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10th Annual Immunology Scientific Retreat

Program and Abstracts

October 18, 2018

Volume 10
Blood Research Institute
8733 Watertown Plank Road
Milwaukee, WI 53226

Organized by
Bonnie Dittel, PhD
Nita Salzman, PhD
Michael Dwinell, PhD
and the Center for Immunology

Immunology Scientific Retreat Schedule

Thursday, October 18, 2018
BRI Seminar Room – Ziegler B

8:00-8:30 a.m.	Reception/continental breakfast
8:30-9:00 a.m.	Introduction
9:00-9:30 a.m.	**Innate Immunity** – Michael Dwinell, PhD, Director, Center for Immunology and Professor Microbiology & Immunology, Medical College of Wisconsin *Strategies to reignite suppressed cancer immunity: STINGing pancreatic cancer*
9:30-9:45 a.m.	Ben Gantner, PhD Assistant Professor Department of Medicine, Division of Endocrinology *Watching the Watchers: Intravital Imaging to Study Neutrophil Polarization*
9:45-10:00 a.m.	Abstract #18 – Jason Siebert, (Malarkannan lab) The Role of Carma1 in IL-15 Priming of NK Cells
10:00-10:15 a.m.	**Break**
10:15-10:45 a.m.	**Adaptive Immunity** – Bonnie N. Dittel, PhD Senior Investigator, Blood Research Institute, BloodCenter of Wisconsin, Part of Versiti and Faculty, Department of Microbiology & Immunology, Medical College of Wisconsin *The search for a novel regulatory B cell subset*
10:45-11:00 a.m.	Abstract #7 – Clint Piper, (Drobyski lab) Donor CD4+ T cell-derived GM-CSF potentiates gastrointestinal inflammation during acute graft-versus-host disease
11:00-11:15 a.m.	Abstract #11 – Bardees Foda, PhD, (Y. Chen lab) Investigating the role of CD137 ligand in the pathogenesis of type 1 diabetes

11:15-11:30 a.m.	**Break**
11:30-12:00 p.m.	**ImmunoGenetics/Proteomics** – Sid Rao, MD, PhD Associate Investigator, Blood Research Institute, BloodCenter of Wisconsin, Part of Versiti; Associate Professor, Pediatrics and Cell Biology, Medical College of Wisconsin *Cohesion mutations in cancer*
12:00-12:15 p.m.	Yi-Guang Chen, PhD Associate Professor Department of Pediatrics, Microbiology & Immunology Max McGee Research Center for Juvenile Diabetes Medical College of Wisconsin *Testing human type 1 diabetes genes in the NOD mouse model*
12:15-12:30 p.m.	Abstract #14 – Carlie Aurubin, (Tarakanova lab) Protein Prenylation in the Context of Gammaherpesvirus Infection
12:30-1:45 p.m.	**Catered Lunch – Ziegler B**
1:45-2:15 p.m.	**ImmunoOncology/Therapeutics** – Subra Malarkannan, PhD Senior Investigator and Gardetto Chair for Molecular Immunology and Immunotherapy, Blood Research Institute, BloodCenter of Wisconsin, Part of Versiti and Professor in Departments of Medicine-Hematology and Oncology, Microbiology & Immunology, and Pediatrics, Medical College of Wisconsin *MicroRNA-mediated regulation of inflammatory responses*
2:15-2:30 p.m.	Matthew Riese, MD, PhD Associate Professor Departments of Medicine - Division of Hematology/Oncology, Microbiology & Immunology, and Surgery Associate Investigator, Blood Research Institute, Blood Center of Wisconsin, Part of Versiti *Intracellular Targets in T cell Cancer Immunotherapy*
2:30-2:45 p.m.	Abstract #17 – Gang Xin, (Cui lab) A novel immunotherapy overcome antigen escape and provides long term protection against relapse
2:45-3:00 p.m.	**Closing Statement** **Introduction to Poster session**
3:00-5:00 p.m.	**Poster presentations and Reception**

Abstract #: 1

Title: Regulatory T cells control PF4/heparin antibody production in mice

Author/s: Yongwei Zheng,[1] Wen Zhu,[1] Dipica Haribhai,[2,] Calvin B. Williams,[2] Richard H. Aster,[1] Renren Wen,[1] and Demin Wang[1,3]

Affiliation: [1]Blood Research Institute, BloodCenter of Wisconsin, *part of Versiti,* Milwaukee, WI 53226; [2]Division of Rheumatology, Department of Pediatrics, [3]Department of Microbiology & Immunology, Medical College of Wisconsin, Milwaukee, WI 53226

Text: Heparin-induced thrombocytopenia (HIT) is an immune-mediated disorder that can cause fatal arterial or venous thrombosis/thromboembolism. Immune complexes consisting of heparin, platelet factor 4 (PF4) and PF4/heparin-reactive antibodies are central to the pathogenesis of HIT. Regulatory T (Treg) cells are a subpopulation of CD4 T cells that modulate immune system by suppressing or down-regulating immune response and play an important role in immune homeostasis. However, the role of Treg cells in controlling PF4/heparin-specific antibody production is not known. Here we found that FoxP3-deficient mice, which have no functional Treg cells, spontaneously generated PF4/heparin-specific antibodies as early as three weeks after birth. Similarly, following transplantation with bone marrow cells from FoxP3-deficient mice, Rag1-deficient mice that have no endogenous B cells and T cells spontaneously produced PF4/heparin-specific antibodies. In contrast, Rag1-deficient mice that received wild-type bone marrow cells failed to produce PF4/heparin-specific antibodies. In addition, adoptively transferred Treg cells prevented spontaneous production of PF4/heparin-specific antibodies in FoxP3-deficient mice. Adoptively transferred Treg cells, not conventional CD4 T cells, also suppressed PF4/heparin complex-induced production of PF4/heparin-specific IgGs in wild-type mice. Treg cells suppress immune response mainly through releasing anti-inflammatory cytokines, such as interleukin-10 (IL-10). Deficiency of IL-10 led to spontaneous production of PF4/heparin-specific antibodies in mice. Moreover, BM chimeric mice with CD4 T cell-specific deletion of IL-10 increased PF4/heparin-specific IgG production following PF4/heparin complex challenge. Taken together, these findings demonstrate that Treg cells play an important role in suppressing PF4/heparin-specific antibody production in mice.

Abstract #: 2

Title: The development and heterogeneity of human natural killer cells defined by single-cell transcriptome

Author/s: Chao Yang[1,7], Jason Siebert[1,7], Robert Burns[2], Benedetta Bonacci[3], Mary Rau[8], Matthew Riese[4,7,9], Sridhar Rao[5,10,11], Karen-Sue Carlson[6,12], Martin Carroll[13,14], James Verbsky[10], Monica Thakar[1,10], Subramaniam Malarkannan[1,7,9]

Affiliation: [1]Laboratory of Molecular Immunology & Immunotherapy, [2]Bioinfomatics Core, [3]Flow Cytometry Core, [4]Laboratory of Lymphocyte Biology, [5]Laboratory of Stem Cell Transcriptional Regulation, [6]Laboratory of Coagulation Biology, Blood Research Institute, Blood Center of Wisconsin; Departments of [7]Microbiology & Immunology, [8]Surgery, [9]Medicine [10]Pediatrics, [11]Cell Biology, Neurobiology, Anatomy, and [12]Hematology and Oncology, Medical College of Wisconsin; [13]Division of Hematology and Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [14]Veterans Administration Hospital, Philadelphia, PA

Text: Natural killer (NK) cells are innate lymphoid cells critical to innate and adaptive immunity. However, the heterogeneity of human NK cells has not been fully appreciated. Using single-cell RNA sequencing technology, we assessed the heterogeneity of NK cells from marrow and blood of healthy donors and patients with germline GATA2 mutation. We found greater heterogeneity of human NK cells than previously defined. We identified a population that is in an active state marked by CXCR4 expression. The functional matured NK cells further acquire high CX3CR1 and TIM3 expression and become terminal matured/exhausted NK cells. We also obtained a gene signature unique to adaptive NK cells (NKG2C□). The transcriptome-based differentiation analyses support that $CD56^{bright}$ NK cells are precursors of $CD56^{dim}$ NK cells with identification of a potential transition population between $CD56^{bright}$ and $CD56^{dim}$. For GATA2 mutation patients, one donor, though clinically asymptomatic, demonstrated loss of $CD56^{bright}$ NK cells typically seen in individuals with GATA2 mutation. Interestingly, another donor from the same family with unusual early onset of the disease has more $CD56^{bright}$ NK cells than the $CD56^{dim}$ NK cells. scRNA-seq data from these patients are currently under extensive analyses. These data greatly expand our understanding of the heterogeneity and development of human NK cells.

Abstract #: 3

Title: CCL28 antimicrobial properties and defense from oropharyngeal candidiasis

Author/s: **Jie He**[1], Monica A. Thomas[2], Mitchell H. Grayson[3], Brian F. Volkman[2], Anna R. Huppler[1]

Affiliation:

[1] Department of Pediatrics, Medical College of Wisconsin
[2] Department of Biochemistry, Medical College of Wisconsin
[3] Department of Pediatrics, Nationwide Children's Hospital - The Ohio State University

Text:

Background

Oropharyngeal candidiasis (OPC) is an opportunistic infection caused by the commensal fungus *Candida albicans*. The T helper 17 (Th17)-associated cytokines IL-23 and IL-17 are crucial for immunity to OPC, but IL-17- mediated defense is insufficient to fully explain susceptibility to OPC. CCL28, a CC chemokine that is abundant in saliva and has *in vitro* antimicrobial activity, but Little is known about the function or regulation of CCL28 in defense from *Candida* infection. We hypothesize that CCL28 plays a role in natural defense from OPC and can be exploited as a therapeutic agent.

Methods

In the current study, we explore the antimicrobial activity of CCL28 variants of lacking motifs or structural components with an *in vitro Candida* killing assay. We investigate the *in vivo* role of CCL28 with quantitative PCR and ELISA for the expression of CCL28 in the presence or absence of OPC and exogenous CCL28 treatment for OPC.

Results

We demonstrate that both structured and unstructured CCL28 proteins show rapid and sustained fungicidal at physiologic concentrations. While the truncation of the C-terminus eliminated the killing, the N-terminus are dispensable for anti-fungal activit. While the expression of *Ccl28* is up-regulated in both tongue and salivary gland in mice with high *Il17a* expression (neutrophil receptor *Cxcr2*-/- mice), the expression of *Ccl28* in tongue of *Il17ra*-/-mice is suppressed. In an *in vivo* model of OPC, application of recombinant CCL28 reduces oral fungal burden in immune suppressed mice.

Conclusions

Our results indicate that the antimicrobial properties of CCL28 require distinct functional components from receptor-mediated chemotaxis. *Ccl28* oral expression correlates with functional IL-17A signaling. Finally, CCL28 treatment reduces mucosal fungal infection *in vivo*. Suggesting that a version of CCL28 with antimicrobial properties alone could be developed as an anti-*Candida* therapeutic agent without inflammatory side effects.

Abstract #: 4

Title: A novel proviral function for IL-17 during gammaherpesvirus infection

Author/s: Christopher Jondle, Kaitlin Johnson, Wadzanai Mboko, & Vera L. Tarakanova

Affiliation: Microbiology & Immunology

Text: Gammaherpesviruses are ubiquitous pathogens that establish lifelong infection and are associated with B cell lymphomas. Gammaherpesviruses have a viral tropism for B cells and infection drives a polyclonal germinal center (GC) response to establish latency in memory B cells. Our lab has shown that the antiviral and tumor suppressor transcription factor interferon regulatory factor 1 (IRF1) selectively controls the GC reaction driven by gammaherpesviruses. Viral infection in the peritoneal cavity is not regulated by the GC response. Therefore, to define the impact IRF1 has in the peritoneal cavity, the viral reservoir was examined in BL6 and IRF1$^{-/-}$ mice infected with murine gammaherpesvirus 68 (MHV68). The frequency of infected cells and viral reactivation in peritoneal cavity was significantly higher in IRF1$^{-/-}$ mice, suggesting that IRF1 function in T cells could play a role in controlling the viral infection. Both naïve and infected IRF1$^{-/-}$ mice have a significant decrease in the number of CD8+ T cells. Despite fewer overall numbers of CD8+ T cells in IRF1$^{-/-}$ mice, the number of viral specific CD8+ T cells was similar in IRF1$^{-/-}$ and BL6 infected mice. Next, the CD4+ T helper response was explored. Interestingly, there was a significant increase in IL-17A producing cells in both mock and infected IRF1$^{-/-}$ mice compared to BL6 mice. Herpesvirus saimiri (HVS), a simian gammaherpesvirus, encodes a viral IL-17, posing an intriguing possibility that IL-17 is proviral. MHV68 does not have a discernible IL-17 homologue, suggesting that MHV68 may rely on a host derived IL-17. Intriguingly, the frequency of both infected cells and MHV68 reactivation was significantly decreased in IL-17RA$^{-/-}$ mice compared to BL6 mice, indicating that host IL-17 signaling promotes viral infection. Given the viral tropism for B cells, the GC response in infected IL-17RA$^{-/-}$ mice was defined. Interestingly, there was a significant decrease in GC B cells in infected IL-17RA$^{-/-}$ mice. Overall, this data suggests that IRF1 suppresses Th17 differentiation and expression of IL-17A, with the latter being proviral in the context of chronic gammaherpesvirus infection.

Abstract #: 5

Title: Inhibition of Myeloperoxidase through the KYC Tripeptide Shows Reduced Severity in Inducible Psoriatic Inflammation

Author/s: Savannah Neu, Tien Vo, Cody Gurski, Kirkwood Pritchard, Bonnie Dittel

Affiliation: BloodCenter of Wisconsin, Blood Research Institute & Medical College of Wisconsin

Text: Over 100 million people suffer from psoriasis. This disease is characterized by unsolicited, constant, and sterile inflammation in the skin across large regions of the body due to overgrowth of epithelial cells. At initiation of inflammatory responses, the first responding cell types are neutrophils that localize to the site of action and begin vast recruitment of immune cells through cytokine secretion and tissue breakdown. Myeloperoxidase (MPO), an enzyme found in neutrophilic granules and released during the inflammatory response, can cause tissue damage through many mechanisms. KYC was developed to inhibit MPO by binding to and blocking its enzymatic site and has shown efficacy in reducing enzymatic activity. We wanted to explore the role and magnitude of MPO activity on the severity of psoriatic inflammation. To test this, we used the Imiquimod (IMQ) model of inducible psoriasis in C57BL/6J (WT) mice in combination with dose-dependent inhibition of MPO through KYC. Psoriatic lesion severity of the exposed back was scored from the day after shaving the animals (Day 0) throughout disease course using a modified PASI scale. We then compared disease outcomes of wildtype animals to those deficient in MPO. We observed that KYC (0.3 mg/kg) significantly decreased psoriasis severity ($p = 0.0235$) in WT mice. Mice with a deficiency in MPO had markedly decreased psoriatic inflammation as compared to their WT counterparts and severity was largely unaffected by KYC treatment. The mechanism whereby MPO contributes to psoriasis remains unknown, but evidence from experiments in other models of sterile inflammation suggests that MPO contributes to vascular permeability, allowing migration of immune cells into inflamed tissues.

Abstract #: 6

Title: Effects of zeros on analysis in the microbiome data

Author/s: Amy Pan, Nita Salzman, T. Hang Nghiem-Rao, Martin Hessner, Pippa Simpson

Affiliation: Pediatrics, Medical College of Wisconsin, Milwaukee, WI

Text:

Introduction: Some would argue that the human microbiome is the key to immunology. Certainly, it is likely that human microbiomes are involved in the pathogenesis of many diseases. Despite intense investigation in human microbiome research, the statistical methods for microbiome data are still being developed and refined. Operational Taxonomic Units (OTUs) are obtained via read error correction, filtering, clustering at 97% sequence similarity. Many OTUs are sparsely represented resulted in a predominance of zero in the data and analyses does not count for these zeros.

Objective: To investigate the effect of zeros on analyses in the microbiome data.

Methods: Simulation studies were performed to evaluate the appropriateness and accuracy of statistical tests.

Results: The Poisson model, typically used, causes high inflation and this gets worse as the mean, lamda, increases although it has high power.

Conclusion: ZIP and ZINB perform similarly with regards to power and they have higher power for smaller lambdas (3 vs 5, 16 vs 20, and 90 vs 100). For larger lambdas (485 vs 500) and larger samples (50 per group), NB has high power.

Abstract #: 7

Title: Donor $CD4^+$ T cell-derived GM-CSF potentiates gastrointestinal inflammation during acute graft-versus-host disease

Author/s: **Clint Piper[1]**, William Drobyski[2]

Affiliation: [1]Department of Microbiology & Immunology and [2]Department of Medicine - Medical College of Wisconsin

Text: Damage to the gastrointestinal tract is the major cause of morbidity during acute graft-versus-host disease (GVHD). While T cells are the proximate drivers of GVHD, disease induction and amplification rely on crosstalk between innate and adaptive immune populations. We previously observed that in an established model of acute GVHD, donor $CD4^+$ T cells are robust producers of granulocyte-macrophage colony stimulating factor (GM-CSF). Given the documented proinflammatory role of GM-CSF in autoimmunity, we sought to define the potential for this cytokine to act as a bridge between innate and adaptive immunity in GVHD. To determine the functional significance of donor T cell-derived GM-CSF, recipients were transplanted with MHC-mismatched grafts from WT or GM-$CSF^{-/-}$ animals. Recipients of GM-$CSF^{-/-}$ grafts had significantly prolonged survival and a reduction of pathological damage to the colon specifically. Restricting our analysis to $CD4^+$ T cells, Balb/c recipients were transplanted with Rag-$1^{-/-}$ BM alone or together with purified $CD4^+$ T cells from WT or GM-$CSF^{-/-}$ mice. Again, mice that received GM-$CSF^{-/-}$ $CD4^+$ cells developed less severe GVHD than those that received WT $CD4^+$ cells. Given that GM-CSF acts on a diverse subset of innate immune cells, we then examined which myeloid populations were responsive to GM-CSF two weeks post-transplantation, a time point at which antigen presenting cells (APCs) are primarily donor-derived. Using established markers for macrophages, neutrophils, and dendritic cells, we observed no perturbation in the numbers of cells from these major lineages between groups. However, there was a significant shift in the composition of classical DCs in the colonic lamina propria, and donor-derived DCs were virtually absent from the mesenteric lymph nodes, indicating that GM-CSF could influence the migratory capacity of certain DC subsets. Collectively, these studies demonstrate that GM-CSF potentiates damage to the GI tract during GVHD through an unresolved mechanism that could involve the manipulation of donor APC populations.

Abstract #: 8

Title: PIM Kinases Boost CD8 T Cell Metabolism to Control Chronic Viral Infection

Author/s: Peter Volberding, Gang Xin, David Schauder, Weiguo Cui

Affiliation: MCW Graduate Student

Text: CD8 T cells are potent killers of virally infected and transformed cells. However, during chronic viral infections and in the tumor microenvironment, T cells are continuously exposed to antigen causing the development of T cell exhaustion. T cell exhaustion is typified by a gradual loss of effector function and the expression of co-inhibitory molecules like PD-1, LAG3, and TIM3. Furthermore, exhausted CD8 T cells have metabolic defects, especially with regard to PI3K-Akt-mTOR signaling.

To control viral replication during chronic viral infections, CD8 T cells require “help” from CD4 T cells in the form of IL-21. IL-21R is a potent trigger of STAT3 signaling, and a poor trigger of PI3K-Akt-mTOR. STAT3 signaling in CD8 T cells causes robust expression of PIM kinases, which have been shown to regulate cellular metabolism in cancer cells and cardiomyocytes. Additionally, little is currently known about how CD8 T cells maintain cellular metabolic fitness during chronic viral infections. We hypothesize that because of limited PI3K-Akt-mTOR signaling, PIM kinase expression downstream of IL-21 is required for the metabolic fitness of CD8 T cells during chronic viral infection.

Mitochondrial function is essential for the functionality of CD8 T cells. Intriguingly, mitochondria are highly dynamic organelles, and the correlation of morphology to function has long been appreciated. For example, elongated, fused mitochondrial networks are known to be more efficient at oxidative phosphorylation than smaller punctate mitochondria. One of the primary regulators of mitochondrial morphology is DRP-1, a mechanoenzyme which is required for the fission of mitochondria. We have shown that PIM kinases phosphorylate DRP1 on S637, a posttranslational modification that inactivates DRP1. Therefore, PIM kinases increase the fusion of mitochondria, and by extension, the oxidative capacity and metabolic fitness of CD8 T cells during chronic viral infection. By understanding the molecular mechanisms by which exhausted CD8 T cell metabolism is regulated, we may be able to engineer methods to re-energize exhausted CD8 T cells to fight chronic viral infections and cancer.

Abstract #: 9

Title: Interleukin 27 signaling in T cells is essential for the development of type 1 diabetes

Author/s: Ashley E. Ciecko[1], Bardees M. Foda[2], Aron M. Geurts[3], Yi-Guang Chen[1,2]

Affiliation: [1]Department of Microbiology & Immunology, [2]Department of Pediatrics, [3]Department of Physiology Medical College of Wisconsin, Milwaukee, WI

Text: Type 1 diabetes (T1D) results from the T cell-mediated destruction of pancreatic β-cells. Human genome wide association studies have identified a region on chromosome 16 significantly linked to the development of T1D and *IL27* is proposed to be the causal gene. *IL27* encodes the p28 subunit of the heterodimeric cytokine interleukin 27 (IL-27). Human eQTL studies suggest a disease promoting role for IL-27 in the pathogenesis of T1D. However, the function of IL-27 in T1D has not been completely defined. The focus of this study was to test the role of IL-27 in the progression of T1D using the NOD mouse model. We generated NOD mice deficient in IL-27 or IL-27 receptor (IL-27Rα). In sharp contrast to wildtype NOD mice, both NOD.*Il27*-/- and NOD.*Il27ra*-/- strains were completely protected from T1D. Histological examination of pancreatic sections revealed that both NOD.*Il27*-/- and NOD.*Il27ra*-/- mice also had significantly less insulitis compared to the wildtype NOD control. We further show that IL-27 production by non-T and non-B cells was sufficient and required to drive T1D progression. However, the ability of non-T and non-B cells to respond to IL-27 was dispensable for T1D progression. Furthermore, adoptive transfer of IL-27 deficient T cells into NOD.*Rag1*-/- recipients resulted in diabetes development indicating that IL-27 deficient mice still harbor diabetogenic T cells. Conversely, NOD.*Rag1*-/- recipients of IL-27Rα deficient T cells did not develop diabetes demonstrating that direct IL-27 signaling is essential for their diabetogenic activity. Moreover, we found IL-27 signaling in both CD4 and CD8 T cells is important for T1D development. We further demonstrated that direct IL-27 signaling promoted the accumulation and effector function of CD8 T cells and altered CD4 T cell subsets (reducing Foxp3+ but increasing T-BET+ frequency) in pancreatic islets. Our observations reveal that IL-27 signaling in T cells is required for diabetes development in the NOD mouse and provide additional evidence to support its potential role in human T1D.

Abstract #: 10

Title: CD4 help is required for the generation of a transcriptionally distinct cytolytic CD8 T cell subset that arises during chronic viral infection

Author/s: **Ryan Zander[1]**, David Schauder[2], Gang Xin[1], Christine Nguyen[2], Xiaopeng Wu[1], Allan Zajac, & Weiguo Cui[1]

Affiliation: 1. Blood Research Institute, BloodCenter of Wisconsin, Milwaukee, WI 53213
2. Department of Microbiology & Immunology, Medical College of Wisconsin, Milwaukee, WI 53226
3. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35233

Text: During chronic viral infections, CD8 T cells are characterized by a dysfunctional state that is associated with their upregulation of multiple co-inhibitory receptors. Nevertheless, CD8 T cells remain indispensible for viral control, indicating that some CD8 T cells retain their cytotoxic potential. A better understanding of the mechanisms that regulate CD8 T cell differentiation during persistent infection is required to improve immunotherapies aimed at restoring function in exhausted CD8 T cells. Here, using single cell RNA-sequencing (scRNA-seq) we show that CD8 T cells responding to chronic viral infection are more heterogeneous than previously appreciated, with three transcriptionally distinct subsets dominating the late phase of the antiviral response. Importantly, our findings uncover the formation of a previously unrecognized CD8 T cell subset that exhibits potent cytolytic function. This subset is characterized by high expression of killer cell lectin-like receptors Klre1 and Klra9, the chemokine receptor CX_3CR1, and the transcription factors T-bet and Zeb2. Notably our data further demonstrate that the formation of this cytotoxic subset is critically dependent on CD4 help via IL-21 production, and that exploitation of this developmental pathway may be used therapeutically to enhance the killer function of CD8 T cells infiltrated into the tumor. These findings provide a better understanding of CD8 T cell differentiation during persistent infection and have implications towards optimizing the generation of protective CD8 T cells during chronic viral infection and cancer.

Abstract #: 11

Title: Investigating the role of CD137 ligand in the pathogenesis of type 1 diabetes

Author/s: **Bardees Foda[1]**, Matthew Forsberg[2], Ashley Ciecko[2], Kevin Mueller[1], Aron Geurts[3] Yi-Guang Chen[1, 2]

Affiliation: [1]Department of Pediatrics, Max McGee National Research Center for Juvenile Diabetes,[2]Department of Microbiology & Immunology,[3]Department of Physiology Medical College of Wisconsin, Milwaukee, WI

Text: The gene encoding the co-stimulatory molecule CD137 is located within the *Idd9.3* T1D susceptibility locus and contributes to diabetes progression in NOD mice. We have previously shown that CD137 expression in T cells has dual functions: CD4+CD137+ T cells negatively regulate T1D development while CD8+CD137+ T cells showed potent diabetogenecity. The protective function of CD137 in CD4+ T cells is likely due to the significant amounts of soluble CD137 (sCD137) produced by Foxp3+ Tregs. The interaction between CD137 and its ligand (CD137L) induces two signaling pathways, forward one driven by CD137 and the reverse signaling mediated by CD137L, both of which modulate T cell function. Here, we study the impact of CD137L deficiency on T1D to gain further insight into disease pathogenesis. We successfully generated a strain of NOD mouse knockout of the gene encoding CD137L (*Tnfsf9*) using CRISPR/Cas9 technology. Relative to NOD, CD137L deficient mice showed significant delay in T1D development, less inflamed islets, and less islet-infiltrating autoreactive CD8 T cells, reduced autoreactive CD8 T cells in spleen and pancreatic lymph node. Interestingly, we detected significant elevated levels of sCD137 in sera of CD137L deficient mice and in the supernatant of cultured Tregs. Furthermore, we could not detect differences in the frequency of CD137+ Treg between NOD and *Tnfsf9*$^{-/-}$ mice. Bone marrow transfer experiments showed that both hematopoietic and non-hematopoietic cells deficient in CD137L are able to suppress T1D development. However, lacking the expression of CD137 ligand does not affect the capability of T cells to induce T1D in NOD.*Rag1*$^{-/-}$ recipients. We are working on identifying the cellular source of increased sCD137 and the mechanism behind this elevation. We direct our efforts to understand how CD137-CD137L interaction can modulate T1D pathogenesis and how to translate the data from NOD mouse to understand the pathogenesis of human diabetes.

Abstract #: 12

Title: A Novel Population of Mature B Cells That Exhibit Regulatory B Cell Activity

Author/s: **Mohamed Khalil**, Cody Gurski and Bonnie Dittel

Affiliation: Blood Research Institute, BloodCenter of Wisconsin

Text: Regulatory B cell (Breg) function was first observed in the mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) where it was shown that they attenuated disease severity. Subsequently, we demonstrated that Breg activity was indirect by driving the homeostatic expansion of $CD4^+FoxP3^+$ regulatory T cells (Treg), in a tumor necrosis factor ligand superfamily member 18 (TNFSF18) or GITRL-dependent manner. Using a phenotyping strategy, we found that Breg activity resided within the mature splenic follicular (FO) phenotype ($B220^+IgM^+CD23^+$), but not, marginal zone B cells. However, unlike FO B cells, Breg activity was associated with a low expression level of IgD. We designated these B cells expressing IgD (D) at a low (L) level (BD_L). Due to their role in controlling autoimmune disease and their potential for therapeutic application, we further studied their development and functional phenotype. Since FO B cells develop from the transitional (T) 2 stage in the spleen, we also asked whether BD_L also derive directly from the T2 stage. Our developmental experiment showed that both FO and BD_L cells originated from the T2 stage. We determined the expression level of surface markers differentially expressed during B cell development. We found that BD_L display a distinct expression profile from the T1 and T2 and the mature B cells (FO and MZ) for the surface markers B220, IgD, CD21, CD23, CD9, CD1d, CD9, CD62L, CCR7 and CD93. RNA seq data confirmed and supported this finding by showing that there were 342 genes upregulated in BD_L and 122 genes downregulated compared to FO B cells. By studying the role played by BD_L during the EAE disease course, we found that BD_L are a stable population during the 30 days of EAE disease and enhanced Tregs expansion. The expression of CCR7 by BD_L suggests that they may preferentially migrate to the T cell zone in the spleen, which requires CCR7 expression. Using immunofluorescence, we found that BD_L cells are present in the T cell zone in contact with Tregs in the spleen. These results provide insight into how BD_L develop and they regulate immune responses via their interaction with Tregs.

Abstract #: 13

Title: Characterizing IL-12p40 and Hemorrhage in Spinal Cord Injured Mice

Author/s: **Jose Rosas**, Brandy Aperi, Kyle Stehlik, Natasha Wilkins, Matthew Budde PhD, Antje Kroner MD, PhD

Affiliation: MCW Depts. of Neurosurgery, Microbiology & Immunology, Neuroscience Doctoral Program, Zablocki VAMC

Text: Traumatic spinal cord injury (SCI) is a relatively frequent event that imposes a massive burden on the health, quality of life and socioeconomic situation of affected people. The National Spinal Cord Injury Statistic Center reported in 2018 that estimated 288,000 people in the U.S. live with SCI with 17,700 new cases a year. Tissue damage during SCI occurs in two phases: primary and secondary damage. Primary damage is caused by the initial trauma that disrupts the structural integrity of the spinal cord resulting in tissue damage and cell death. Inflammation and hemorrhage are two secondary damage processes that promote subsequent tissue damage. Many cell types contribute to inflammation after SCI, including microglia and macrophages, which phagocytose cellular debris including red blood cells at the injury site. Uptake of blood breakdown products and iron can induce a pro-inflammatory phenotype of macrophages to release pro-inflammatory cytokines like IL-12 and IL-23. IL-12 and IL-23 are two key cytokines that share the IL-12p40 subunit and modulate the innate and adaptive immune response.

We are using a contusion model of SCI in C57BL/6 mice, IL-12p40 knockout mice (deficient for IL-12 and IL-23) and IL-23p19 knockout mice (deficient in IL-23). A laminectomy is performed at T11 and a contusion is induced with the Infinite Horizon Impactor.

Using RT-qPCR, immunofluorescence and Western blot, we can show an upregulation of IL-12p40, IL-23p19 and IL-12 receptors at various time points after SCI in wildtype(wt) mice. Higher amounts of hemoglobin are present in injured tissue compared to uninjured tissue, which correlate to hemorrhage volumes measured by MRI. IL-12p40 KO mice show improved recovery after SCI compared to wt mice, while recovery in IL-23p19 knockout mice is similar to wt in the Basso Mouse Score (BMS).

Our data indicate that IL-12 factors are modulated after SCI. Absence of IL-12p40 resulted in better locomotor recovery, which may be attributed to IL-12 alone or its combination with IL-23. The absence of IL-23 alone was not beneficial after SCI.

Abstract #: 14

Title: Protein Prenylation in the Context of Gammaherpesvirus Infection

Author/s: Carlie Aurubin [Δ]**,** Phil Lange[Δ], Kiall Suazo[˚], Mark Distefano[˚] PhD, Vera Tarakanova[Δ] PhD

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Text: Gammaherpesviruses are ubiquitous pathogens that establish lifelong infections in the host and are associated with multiple cancers, particularly lymphomas. Factors that contribute to lymphomagenesis are relatively understudied, therefore to gain insight into viral pathogenesis it is important to study cellular responses to viral infection. We have previously demonstrated that protein prenylation, a host process, supports gammaherpesvirus replication. Prenylation is an important post translational modification that covalently attaches a lipid moiety, onto a select group of proteins. This lipid modification increases the hydrophobicity of proteins thereby altering their localization, stability, and interaction with other proteins. While the enzymes responsible for prenylation have been identified, little is known about how they are regulated, particularly in the context of infection. We aim to identify what proteins are prenylated in primary macrophages during gammaherpesvirus infection, and the extent to which infection induced type 1 interferon signaling alters the expression and activity of the prenylation machinery. Utilizing novel labeling technology, we have identified proteins that are differentially prenylated during infection. Further, we observed that type 1 interferon increases transcription of certain prenyltransferases suggesting a complex interplay between IFN-mediated changes in the prenylation machinery and optimal gammaherpesvirus replication. Defining such interplay is likely to offer a unique perspective on gammaherpesvirus infection and lymphomagenesis.

Abstract #: 15

Title: Immune Modulation of The Pancreatic Cancer Tumor Microenvironment Via Sting Agonist

Author/s: **Donna McAllister**, Weiqing Jing, Bryon Johnson, and Michael B. Dwinell

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Text: Pancreatic ductal adenocarcinoma (PDA) is one of deadliest cancers with a five-year survival rate of approximately 7%. PDA's dense stroma is comprised of collagen, cancer associated fibroblasts (CAFs) and myeloid and lymphoid immune suppressive cells. The dense stroma and immune suppression creates a barrier preventing tumor-reactive immune cell infiltration. Recently, treatment with 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a ligand of the STimulator of Interferon Genes (STING), decreases tumor mass, collagen deposition and increases immune cell infiltrates (CD3) in other cancer models. We were interested in testing DMXAA's effectiveness against the immune restrictive stroma of PDA. We employed a transgenic murine model that recapitulates the human pancreatic cancer to test the ability of STING agonist to re-ignite the immunologically cold tumor. We have shown that DMXAA treatment potently changed the architecture of the tumor, the immune profile and increased survival of tumor-bearing mice. Notably, DMXAA elevated levels and activation of cytotoxic T cells within the tumor, concomitant with decreased levels of suppressive regulatory T cells. Further, DMXAA reprogrammed macrophages within the tumor from a suppressive to an inflammatory profile, simultaneous with increased levels of cross-presenting dendritic cells. Consistent with the change in tumor immune landscape, DMXAA inflamed the tumor, with elevated levels of and proinflammatory cytokines and chemokines produced by macrophages and dendritic cell. Moreover, we determined that pancreatic cancer cells are also activated by STING agonist, increasing TBK-1, and STAT-6 signaling leading to secretin of T cell chemoattractants. Cumulatively, these data demonstrate that pancreatic cancer progression is potently inhibited by STING agonist, STING agonist inflamed the immunologically cold tumor to promote trafficking and infiltration of tumor-killing T cells.

Abstract #: 16

Title: Ceftriaxone-mediated dynamics between the host, microbiota and enterococci in mouse gastrointestinal tract

Author/s: **Rajrupa Chakraborty**[a], Vy Lam[a], Sushma Kommineni[a], Jeremiah Stromich[a], Michael Hayward[a], Christopher Kristich[b], Nita Salzman[a]

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Text: The mammalian gastrointestinal (GI) tract is colonized by complex microbial communities, collectively termed as the microbiota, which constitutes symbionts, commensals and opportunists. The host anatomically restricts the microbiota in the intestinal lumen to establish compartmentalization and limit microbial access to the mucosa. An intact microbiota and a balanced host-microbiota interaction at the mucosal interface is essential for the maintenance of homeostasis. However, disruption of this homeostasis by broad-spectrum antibiotics like cephalosporins, allows increased intestinal proliferation and disseminated infections by opportunists such as enterococci in hospitalized patients.

Enterococci are Gram-positive residents of the mammalian GI tract that harbor intrinsic resistance to broad-spectrum cephalosporins like ceftriaxone and have also acquired resistance to several other antibiotics by their ability to exchange mobile genetic elements. We used *Enterococcus faecalis* (EF) colonized mice to study the dynamics of enterococci, commensal microbiota and host in response to systemic ceftriaxone administration. We found that the mouse model recapitulates enterococcal dynamics described in humans. However, investigation of the impact of ceftriaxone treatment on the mucosal barrier defenses and integrity suggested that translocation of enterococci across the intestinal mucosa was not associated with intestinal pathology or increased permeability. Ceftriaxone-induced alteration of intestinal microbial composition was associated with transient increase in the abundance of multiple bacterial Operational Taxonomic Units (OTUs) in addition to enterococci, for example, lactobacilli, which also disseminated to the extra-intestinal organs. Collectively, these results emphasize that ceftriaxone-induced disruption of mucosal homeostasis, facilitate increased intestinal abundance of a limited number of commensals along with enterococci, allowing their translocation and systemic dissemination in a healthy host.

Abstract #: 17

Title: A novel immunotherapy overcome antigen escape and provides long term protection against relapse

Author/s: **Gang Xin**, David Schauder, Peter Volberding, Weiguo Cui

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Text: One of the major hurdles limiting the efficacy of adoptive cell transfer (ACT) based immunotherapies is antigen escape, a mechanism exploited by cancer cells to allow the outgrowth of tumor variants that are not targeted by the therapies. Thus, inducing a more broadly targeted antitumor response involving endogenous tumor-specific T cells against the escaped tumor cells is critical to improve the efficacy of ACT. The endogenous immune system is capable of antigen spreading, which is the generation of immune responses against antigens distinct from the initial targeted one by cross presentation. To employ this mechanism for preventing antigen escape, we propose to combine ACT with bacterial infection, which is known to effectively induce the cross priming via activation of Batf3-dependent dendritic cells (DCs). In this vein, the ACT mediated initial tumor destruction will release tumor antigens, which will be taken by pathogen induced DCs to prime secondary tumor-specific T cells for broader antigen coverage. To induce the antigen spreading, we utilized our previous published ReACT therapy which capitalizes on the synergistic effect of pathogen-based immunotherapy and ACT for treating melanoma tumors. In the present study, we evaluate whether ReACT can eliminate escape mutants using a model we generated by knocking out the targeted antigen in melanoma tumor cells. Compared to conventional ACT, ReACT could significantly boost the Batf3-driven DC and endogenous antitumor T cell responses, which results in eradication of tumors with targeted antigen loss. More interestingly, these endogenous CD8 T cells can differentiate into memory cells for protection against relapse. Overall, the ReACT can generate durable and broad targeted antitumor response to prevent antigen escape and relapse. Our findings present an effective solution to the challenge of antigen escape, which broadens the applicability of T-cell therapy for solid tumor. Furthermore, applying this knowledge to improving the ACT will translate into long-term survival for treated patients.

Abstract #: 18

Title: The Role of Carma1 in IL-15 Priming of NK Cells

Author/s: **Jason R Siebert**[1,5]., K Dixon[1,5]., M Thakar[2,4]., S Malarkannan[1,3,5].

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Text: Natural Killer (NK) cells are the largest lymphocyte component of the cellular innate immune response and are important for the clearance of transformed and virally infected cells. NK cells require a two-step process for complete effector function: an initial priming step commonly through a cytokine receptor and a second activation step through interaction with a target cell. One cytokine that is critical in NK cell priming is the common-gamma cytokine IL-15. Scaffold proteins regulate cellular signaling through a variety of mechanisms. CARD-containing MAGUK protein 1 (Carma1) is a 130 kDa scaffold protein essential for NK cell effector functions. Recent work has shown a multi-faceted role for Carma1 in lymphocyte signaling pathways. Here we explore the Carma1-dependent signaling pathways downstream of IL-15R necessary to prime NK cells during an innate immune response. The specific aims we focused on to evaluate the role of Carma1 are *1*) the *in-vivo* NK cell priming and effector function*,* 2) the *ex-vivo* priming, and 3) the molecular mechanisms of priming. To study these aims we used two different mouse models of Carma1, a mutant model lacking the CARD domain (□CARD) and a null mutant (*Carma1*□□□). We examine the functional role of Carma1-dependent priming using a murine Cytomegalovirus (mCMV) *in-vivo* and an IL-15 priming system *ex-vivo.* To decipher the Carma1-dependent molecular mechanisms we use immunoblotting, RNA-sequencing, and ATAC-Sequencing. We identified a role for Carma1 in regulating the proliferative capacity and effector function of NK cells during a viral infection, and this is explained by Carma1's role in regulating IL-15R signaling *ex-vivo*. *Carma1*□□□ NK cells demonstrate a lack of upregulation in Irf3 dependent genes downstream of IL-15R. In conclusion these results demonstrate that Carma1 is essential for IL-15 based NK priming through the activation of a Carma1→TBK1→Irf3 pathway. In the future I hope to further define the role of Irf3 in NK cell priming and NK cell effector function.

Abstract #: 19

Title: The Double Identities of IRF-3 and IRF-7 During Gammaherpesvirus Infection

Author/s: Kaitlin Johnson[1], Christopher Jondle[1], Nicole Minton[1], Vera Tarakanova[1]

Affiliation: [1]The Medical College of Wisconsin

Text: Gammaherpesviruses are ubiquitous pathogens that establish lifelong infections. These viruses undergo two distinct lifecycles, lytic replication, which largely occurs during the first 10-12 days following *de novo* infection, and latency. Type I Interferons (IFNs) are widely known as innate antiviral factors and have been widely studied in the context of acute viral infection. Type I IFN signaling is critical to the control of acute gammaherpesvirus infection, as deficiency in the type I IFN receptor (IFNAR) results in elevated viral titers, and, under high doses of infection, host mortality. This signaling pathway is initiated through the transcription factors Interferon Regulatory Factors (IRFs) 3 and 7. Upon activation, IRF-3 and IRF-7 will stimulate the production of interferons α and β, which then signal through IFNAR to stimulate antiviral gene production. Deficiency in IRF-3 and IRF-7 also results in elevated viral titers during acute gammaherpesvirus infection. Despite their well-established importance during acute viral infection, few studies have examined the role of the key components of the type I IFN signaling pathway during chronic latent gammaherpesvirus infection of an intact host.

Our current studies have highlighted intriguing roles for the IRF-3 and IRF-7 during chronic gammaherpesvirus infection. We found that in the absence of IRF-7, infection drives an exaggerated germinal center response in the spleens of chronically-infected hosts. Additionally, $IRF7^{-/-}$ hosts are unable to restrict viral reactivation in the peritoneum. Curiously, we observed a contrasting phenotype in $IRF\text{-}3^{-/-}$ mice, wherein the germinal center response was similar to WT, but viral latency is reduced in spleens. Further studies will utilize these mouse models, as well as IFNAR-deficient mice, to further examine the importance of type I IFN signaling during chronic gammaherpesvirus infection.

Abstract #: 20

Title: BATF Metabolically Reprograms CD8 T cells for ACT Therapy in Melanoma

Author/s: Paytsar Topchyan, Gang Xin, Weiguo Cui

Affiliation: MCW Medical Scientist Training Program Student

Text: Adoptive cell transfer (ACT) therapy has emerged as a therapeutic approach to improve cancer patient outcomes; however, increasing evidence suggests that a major hurdle in ACT therapy is the bioenergetic stress caused by the tumor microenvironment. While the effects of metabolic demand on T cell function have garnered recent interest, the underlying transcriptional mechanism of their metabolic regulation remains unclear. Recent studies reveal that BATF targets several important molecules in metabolic pathways and is highly expressed in tumor infiltrating lymphocytes. Our previous studies also revealed that BATF sustains T cell function during chronic viral infection, which induces similar bioenergetic stress on T cells, as seen in the tumor. Inspired by these studies, we hypothesize that BATF overexpression in $CD8^+$ T cells increases T cell metabolic fitness through increased fatty acid oxidation via the PPAR-gamma pathway, resulting in increased effector function and reduced tumor burden. To test this idea, we employed T cell receptor transgenic $CD8^+$ T cells (Pmel-1) that specifically recognize the melanocyte differentiation antigen gp100 and B16-F10 melanoma tumor cells. The *in vitro* overexpression of BATF in activated Pmel cells results in enhanced fatty acid uptake and mitochondrial respiration with elevated level of PPAR-gamma, which is a master regulator of fatty acid oxidation. More intriguingly, we also found that the adoptive transfer of these BATF overexpressing Pmel cells induces significant tumor regression in tumor-bearing mice, compared with ACT of empty-vector Pmel cells. Overall, we metabolically reprogram the tumor-reactive T cells via manipulation BATF to reinvigorate their effector function. Such an approach might represent a promising strategy with superior long-lasting anti-tumor immune effects, resulting in better outcomes of patients.

Abstract #: 21

Title: Single B cell Analysis of Heparin Induced Thrombocytopenia patients

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Text: Heparin-induced thrombocytopenia (HIT) is characterized by the platelet count decrease of more than 50% beginning 5-10 days after initial heparin administration, with or without thrombosis. HIT is induced by antibodies recognizing PF4/heparin complexes. Clinical studies have found that the PF4/heparin-reactive antibodies have two types, one of which can activate platelets (pathogenic) and the other ones fail to active platelets (non-pathogenic). However, the features of these antibodies that determine the pathogenicity are not known. To understand the molecular basis for the HIT antibody pathogenicity, we decided to clone PF4/heparin-reactive antibodies from the HIT patients. We utilized the combined technologies of single-cell sorting, culture and PCR. Single B cells were sorted from peripheral blood mononuclear cells (PBMCs) of HIT patients into 96-well PCR plates, followed by two rounds of PCR to amplify immunoglobulin heavy chain (VH) and light chain (VL) genes. Successfully amplified VH and VL genes from individual B cells were cloned into an immunoglobulin expression vector and expressed in 293T cells. Supernatants from cultured 293T cells were subjected to an enzyme-linked immunosorbent assay (*ELISA*) to screen for PF4/heparin-specific antibodies. Alternatively, single B cells were sorted from PBMCs of HIT patients into 96-well plates and cultured for about 3 weeks. Supernatants from each well were subjected to the same screening method. Using both strategies, we screened total around 5000 single B cells from 9 HIT patients and successfully obtained around 1500 monoclonal antibodies. Among these monoclonal antibodies, 17 clones were PF4/heparin-reactive. Importantly, 5 clones of the PF4/heparin-reactive antibodies were able to activate platelets. Taken together, we can successfully clone PF4/heparin-reactive and platelet-activating HIT antibodies from HIT patients. Now we are in the process of characterizing these PF4/heparin-reactive and platelet-activating antibodies.

Abstract #: 22

Title: The NK cell response to MCMV reveals a novel role for MyD88 in Ly49H-mediated signaling and inflammatory cytokine generation.

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Text: Natural killer cells are innate lymphocytes responsible for providing anti-viral immunity. The adaptor protein, MyD88, mediates TLR signaling and promotes a global anti-viral immune response. Studies using MCMV found that both NK cells and MyD88 are required for viral clearance and host survival. NK cells respond to MCMV via Ly49H recognition of the MCMV protein, m157. Ly49H signals via the adaptor, DAP10, and MyD88 propagates signaling downstream of DAP10-assiciated receptors in keratinocytes. Therefore, we hypothesize that MyD88 functions as a signaling adaptor for Ly49H in NK cells. Using a novel NK cell-conditional MyD88 knockout mouse model, we aim to 1) determine the NK cell intrinsic role of MyD88 during MCMV infection, and 2) investigate the role of MyD88 in Ly49H signaling and function. Initial results demonstrate that NK cells in *Myd88*$^{fl/fl}$*Ncr1*$^{Cre/+}$ mice fail to initiate a robust Ly49H-mediated response to MCMV and NK cells lacking MyD88 produce significantly less IFNg upon Ly49H stimulation. These findings suggest a novel role for MyD88 in NK cell function and further investigation will provide valuable insights into the molecular mechanism(s) that govern NK cell signaling in the context of anti-viral immunity.

Abstract #: 23

Title: Probing the unique structure of CCL21 to resolve function

Author/s: **Natasha A. Moussouras**,[1] Christopher T. Veldkamp,[2] Brian F. Volkman,[3] Michael B. Dwinell[1]

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Text: The CCL21/CCL19/CCR7 axis is responsible for migration to lymph nodes, classically of naïve T cells and dendritic cells to facilitate antigen presentation. CCR7 has also been linked to the pathologic migration and metastasis of cancer cells to lymph nodes. Interestingly, CCL21 has a unique structure due to its extended 30 residue C-terminal tail. Studies have shown the tail regulates an autoinhibitory effect that is relieved by either C-terminal truncation or the post-translational modification polysialic acid. We hypothesize the extended tail region of CCL21 mediates this phenomenon by interacting with the chemokine core to impede signaling required for chemotaxis. Using NMR spectroscopy, we probed the unique structural features of CCL21 in order to determine the residue-specific interactions responsible for autoinhibition. Serial C-terminal tail truncations monitored via NMR spectroscopy suggest the region between residues 80-91 is responsible for the majority of the structural differences observed between the wild type and fully tail-truncated chemokine. Heteronuclear NOE experiments reveal a dynamic tail region that suggests a weak, transient interaction between the C-terminal tail and chemokine core. Furthermore, a CCL21 dilution series produced chemical shift perturbations in the HSQC NMR spectra that point to the possibility of previously unconsidered oligomerization. We further examine the role of polysialic acid in this interplay. Overall, these structural pieces shed light on a likely tissue or receptor biased signaling system and a novel mechanism of chemokine regulation that can then be harnessed for specific targeting in pathologic contexts.

Abstract #: 24

Title: Downregulation of the protein tyrosine phosphatase CD45 in Roseolovirus-infected T cells

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Text: The Roseolovirus genus is comprised of several highly prevalent human-specific herpesviruses, including human herpesvirus-6 & -7 (HHV6 and HHV-7). Like all herpesviruses, HHV-6 & -7 establish life-long infection within their host, requiring these viruses to employ various mechanisms of immune evasion.

Here we show that the protein tyrosine phosphatase CD45 is downregulated in T cells infected with HHV-6 or HHV-7. CD45 is essential for signaling through the T cell receptor and as such, it is necessary for developing a fully functional immune response. Interestingly, three other large dsDNA viruses have separately evolved mechanisms to target CD45, suggesting that CD45 is an important viral target. The functional outcome of virus-mediated inhibition of CD45 is poorly understood, though an increase in IL-10 production has been observed in response to the inhibition of CD45 by other viruses. This change in Il-10 production hints at the downregulation of CD45 as a potential immunoevasive strategy.

To identify the viral protein responsible for downregulating CD45, we deleted a of a block of 4 open reading frames from the HHV-6 genome (ORFs U21-U24). Cells infected with this deletion mutant virus displayed normal CD45 expression, suggesting the downregulation of CD45 in HHV6-infected T cells is mediated by the virus and more specifically, by one of these deleted gene products.

Abstract #: 25

Title: A microbiota-sensitive developmental window for the establishment of long-term mucosal homeostasis

Author/s: **Jonathan C. Jeschke[1*]**, Christopher L. DeCiantis[1*], Jennifer Ziegelbauer[1], Selena Singh[1], and Calvin B. Williams[1]

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Text: Our “Bigenic” mice model ileal homeostasis by combining a TCR transgene with the matching transgenic target antigen expressed in the ileum. Half of Bigenic mice develop failure to thrive and TH17-associated colitis, whereas the remainder exhibit TH17-associated ileal hyperplasia and accumulate ileal-reactive Treg cells. IFN deficiency caused increased TH17 production and more penetrant colitis. The highest incidence of colitis in both WT and IFN-deficient strains occurs in the first weeks after weaning. We hypothesized that newly weaned Bigenic mice are transiently hypersensitive microbial colonization events. *ifng*$^{-/-}$Bigenic mice pretreated with streptomycin & bacitracin remained resistant to spontaneous disease long after antibiotic withdrawal. By contrast, the same antibiotics failed to achieve an equivalent therapeutic effect when started after the onset of symptoms. Only some antibiotics had the capacity to reverse established disease in *ifng*$^{-/-}$Bigenic mice. These findings demonstrate the presence of a microbiota-sensitive regulatory system active soon after weaning that can limit long term hypersensitivity to specific microbial compositions.