interest, which might be involved in disease mechanism (Hunter et al, 2010). ERBB receptor feedback inhibitor 1 (ERRF1), vasoactive intestinal peptide receptor 1 (VIPR1) and SMAD3, a TGF-B signaling molecule, were chosen for further investigation. All three play important roles in the immune system, inflammation and can influence NF-KB activation, which is important in cytokine production and other inflammatory processes. The expression of VIPR1 was analyzed by flow cytometry and quantitative RT-PCR. SMAD3 and ERRF1 expression were characterized by quantitative RT-PCR. This was performed on sorted (CD3+CD4+, CD3+CD4-, CD14+ and CD19+) and unsorted healthy peripheral blood mononuclear cells (PBMC) and patient SFMC. Differential expression of these three genes between cell subtypes was observed. VIPR1 was enriched in CD25hi CD4 T cells, CD19+ B cells and CD14+ monocytes. SMAD3 was highly enriched in CD19+ cells. ERRF1 showed low expression in healthy PBMC. Compared to healthy PBMC a trend towards lower expression of VIPR1 on CD19+ and CD14+ cells on patient SFMC was observed. Further characterization of the expression of VIPR1, SMAD3 and ERRF1 on different cell subsets in healthy and patient samples and functional assays are in progress. These data suggest differential expression of the 3 selected target genes among cell populations in healthy PBMC and synovial cells. A functional analysis of these pathways is underway. This work should ultimately lead to better understanding of how these pathways contribute to severity in inflammation, as well as to novel therapeutic targets for childhood arthritis and biomarkers with which to predict disease course and outcome.

The danger signal adenosine induces persistence of chlamydial infection through stimulation of A2b receptors on epithelial cells

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University of California, Merced

Infections with intracellular bacteria such as chlamydiae affect the majority of the world population. Infected tissue inflammation and granuloma formation help contain the short-term expansion of the invading pathogen, leading also to local tissue damage and hypoxia. However, the effects of key aspects of damaged inflamed tissues and hypoxia on continued infection with intracellular bacteria remain unknown. We find that development of *Chlamydia trachomatis* is reversibly retarded by prolonged exposure of infected cells to extracellular adenosine, a hallmark of hypoxia and advanced inflammation. In epithelial cells, this effect was mediated by the A2b adenosine receptor, unique in the adenosine receptor family for having a hypoxia-inducible factor (HIF1- α) binding site at its promoter region, and was dependent on an increase in the intracellular cAMP levels, but independent of cAMP-dependent protein kinase (PKA). Further study of adenosine receptor signaling during intracellular bacterial infection could lead to significant breakthroughs in our understanding of persistent infections with these ubiquitous pathogens.

Pierson, Emily

Role of B cells as antigen presenting cells in experimental autoimmune encephalomyelitis

Emily Pierson, Ingunn Stromnes, Joan Goverman

University of Washington

Abstract not to be posted.

Prinz, Marco

CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system

Marco Prinz, Alexander Mildner; Mathias Mack; Hauke Schmidt; Wolfgang Brück; Marija Djukic; Mark D. Zabel; Andrea Hille; Josef Priller

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The chemokine receptor CCR2 plays a vital role for the induction of autoimmunity in the central nervous system (CNS). However, it remains unclear how the pathogenic response is mediated by CCR2 bearing cells. By combining bone marrow chimerism with gene targeting we detected a mild disease-modulating role of CCR2 during experimental autoimmune encephalomyelitis (EAE), a model for CNS autoimmunity, on radioresistant cells that was independent from targeted CCR2 expression on endothelia. Interestingly, absence of CCR2 on lymphocytes did not influence autoimmune demyelination. In contrast, engagement of CCR2 on accessory cells was required for EAE induction. CCR2+Ly-6Chi monocytes were rapidly recruited to the inflamed CNS and were crucial for the effector phase of disease. Selective depletion of this specific monocyte subpopulation through engagement of CCR2 strongly reduced CNS autoimmunity. Collectively, these data indicate a disease-promoting role of CCR2+Ly-6Chi monocytes during autoimmune inflammation of the CNS.

Reem, Mohamed

The Role of Immunoglobulin E antibodies in protection against Plasmodium falciparum

Mohamed Reem, Mohamed R1, Eltayeb M1, Iriemenam NC2, Elhassan I1

Institute of Endemic Diseases, University of Khartoum

The Role of Immunoglobulin E antibodies in protection against Plasmodium falciparum
Mohamed R1, Eltayeb M1, Iriemenam NC2, Elhassan I1. 1-Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan. 2- Department of Immunology, Stockholm University, Sweden. Department of Medical Microbiology & Parasitology, College of Medicine University of Lagos, Nigeria
Introduction: Malaria occurs in over 100 countries and territories. More than 40% of the people in the world are at risk of getting malaria. Infection with malaria is associated with elevation of strong specific and non-specific antibody responses with the humoral immune responses. In endemic malaria areas, there is an increase of serum levels of IgE during Plasmodium falciparum infections. It is well known that IgE mediates activation of various effector cells such as monocytes/macrophages), this may suggest that IgE may also play a role in protection against malaria. The main objective of my research project is to determine the role of IgE in protection against acute P. falciparum malaria in an area characterized by highly seasonal but stable malaria transmission in Sudan. Materials and Methods: Blood samples were collected from malaria patients with different clinical presentations, severe cases, asymptomatic, mild and controls. Total and specific IgE levels were determined by ELISA. IL10, IL4 and TNF expressions were determined with Real Time PCR. Results: Total IgE levels were confirmed to increase upon infection. Plasma IgE levels correlated with those of IL-10 in uncomplicated malaria patients. TNF and IL-10 levels were significantly correlated with IgE concentrations in all groups. Conclusion: Circulating levels of total IgE do not appear to correlate with protection or pathology. On the contrary, the P. falciparum-specific IgE response seems to contribute to the control of parasites, since functional activity was higher in asymptomatic and mild malaria patients than in severe malaria group.

Micropatterned Surfaces for Detection of Cytokine Release from Individual T-cells

Alexander Revzin, Gulnaz Stybayeva, Jaime Silnagcruz

University of California, Davis

The high throughput analysis of immune cells at the single cell level is crucial in understanding cellular function in response to viral and bacterial infection. In particular, the analysis of cytokines produced by immune cells in the presence of pathogens provides significant diagnostic value. The present study describes the development of a microfabricated cytometry platform used to determine the interferon (IFN)- γ cytokine production of individual CD4⁺ T-lymphocytes (T-cells) isolated from human blood. A mixture of cell and cytokine-specific antibodies was co-printed onto a poly(ethylene glycol) (PEG) hydrogel coated glass slide and superimposed with an array of PEG microwells via photolithography. This engineered surface was then enclosed in a microfluidic device to minimize blood volume requirement. Introduction of red blood cell (RBC) depleted whole human blood into the microfluidic chamber followed by washing at a pre-defined shear stress resulted in the isolation of individual CD4⁺ T-cells in polymer microwells. Mitogenic activation of the captured T-cells followed by immunofluorescent staining in the microfluidic chamber revealed a localized IFN- γ cytokine signal around individual cells. Significantly, IFN- γ secretion profiles for hundreds of live CD4 T-cells could be characterized at single cell resolution. The device and process presented herein marks the first step towards multiplexed analysis of live immune cells organized into high density single cell arrays.

Rothausler, Kristina

Tissue-resident effector B cell populations provide lifelong antibody production after influenza infection in the absence of memory formation

Kristina Rothausler, Nicole Baumgarth

University of California Davis, Center for Comparative Medicine

Life-long protective systemic humoral immunity to influenza virus is generated from germinal centers that develop in regional lymph nodes of the respiratory tract. These antiviral antibodies are either provided by long-lived bone marrow-resident plasma cells and / or circulating memory B cells. In contrast, early local antibody production following influenza infection is provided mainly from extrafollicular foci-derived plasma cells. These cells are thought to be short-lived and secrete the bulk of early-secreted antibodies, thereby importantly contributing to protection from primary infection. Whether and to what degree local protection is provided also by long-lived plasma cells in the respiratory tract is currently unknown. To address this question we followed a prototypic extrafollicular foci response that develops in BALB/c mice following influenza A/PR/8/34 infection and that is encoded by the C12 idiotype (Id) (EJI in press). Virus-specific ELISPOT analysis showed that C12Id does not contribute to long-term antibody secretion in the bone marrow or spleen, and prime / boost experiments in conjunction with limiting dilution analysis indicated a lack of C12Id-encoded splenic memory B cell formation following infection. Instead, we found C12Idpos antibody-secreting cells locally in the lung tissue for over 16 months following infection. In addition, in vivo BrdU-labeling studies showed that the lungs of mice infected for over 400 days contain C12Idpos HA-specific cells expressing a plasmablast phenotype that rapidly turn over. Taken together our data demonstrate that influenza infection induces populations of tissue-restricted antibody-secreting cells. They further suggest that this life-long humoral response is provided by a population of respiratory tract-resident plasma blasts with self-renewal capacity.

[b]-catenin/TCF-1 Regulates Thymocyte Survival via ROR[γ]t-dependent Pathway

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Mechanisms that regulate the survival and apoptosis of CD4+CD8+ double positive (DP) thymocytes play critical role in shaping the peripheral T cell repertoire. However, the mechanisms of regulation of DP thymocyte lifespan remains poorly understood. Both the B-catenin/TCF-1 pathway and ROR γ t were found to be required for regulation of DP thymocyte survival through modulation of Bcl-xL expression. In this study, we demonstrated that ROR γ t expression is significantly reduced in TCF-1^{-/-} thymocytes but increased in B-catenin transgenic thymocytes. In addition, the transgenic expression of B-catenin was not able to rescue the massive thymocyte apoptosis phenotype of ROR γ ^{-/-} mice when ROR γ ^{-/-} mice were crossed with B-cat^{Tg} mice. Furthermore, ROR γ t is regulated by B-catenin/TCF-1 at a transcriptional level since overexpression of B-catenin stimulates the activity of a reporter containing the 1kb region immediately upstream of the ROR γ t translation starting site. Our data suggest that B-catenin/TCF-1 acts upstream of ROR γ t in the regulation of Bcl-xL expression and DP thymocyte survival.

Santer*, Deanna M.

C1q deficiency leads to the defective suppression of IFN-alpha in response to nucleoprotein containing immune complexes

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Abstract not to be posted.

The Varicella Zoster Virus Immediate Early Protein, IE62, Inhibits the Type-I Interferon Response by Restricting IRF3 Phosphorylation

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Varicella zoster virus (VZV) is a member of the alpha-herpesvirus family with a 125-kb dsDNA genome encoding approximately 70 unique proteins made during the virus life cycle. Primary VZV infection causes varicella (chicken pox) while reactivation from latency causes zoster or shingles. VZV is a human restricted pathogen and infects T cells that aid virus dissemination to skin and consequent establishment of latency via spread to neurons. Successful VZV infection and establishment of latency likely require well controlled evasion of the host primary immune response and limited secretion of various cytokines including interferons (IFN) and interferon stimulated anti-viral factors. Infection of human primary fibroblasts with VZV does not elicit IFNB production and IFNB pre-treatment of cells blocks infection with VZV indicating that VZV encodes mechanism(s) to evade the Type-I IFN (IFNA/B) response. VZV has been shown earlier to block NFkB activation during infection (Jones, JO, et al., 2006) and our current study revealed that VZV inhibits the activation of IRF3, a transcription factor critical to early IFNB induction. Infection of cells with VZV downregulated interferon stimulated genes (ISG) like ISG56 and ISG54 that are transcribed by activated IRF3. Similar downregulation of ISGs was observed when cells were infected with replication deficient (UV-inactivated) virus indicating that inhibition of IRF3 is a replication-independent process. A preliminary screening of various VZV ORF expression plasmids using an IRF3-responsive ISRE-Luciferase assay revealed that the immediate early protein, IE62, is a potent antagonist of the IFNB pathway. While IE62 efficiently blocked ISRE-Luciferase activation mediated by TBK1 it failed to block luciferase reporter activation mediated by IRF3-5D (a constitutively active IRF3 mutant). ELISA and immunoblot analysis confirmed results from the luciferase reporter assay and revealed that IE62 blocks TBK1-mediated IRF3 activation at a stage upstream of IRF3 phosphorylation. Since IRF3 phosphorylation at the five C-terminal Ser/Thr residues occurs in a sequential fashion, we introduced systematic Ser/Thr to Asp mutations and identified Ser-402 as being critical for IE62 mediated inhibitory function. Mutation of Ser-402, but not Ser-396 or -398, to the phosphomimetic Asp residue, abrogated IE62 mediated IRF3 inhibition. IE62 failed to co-immunoprecipitate either TBK1 or IRF3 and further did not perturb TBK1-IRF3 complex formation. However, the presence of IE62 appeared to enhance the stability of the TBK1-IRF3 complex. Based on our findings, we postulate that IE62 by its ability to block phosphorylation of IRF3 at a discrete Ser residue inhibits the disassociation of TBK1-pIRF3 complexes thus limiting availability of active pIRF3 for transcription of the IFNB gene. IE62 is the first identified VZV-encoded IFN antagonist that is also an abundant tegument protein and a promiscuous viral transactivator.

Seong, Rho H.

**The SWI/SNF chromatin remodeling complex is an essential transcriptional regulator
in early B cell development**

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Seoul National University

Abstract not to be posted.

Shainheit, Mara

Dendritic Cell IL-23 and IL-1 Production in Response to Schistosome Eggs Induces Th17 Cells in a Mouse Strain Prone to Severe Immunopathology

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Infection with schistosomes results in a CD4 T cell-mediated inflammatory reaction against parasite eggs that varies greatly in magnitude both in humans as well as among mouse strains. In the murine disease, severe immunopathology correlates with high levels of interleukin 17 (IL-17), whereas milder forms of schistosomiasis develop in the context of a largely anti-inflammatory immune response. We now report that live schistosome eggs stimulate dendritic cells (DCs) from high pathology-prone CBA mice to produce IL-12p40, IL-6 and TGF- β , whereas those from low pathology-prone BL/6 mice only make TGF- β . Moreover, egg-stimulated, syngeneic DC-CD4 T cell co-cultures using cells from BL/6 mice resulted in enhanced expression of IL-4, IL-5, IL-10 and the transcription factor Foxp3. Conversely, identically treated CBA co-cultures instead produced higher levels IL-6, IL-23, IL-1 β , as well as IL-17 and the chemokines CXCL1, CXCL2 and CCL2. Neutralization of IL-23 and IL-1, but not of IL-6 or IL-21, profoundly inhibited egg-induced IL-17 production in the CBA co-cultures. Furthermore, in DC-CD4 T cell co-cultures using cells from novel IL-12p40^{-/-} and Sm-p40 egg antigen-specific TCR transgenic mice, both on the high pathology-prone CBA background, IL-23 and IL-1 β were shown to be essential for the differentiation of egg antigen-specific Th17 cells. Additionally, we conclude that soluble egg-derived molecule(s) mediate the secretion of IL-17 in CBA DC-CD4 T cell co-cultures. Microarray analysis of unstimulated DCs revealed markedly enhanced expression of C-type lectin receptors in CBA DCs, suggesting a glycan-sensing gene profile, whereas BL/6 DCs demonstrated a broader, anti-inflammatory-skewed phenotype. Additionally, egg-stimulated CBA DCs exhibited a proinflammatory gene profile punctuated by augmented IL-1 β expression, while the enhancement of general anti-inflammatory genes was observed in BL/6 DCs. These findings suggest that DCs derived from genetically susceptible hosts differentially recognize schistosome egg-derived molecules that precipitate a proinflammatory cascade driven by IL-23 and IL-1 β , ultimately resulting in the differentiation of pathogenic Th17 cells and exacerbation of disease.

Lipopolysaccharide Primes Macrophages for Enhanced Zymosan Phagocytosis

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Zymosan particles are cell wall derivatives of the fungus *Saccharomyces cerevisiae*. Zymosan is a useful model for investigating the role of macrophage cell surface receptors in fungal recognition and subsequent phagocytosis. We used the RAW 264.7 macrophage cell line to examine the importance of macrophage lectin receptors in the phagocytosis of zymosan. Our results confirmed the essential role of the carbohydrate beta-glucan, present on zymosan surfaces, as a significant ligand for macrophage recognition and phagocytosis of these particles. Zymosan ingestion was inhibited by the presence of laminarin in cell cultures, but not by the addition of either mannan or galactomannan. Furthermore, we demonstrated that lipopolysaccharide (LPS) from *E. coli* primed macrophages for enhanced zymosan phagocytosis in a dose-dependent manner. LPS pretreatment of cells prior to zymosan addition significantly augmented the number of zymosan particles ingested by cells. Additional studies suggested that the increased phagocytosis mediated by LPS was likely due to direct cell surface receptor activation, and not via secreted opsonins, since the transfer of cell culture supernatants from LPS exposed macrophages to naïve cells, failed to induce enhancement of phagocytosis in the latter.

Swainson, Louise

Expression of the autoimmune susceptibility gene FcRL3 on human regulatory T cells is associated with dysfunction and high levels of PD-1

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UCSF

CD4⁺FoxP3⁺ regulatory T cells (Treg) play a critical role in maintaining self tolerance and inhibiting autoimmune disease. Despite being a major focus of modern immunological investigation, many aspects of Treg biology remain unknown. In a screen for novel candidate genes involved in human Treg function, we detected the expression of an autoimmune susceptibility gene, FcRL3, in Treg but not in conventional CD4⁺ T cells. FcRL3 is an orphan receptor of unknown function with structural homology to classical Fc receptors. Numerous genetic studies have demonstrated a link between a single nucleotide polymorphism in the FCRL3 promoter and both overexpression of FcRL3 and autoimmune diseases such as rheumatoid arthritis. Given the critical role of Treg in suppressing autoimmunity, we sought to ascertain how expression of FcRL3 relates to the phenotype, differentiation, and function of Treg. We show here that FcRL3 is expressed on a population of thymically derived Treg that exhibits a memory phenotype and high levels of programmed cell death-1 (PD-1). Purified FcRL3⁺ Treg are less responsive to antigenic stimulation in the presence of IL-2 than their FcRL3⁻ counterparts, despite intact proximal and distal IL-2 signaling as determined by phosphorylation of Stat-5 and upregulation of Bcl2. In vitro suppression assays demonstrated that FcRL3⁺ Treg have reduced capacity to suppress the proliferation of effector T cells. These data suggest that FcRL3 expression is associated with Treg dysfunction that may, in turn, contribute to the loss of self tolerance and the development of autoimmunity.

Tait, Elia D.

A role for IL-27 in controlling T regulatory cell homeostasis

Elia D. Tait, Elia D. Tait, Jason S. Stumhofer, Nancy Hosken, Aisling C. O'Hara, Qun Fang, Steven D. Levin, and Christopher A. Hunter

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A role for IL-27 in controlling T regulatory cell homeostasis Elia D. Tait¹, Jason S. Stumhofer¹, Nancy Hosken², Aisling C. O'Hara¹, Qun Fang¹, Steven D. Levin², and Christopher A. Hunter¹ ¹Department of Pathobiology, University of Pennsylvania, Philadelphia, PA, ²Department of Immunology, ZymoGenetics Inc., Seattle, WA IL-27 is a heterodimeric cytokine composed of the p28 and Epstein-Barr virus-induced 3 (EBI3) subunits. This pleiotropic factor has both pro- and anti-inflammatory properties. Its role in regulating inflammation has been characterized in numerous models, and previous studies have shown that IL-27 inhibits upregulation of Foxp3 on inducible T regulatory (Treg) in vitro. However, IL-27's role in immuno-regulation under homeostatic conditions in vivo remains unclear. We utilized mice that transgenically overexpress the two subunits of IL-27 (IL-27 tg mice) to dissect this cytokine's function in controlling the Treg population during steady state. We found that IL-27 tg mice have significantly decreased frequencies of Treg in lymphoid organs. These mice develop spontaneous inflammation in multiple tissues, have activated T cells, and succumb to this condition at 8-11 weeks of age. While exposing mature Treg to IL-27 does not cause Foxp3 downregulation, the presence of IL-27 during Treg generation limits the size of this population. These effects may be mediated through IL-27's ability to dampen IL-2 production, a vital cytokine for normal Treg homeostasis, as IL-27 tg mice are IL-2-deficient. Together, these data indicate that IL-27 is an important factor in regulating inflammation and shaping the Treg pool under homeostatic conditions.

Tyznik, Aaron J.

The Mechanism of Invariant NKT Cell Responses to Microbial-Associated Molecular Patterns

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Invariant NK T (iNKT) cells influence the response to viral infections, although the mechanisms are poorly defined. In this study we show that these innate-like lymphocytes secrete IFN- γ upon culture with CpG oligodeoxynucleotide-stimulated dendritic cells (DCs) from mouse bone marrow. This requires TLR9 signaling and IL-12 secretion by the activated DCs, but it does not require CD1d expression. iNKT cells also produce IFN- γ in response to mouse CMV infection. Their mechanism of mouse CMV detection is quite similar to that of CpG, requiring both TLR9 signaling and IL-12 secretion, while the need for CD1d expression is relatively minor. Consequently, iNKT cells have the ability to respond to a variety of microbes, including viruses, in an Ag-independent manner, suggesting they may play a broad role in antipathogen defenses despite their limited TCR repertoire.

Cytokine Production by Induced Versus Natural Regulatory T Cells

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Most resting CD4⁺CD25⁺ T cells express FOXP3 at baseline, and are termed "natural" regulatory T cells (nTregs). However, FOXP3 expression can also be induced de novo upon activation of CD4⁺CD25⁻ T cells, which also have inhibitory potential, and are termed "induced" regulatory T cells (iTregs). This FOXP3 induction is at least partly dependent upon TGF- β , but can be inhibited by the presence of other Tregs. We find that both naïve (CD45RA⁺) and antigen-experienced (CD45RA⁻) CD4⁺ T cells can up-regulate FOXP3 expression to similar levels after activation, although not as high as nTregs can. iTregs and nTregs are both able to inhibit the proliferation of other T cells. However, iTregs are able to produce IL-2, which nTregs are not., and only IL-2 if produced from CD45RA⁺ cells. iTregs appear to retain a similar cytokine-secreting profile to the cells from which they were derived, producing IL-2, 17, and IFN- γ if derived from CD45RA⁻ cells, and only IL-2 if produced from CD45RA⁺ cells.

Vercoulen, Yvonne

Human polyclonally induced CD4+CD25+FOXP3+ regulatory T cells suppress effector immune responses in vitro, but not in vivo.

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Abstract not to be posted.

Post-transcriptional regulation of effector cytokine mRNA underlies the anergic phenotype of auto-reactive helper T cells

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To prevent the development of autoimmune disease, self-reactive T cell clones that escape negative selection are either deleted (apoptosis) or rendered functionally unresponsive (anergic) when they encounter cognate antigens. Both cooperate in the maintenance of peripheral T cell tolerance but only apoptosis is well understood at the molecular and cellular levels while the process of T cell anergy remains ill-defined, in part, due to the fact that it is an in vivo phenomenon which, unlike apoptosis, is not accurately reproduced in vitro. Using a mouse model where naive, monoclonal T cells are adoptively transferred into transgenic hosts expressing their corresponding antigen in the context of a normal immune compartment (i.e. as a neo-self antigen), we demonstrate that, despite a characteristic inability to secrete effector cytokines, anergic T cells express high levels of effector cytokine mRNA. As with effector T cells, the appearance of cytokine transcripts in anergic T cells is accompanied by proliferation and classic activation markers like CD44 and CD25, suggesting that the relationship between these two fates is closer than previously appreciated. However, unlike effectors, which tend to polarize into distinct subsets with restricted cytokines profiles, anergic T cells express mRNA for cytokines associated with all known effector subsets (Th1/Th2/Th17/Tfh), likely reflecting the fact that they were primed in the absence of overt inflammation and attendant polarizing stimuli. A large degree of heterogeneity is apparent among anergic T cells, with some bearing higher amounts of cytokine transcripts than others, but even those with the highest mRNA levels are unable to produce functional protein when re-stimulated ex vivo, thus indicating a true disconnect between the transcriptional and translational programs. Cytokine transcripts were also detected in T cells exposed to cognate self-antigen throughout development (i.e. double transgenic mice), suggesting that this phenotype is not unique to our adoptive transfer system and, more importantly, that it may be a general feature of anergic T cells that have evaded thymic selection and subsequently encountered cognate antigen. Taken together, the data presented here support the idea that post-transcriptional regulation of effector cytokine mRNA is a key underlying mechanism for the induction/maintenance of T cell anergy and immune tolerance.

Waffarn, Elizabeth

IL-1 ices the cake: Innate regulation of B-1 cell responses to local influenza virus infection

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Abstract not to be posted.