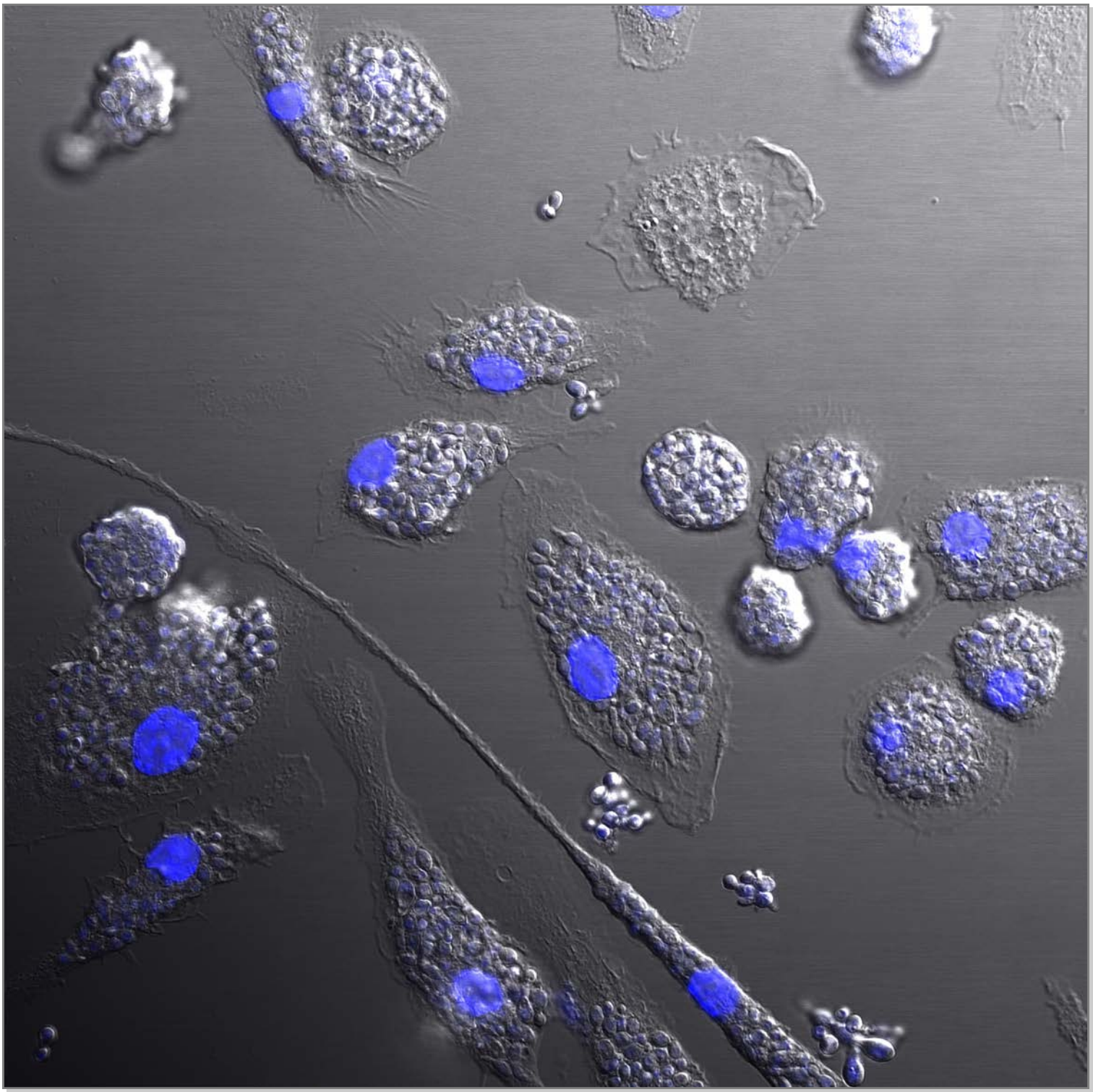
Annual Immunology Retreat

October 9-10, 2015 · Deer Creek Lodge & Conference Center



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From the cover: Brightfield microscopy of bone marrow derived macrophages infected with yeast cells of the pathogenic fungus *Histoplasma capsulatum*. Cells obtained from mice lacking hypoxia inducing factor 1a. Cell nucleus is stained by DAPI.

Annual Immunology Retreat 2015

Friday

10:00 **Welcome Remarks** - Harinder Singh

Session I Session Chair: Harinder Singh

10:10 **Michael Jordan**, *Associate Professor, Division of Immunobiology*
Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy.

10:30 **Andrew Patterson**, *Graduate Student, Division of Immunobiology*
Gimap5-regulated inhibition of GSK3 is essential for CD4+ T cell proliferation and peripheral tolerance

10:50 **Carolyn Rydyznski**, *Graduate Student, Center for Autoimmune Genomics and Etiology*
Is bigger really better: consequences of natural killer cell suppression of the germinal center

11:10 **Break**

11:20 **Jared Klarquist**, *Graduate Student, Division of Immunobiology*
STING promotes immunity towards dying cells

11:40 **Shouxiong Huang**, *Assistant Professor, Department of Environmental Health*
Structures and functions of CD1-associated self lipids

12:00 **Lunch**

1:00 **Free Time**

Session II Session Chair: Marc Rothenberg

3:15 **Matthew Flick**, *Assistant Professor, Division of Experimental Hematology*
The coagulation factor fibrinogen is at the interface of pathogen virulence and host defense following S. aureus infection

3:35 **Hansraj Bangar**, *Post-Doctoral Fellow, Division of Infectious Diseases*
Intestinal stem cell injury caused by Clostridium difficile is averted by Bacteroidales-induced crypt defenses

3:55 **Kate Carroll**, *Graduate Student, Division of Immunobiology*
Prolongation of the Remission Period and Reversal of New Onset Type 1 Diabetes with the Selective Elimination of Autoreactive T Cells

4:15 **Yrina Rochman**, *Instructor, Division of Immunobiology*
Thymic Stromal Lymphopoietin acts in concert with IL-4 to induce a robust TH2 state with pathogenic potential

4:35 **Break**

Session III Session Chair: Jonathan Katz

4:45 **Sara Stoffers**, *Graduate Student, Division of Immunobiology*
Dissecting Molecular Mechanisms of Synergy Between IL-13 and IL-17A in Severe Asthma

5:05 **Shannon Rapovy**, *Graduate Student, Division of Infectious Diseases*
Contribution of L-arginine biosynthesis for antimycobacterial T cell function

5:25 **Joerg Koehl**, *Professor, University of Lübeck*
Immunoregulation of inflammation in allergy and infection - the International Research Training Group between Cincinnati and Lübeck

6:00 **Dinner**

7:30 - 9:30 Poster Session - Odd numbered abstracts will present 7:30-8:15
Even numbered abstracts will present 8:30-9:15

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Saturday

8:00 - 9:15 **Breakfast Buffet** (located on the Greater Mezzanine)

Session IV Session Chair: Joe Qualls

9:00 **Simon Hogan**, *Associate Professor, Division of Allergy and Immunology*
Immune- intestinal epithelial Interactions in health and disease

9:20 **Maria Fields**, *Post-Doctoral Fellow, Division of Immunobiology*
Role of the IL-17 Axis in the Progression of Non-alcoholic Fatty Liver Disease

9:40 **Jeremy Kinder**, *Graduate Student, Division of Infectious Diseases*
Durability of pregnancy-primed regulatory T cell memory

10:00 **Heping Xu**, *Post-Doctoral Fellow, Division of Immunobiology*
Visualizing germinal center B cell fate dynamics at single cell transcriptome resolution

10:20 **Break**

10:30 **Leah Kottyan**, *Assistant Professor, Center for Autoimmune Genomics and Etiology*
Dissecting the 11q13 disease risk locus in Eosinophilic Esophagitis

10:50 **Kun-Po Li**, *Graduate Student, Division of Immunobiology*
Temporal expression of Bim limits the development of TCR+ double negative thymocytes and CD8αα intestinal intraepithelial lymphocytes.

11:10 **Michael Borchers**, Associate Professor, Department of Internal Medicine
Effects of Cigarette Smoking on Pulmonary Immune Function

11:30 **Lunch**

Session V Session Chair: David Hildeman

12:30 **Hitesh Deshmukh**, *Assistant Professor, Division of Neonatology and Pulmonary Biology*
Commensal enteric bacteria induce accumulation of innate lymphoid cells in the lungs and reduces susceptibility to pneumonia.

12:50 **Courtney Jackson**, Graduate Student, Division of Immunobiology
Pro-inflammatory immune response of premature infants exposed to intrauterine inflammation

1:10 **Matthew Alder**, *Instructor, Division of Critical Care Medicine*
A potential novel role for olfactomedin 4 in sepsis

1:30 - 2:30 Awards And Closing Remarks - David Hildeman and Harinder Singh

Speaker Abstracts

Michael Jordan - Associate Professor
Division of Immunobiology

Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy.

Mutations in the LRBA gene (encoding the lipopolysaccharide-responsive and beige-like anchor protein) cause a syndrome of autoimmunity, lymphoproliferation, and humoral immune deficiency. The biological role of LRBA in immunologic disease is unknown. We found that patients with LRBA deficiency manifested a dramatic and sustained improvement in response to abatacept, a CTLA4 (cytotoxic T lymphocyte antigen-4)-immunoglobulin fusion drug. Clinical responses and homology of LRBA to proteins controlling intracellular trafficking led us to hypothesize that it regulates CTLA4, a potent inhibitory immune receptor. We found that LRBA colocalized with CTLA4 in endosomal vesicles and that LRBA deficiency or knockdown increased CTLA4 turnover, which resulted in reduced levels of CTLA4 protein in FoxP3(+) regulatory and activated conventional T cells. In LRBA-deficient cells, inhibition of lysosome degradation with chloroquine prevented CTLA4 loss. These findings elucidate a mechanism for CTLA4 trafficking and control of immune responses and suggest therapies for diseases involving the CTLA4 pathway.

Andrew Patterson - Graduate Student
Division of Immunobiology - Kasper Hoebe

Gimap5-regulated inhibition of GSK3 is essential for CD4+ T cell proliferation and peripheral tolerance

The survival and activation of host immune cells is tightly regulated to ensure robust immune responses towards pathogens, while still maintaining immune tolerance towards self-antigens. A disruption in the pathways governing this regulation can lead to loss of immunological tolerance or loss of immune cell function and survival. Ultimately this may lead to autoimmunity and/or severe immune deficiencies. We have previously shown that the GTPase of immunity-associated protein (Gimap)5 plays a key role in the regulation of lymphocyte survival and activation. Gimap5sph/sph lymphocytes fail to proliferate in response to antigen-receptor stimulation and show increased rates of activation-induced cell death. Importantly, Gimap5sph/sph mice exhibit a reduced number of regulatory T cells, while their immunosuppressive function is progressively lost. Ultimately, this results in CD4+ T cell-dependent colitis driven by gut-derived microbial antigens leading to wasting disease and early morbidity. Our recent work shows T cells are characterized by dysregulation of glycogen synthase kinase-3 (GSK3). The GSK3 target cMyc, which is required for optimal T cell activation and proliferation, fails to accumulate in Gimap5sph/sph CD4+ T cells upon TCR stimulation. Interestingly, pharmacologic inhibition of GSK3 activity restores both cMyc accumulation and CD4+ T cell proliferation. Additionally, in vivo pharmacological or genetic inhibition of GSK3 activity in Gimap5sph/sph mice protects against CD4+ T cell loss and the development of colitis, demonstrating that inhibition of GSK3 activity is impaired in the absence of Gimap5. This constitutes a novel regulatory pathway essential for CD4+ T cell activation.

Carolyn Rydyznski - Graduate Student

Center for Autoimmune Genomics and Etiology - Stephen Waggoner

Is bigger really better: consequences of natural killer cell suppression of the germinal center

A major goal of vaccination is the induction of class-switched, high-affinity, pathogen-specific antibodies, which typically arise from germinal center (GC) reactions. There is a pressing need to understand the factors that restrict the GC in order to design improved vaccines. We found that natural killer (NK) cells restrict GC development by suppressing expansion of follicular helper T cells (Tfh) after virus infection. However, our ability to interrogate qualitative changes in antigen-specific B cells, including mutation and affinity maturation of immunoglobulin, is limited in virus infection. Therefore, we employed a hapten-carrier immunogen, NP-KLH (with alum), that stimulates a T cell-dependent NP-specific IgG1 response. NK-cell depletion prior to immunization resulted in increased numbers of Tfh cells, GC B cells, and NP-specific IgG1 class-switched GC B cells within lymphoid tissues. At early time points after immunization, depletion of NK cells stimulated increased levels of high-affinity NP-specific antibodies in sera relative to control mice. Similarly, ablation of NK cells enhanced the early differentiation of plasmablasts. Our current experiments are focused on single-cell sequencing the NP-specific immunoglobulin heavy chain in sorted B cells from control and NK cell-deficient mice in order to assess the effect of NK cells on the accumulation of somatic mutations during a GC reaction. We will also examine mice at later time points to determine the effect of NK cells on B-cell memory and recall responses. To date, out results provide evidence that NK cell suppression of the GC constrains the quality of humoral responses after infection or vaccination.

Jared Klarquist - Graduate Student
Division of Immunobiology - Edith Janssen

STING promotes immunity towards dying cells

Adaptive immune responses to antigens released by dying cells play a critical role in the development of autoimmunity, allograft rejection, and spontaneous as well as therapy-induced tumor rejection. Although cell death in these situations is considered sterile, various reports have implicated type I IFNs as drivers of the ensuing adaptive immune response to cell-associated antigens. However, the mechanisms that underpin this type I IFN production are poorly defined. Here we show that dendritic cells (DCs) can uptake and sense nuclear DNA released by dying cells to induce type I IFN. Remarkably this molecular pathway requires STING but not TLR or NLR function and results in the activation of IRF3 in a TBK1-dependent manner. DCs are shown to depend on STING function in vivo to efficiently prime IFN-dependent CD8+ T cell responses to tumor antigens. Furthermore, loss of STING activity in DCs impairs the generation of follicular helper T (Tfh) and plasma cells as well as anti-nuclear antibodies in an inducible model of systemic lupus erythematosus (SLE). These findings suggest that the STING pathway could be manipulated to enable the rational design of immunotherapies that enhance or diminish anti-tumor and autoimmune responses, respectively.

Shouxiong Huang - Assistant Professor
Department of Environmental Health

Structures and functions of human CD1-associated self lipids

Cellular lipids presented by human CD1 proteins are controlling elements for the development and responses of lipid-reactive T cells in homeostasis, inflammation, and microbial infections. Whereas a few self and microbial lipids for T cell activation have been discovered, the comparative nature of many self lipids binding to four human CD1 proteins and detecting lipid-reactive T cells has not been characterized. We established a comparative lipidomic platform to profile the cellular lipids bound to recombinant human CD1a, CD1b, CD1c, and CD1d proteins during their egress through the secretory pathway. The unbiased lipidomic analysis using separating liquid chromatography combined with sensitive time-of-flight mass spectrometry supports that human CD1 proteins broadly survey cellular lipids through the capture of more than one thousand molecularly distinct phospholipids, lyso-phospholipids, ether-linked phospholipids, sphingolipids, and glycosphingolipids. In an overlapping manner, eighty percent of CD1-associated lipids were detected from multiple CD1 proteins. Surprisingly, phosphatidylcholine and sphingomyelin with shorter alkyl chains favorably associate with CD1b and CD1c proteins with large groove volume in contrast to the association with CD1d protein, supporting a regulatory role of spacer lipids in rendering CD1b and CD1c antigen presentation. Our collision-induced dissociation mass spectrometry identified more than ten families of cellular lipids, including lipid families capable of activating or blocking T cell responses. Thus, the overlapping lipid profiles strongly support a conserve manner of CD1-lipid association and provide a resource to identify self antigens or non-permissive ligands for T cell responses. Functionally, cellular diacylglycerol enhances the presentation of mycobacterial glucose monomycolate to CD1b-restricted T cells and headless skin lipids activate CD1a-restricted T cells, potentially regulating immune responses in mycobacterial infections and tissue inflammation. Meantime, environmental pollutants adversely impact the lipid antigen presentation, posting the importance of lipid metabolites in regulating inflammatory responses in environmentally insulted diseases.

Matthew Flick - Assistant Professor
Division of Experimental Hematology

The coagulation factor fibrinogen is at the interface of pathogen virulence and host defense following *S. aureus* infection

Staphylococcus aureus is a pervasive Gram-positive bacterium that underlies a spectrum of infections ranging from minor skin infections to serious, life-threatening conditions such as endocarditis, pneumonia, and sepsis. The emergence of antibiotic resistant strains of *S. aureus* (*ie,* MRSA and VRSA) has renewed interest in better defining mechanisms of pathogen virulence and host defense. The host coagulation system is at the interface of *S. aureus* virulence and host immune protection. Indeed, the coagulation factors prothrombin and fibrinogen are capable of promoting host protective immunity by directly engaging and activating innate immune cells though cell surface receptors. At the same time, a remarkable number of *S. aureus* virulence factors target host coagulation system components, including two distinct staphylocoagulases that form proteolytically-active procoagulant complexes with host prothrombin and multiple fibrin(ogen) binding proteins. Considering these disparate activities a fundamental question is whether, on balance, host coagulation system activity predominantly supports host defense or *S. aureus* virulence. *Our over-arching hypothesis is that host prothrombin and fibrinogen are context-dependent determinants of host defense/pathogen virulence whose contribution is dictated by the path of infection and tissue microenvironment.* In support, we find that mice that express constitutively low circulating levels of prothrombin (10% of normal) or that are fibrinogen-deficient have improved survival following intravenous *S. aureus* infection (*ie,* bacteremia), but these same mice have significantly worsened survival following an intraperitoneal infection (*ie*, peritonitis). Mechanistic studies have highlighted that fibrinogen-dependent pathogen virulence following intravenous infection is linked to clumping factor A, a *S. aureus* virulence factor that supports fibrinogen binding. In contrast, key elements of fibrinogen-dependent host defense following peritonitis infection include the thrombin-driven fibrin polymer formation, fibrin engagement of leukocytes through the integrin receptor a_Mb_2, and peritoneal macrophage function. Current work in our laboratory centers on further defining the molecular mechanisms of thrombin/fibrinogen-dependent pathogen virulence and host defense following bacteremia and peritonitis challenge as well as defining the contribution of host coagulation system components to *S. aureus*-mediated skin infection and endocarditis.

Hansraj Bangar - Post-Doctoral Fellow
Division of Infectious Diseases - David Haslam

Intestinal stem cell injury caused by Clostridium difficile is averted by Bacteroidales-induced crypt defenses

Clostridium difficile infection (CDI) results from a complex interplay between the pathogen, resident intestinal microbes, and innate host defenses. Altered commensal microbial ecology from antibiotic exposure is the most common risk factor for CDI, whereas crude fecal microbiota transplant remains the most effective treatment. Identifying individual species of protective commensals and establishing the mechanism by which they protect is urgently needed. Here we show that bacteria from the order Bacteroidales protect against CDI by inducing antimicrobial peptides that prevent C. difficile access to intestinal crypts and protect against stem cell injury. Metagenomic analysis revealed that resistance to CDI among genetically identical mice was consistently linked with the presence of individual enteric Bacteroidales species. In susceptible hosts without commensal Bacteroidales, fatal colitis paralleled C. difficile penetration into crypts, causing injury to stem cells that are required for epithelial repair. Oral administration of a mixture of four Bacteroidales species (Bacteroides uniformis, Bacteroides ovatus, Parabacteroides merdae and Parabacteroides distasonis) efficiently blocked C. difficile crypt entry and silenced intestinal inflammation. Complementary loss-of-function studies using mice deficient in Reg3□ demonstrated this crypt-localized antimicrobial protein is essential for Bacteroidales-mediated protection against CDI. Establishing the identity and protective host pathways induced by commensal bacteria that protect against C. difficile colitis represents an important step toward targeted bacteriotherapies replacing crude feces in treatment and prevention of CDI.

Kate Carroll - Graduate Student
Division of Immunobiology - Jonathan Katz

Prolongation of the Remission Period and Reversal of New Onset Type 1 Diabetes with the Selective Elimination of Autoreactive T Cells

Organ-specific autoimmune diseases such as Type 1 Diabetes (T1D) are principally mediated by autoreactive T lymphocytes targeting self-antigens, such as those from insulin-producing pancreatic beta cells. The rapid proliferation and concomitant genomic stress that these autoreactive T cells undergo during their active immune response to self-antigens affords a unique opportunity for their elimination. We have found that autoreactive T cells have a distinct apoptotic sensitivity as a result of intrinsic DNA damage response (DDR) both in vitro and in vivo upon activation. This sensitivity can be manipulated with small molecule drugs that selectively eliminate these pathogenic cells. This can be accomplished with the use of G2/M cell cycle checkpoint inhibitors, which synergize with small molecule inhibitors that regulate the tumor suppressor protein p53, the master regulator of the cell's response to genomic stress. The use of these inhibitors in combination selectively eliminates activated autoreactive T cells while leaving naïve and regulatory T cell populations largely untouched. When such combination therapy is applied at the onset of T1D (≥200 mg/dL blood glucose), we significantly prolong the remission period and reverse new onset T1D in the Nonobese Diabetes (NOD) mouse. Thus, the targeted manipulation of p53 and cell cycle checkpoints may represent a new therapeutic modality with significant translational potential for diverse immune-mediated diseases.

Yrina Rochman - Instructor
Division of Immunobiology

TSLP acts in concert with IL-4 to induce a robust Th2 state with pathogenic potential

Pathogenesis of allergic diseases including asthma is strongly associated with robust responses of allergen-specific Th2 cells, which produce high levels of IL-4, IL-5, IL-9, and IL-13. Despite evidence for pathogenic Th2 cells in the induction and propagation of allergic disorders, signals required for differentiation of such cells *in vivo* remain largely unknown. Thymic stromal lymphopoietin (TSLP) is a cytokine that is expressed upon epithelial injury, dysfunction or infection and is strongly implicated in the pathogenesis of atopic dermatitis (AD) and asthma. TSLP acts on innate and adaptive immune cells including dendritic cells, ILC2s, basophils, and CD4 T cells. TSLP signals through JAK1/JAK2/STAT5 pathway and triggers IL-4 production by CD4 T cells. We show that TSLP acting in concert with IL-4 induces more robust expression of IL-4, IL-5, and IL-13 by human and mouse CD4 cells. This more potent effector state appears to be stably programmed in memory Th2 cells. As part of molecular mechanism, we demonstrate that TSLP and IL-4 signals induce distinctive genome wide alterations in activating histone modification (H3K27Ac). We propose that TSLP acts in coordination with IL-4 to generate a more potent Th2 state that could underlie persistence and propagation of allergic disorders.

Sara Stoffers - Graduate Student

Division of Immunobiology - Ian Lewkowich

Dissecting Molecular Mechanisms of Synergy Between IL-13 and IL-17A in Severe Asthma

Rationale: IL-17A is elevated in mice with more severe asthma, and correlates with disease severity in humans. We hypothesize that signaling events activated downstream of IL-17RA engagement (TRAF6, NF-κB, C/EBPβ, p38) enhance IL-13-induced STAT6 phosphorylation (pSTAT6) and gene expression, exacerbating IL-13-driven asthma pathology. Methods: Synergy was assessed in primary human cells, TRAF6-deficient splenic DCs, NIH/3T3 cells, and wild-type or IL-17RA-/- pulmonary fibroblasts co-cultured in Transwells. Medium was supplemented with IL-13 (100 ng/ml), IL-17A (100 ng/ml) or IL-13+IL-17A in the presence or absence of inhibitors of NF-κB (CAY10512), Erk (U0126), p38 (SB203580), or C/EBPβ (transfection with C/EBPβ-specific siRNA) prior to cytokine stimulation. Results: IL-17A enhanced IL-13-driven pSTAT6 levels in primary human cells, and splenic DCs from TRAF6-deficient mice. Transcriptional synergy was absent in murine IL-17RA-/- fibroblasts and could not be rescued following co-culture with wild-type cells. Inhibition of Erk had no effect on transcriptional synergy, while inhibition of NF-κB and C/EBPβ only partially attenuated IL-17A-mediated enhancement of IL-13 responses. By contrast, p38 inhibition completely abrogated transcriptional synergy, but did not alter IL-17A-mediated enhancement of pSTAT6. Conclusions: IL-17A enhances IL-13 activity in humans and mice, but IL-17A-mediated enhancement of IL-13-driven pSTAT6 and gene expression are regulated by distinct mechanisms. Partial attenuation of transcriptional synergy in the absence of NF-κB and C/EBPβ, and complete abrogation in the absence of p38, suggests that NF-κB and C/EBPβ activation occurs downstream of p38. IL-17A-mediated enhancement of pSTAT6 is regulated by a mechanism independent of p38 and TRAF6, suggesting that this feature of IL-13/IL-17A synergy is regulated by yet unknown signaling intermediates.

Shannon Rapovy - Graduate Student
Division of Infectious Diseases - Joseph Qualls

Contribution of L-arginine biosynthesis for antimycobacterial T cell function

L-Arginine is necessary for T cell function. Diminished L-arginine triggers T cell hypo-responsiveness, which the immune system exploits to down-regulate T cell function. Arginase 1 (Arg1)-expressing macrophages consume extracellular L-arginine, depleting the L-arginine pool available for T cell utilization. Arg1 regulates host responses to Mycobacterium tuberculosis (TB) infection, and its activity correlates with decreased T cell function. Yet, T cells can synthesize L-arginine from L-citrulline, leading us to question the importance of L-citrulline metabolism in T cells during infection. Host defense during TB infection is inhibited in mice lacking L-citrulline metabolism in hematopoietic cells, yet the role of L-citrulline metabolism during anti-mycobacterial T cell responses remains unknown. We hypothesize that L-citrulline metabolism enhances mycobacteria-specific T cell function. We first investigated the effects of L-citrulline availability on T cell function in vitro. Our data show that L-citrulline metabolism augments T cell proliferation by counteracting the effects of Arg1 activity in M. bovis BCG-stimulated macrophages. Employing novel mouse models that lack L-arginine synthesis in immune cells, we observed mycobacteria-specific T cells overcome limited L-arginine availability by mechanisms in addition to intrinsic L-citrulline metabolism. Taken together, we demonstrate L-citrulline enhances mycobacteria-specific T cell proliferation in vitro, warranting further investigation in infected mice lacking L-citrulline metabolism in T cells.

Joerg Koehl - Professor
University of Lübeck

Immunoregulation of inflammation in allergy and infection - the International Research Training Group between Cincinnati and Lübeck

This is a collaborative proposal between the University of Lubeck/Research Center Borstel In Lubeck, Germany and the University of Cincinnati/Cincinnati Children's Hospital, Cincinnati, OH. The major goal of this proposal is to establish an international network of training and research projects that aim at identifying novel pathways that initiate, maintain and resolve chronic infectious and allergic diseases. The research program is divided into two project areas: *(A) Humoral and cellular pathways of allergic inflammation; and (B) Immunoregulation of infection driven inflammation*. The doctoral researchers will benefit from the broad spectrum of *in vitro* systems and *in vivo* models of inflammatory diseases offered by the IRTG and the expertise of the IRTG researchers in both the US and Germany within the fields of allergy and infection research. The research program is supplemented by a structured qualification and cross-border supervisory concept with defined training modules that integrates student exchanges between the German and the US partner institutions. The qualification concept comprises an IRTG-specific seminar that integrates visiting researchers, jointly held courses, a yearly retreat, an international symposium and individualized educational as well as mentoring programs. This framework should convey skills to the doctoral researchers that qualify for the domestic and the international job market within a period of three years.

Simon Hogan - Associate Professor
Division of Allergy and Immunology

Immune- intestinal epithelial Interactions in health and disease

The goal of the Hogan Laboratory is to understand the immune-intestinal epithelial interactions during homeostasis and how alterations in these pathways predispose to the development and maintenance of chronic inflammatory diseases such as food allergy and anaphylaxis, infection-induced diarrheal diseases, cystic fibrosis (CF) and inflammatory bowel disease (IBD).

We take a multidisciplinary approach that integrates in vitro and in vivo models and cell and epithelial biology, transport physiology and mucosal immunology to 1) define fundamentals of epithelial barrier function; 2) understand the role of the epithelial barrier in regulating other mucosal processes, e.g. immune responses; 3) identify immune pathways that modulate epithelial barrier function and how these pathways alter susceptibility and severity to disease; and 4) develop novel approaches to correct barrier dysfunction and restore health.

Maria Fields - Post-Doctoral Fellow
Division of Immunobiology - Senad Divanovic

Role of the IL-17 Axis in the Progression of Non-alcoholic Fatty Liver Disease.

Obesity is a primary risk factor for the development of non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease in the world. Although the etiology of NAFLD is multifactorial and remains largely enigmatic, it is well accepted that inflammation is a central component of NAFLD pathogenesis. Despite its clinical and public health significance, the critical immune signaling pathways and mechanism(s) underlying disease progression remain undefined. Obesity has been shown to favor Th17 bias. Notably, high fat diet (HFD) fed IL-17RA-/- mice were protected from glucose dysmetabolism and hepatocellular damage, despite increased obesity and hepatic steatosis. To determine the critical IL-17RA signaling ligand for this uncoupling, IL-17A-/-, IL-17F-/- and WT mice were fed HFD or chow. Despite similar induction of obesity, adiposity and hepatic steatosis both IL-17A-/- and IL-17F-/- mice were protected from the development of glucose dysmetabolism compared to WT controls. However, only IL-17A-/-, but not IL-17F-/-, mice had a significant protection from obesity-driven hepatocellular damage. Additionally, WT mice on HFD exhibit robust infiltration of pathogenic Th17 cells (CD4+CXCR3highCCR4lowIL-17+TNF+) in the WAT and in the liver. Infiltration of pathogenic Th17 cells correlated with development of glucose dysmetabolism and hepatocellular damage. Importantly, transfer of liver resident CD4+CXCR3highCCR4low cells to WT mice significantly accelerated and exacerbated hepatocellular damage. Our findings suggest that obesity-driven activation of the IL-17 axis is central to the development and progression of obesity-sequeale including, glucose dysmetabolism and NAFLD. Thus, modulation of the IL-17 axis may lead to development of novel therapeutic strategies for treating obesity-associated sequelae.

Jeremy Kinder - Graduate Student
Division of Infectious Diseases - Sing Sing Way

Durability of pregnancy-primed regulatory T cell memory

Maternal exposure to paternal antigens expressed by the fetus during in utero development requires expansion of immune suppressive regulatory T cells (Tregs) to accommodate these immunologically foreign tissues. The importance of maternal Treg expansion is demonstrated in humans where blunted accumulation of maternal Tregs is associated with many pregnancy complications (i.e. preeclampsia, recurrent miscarriage) and in animals where even partial depletion of maternal Tregs cells during pregnancy disrupts fetal tolerance and triggers fetal wastage. Furthermore, using a mating strategy that transforms defined model antigens into surrogate fetal antigen we have shown a selective expansion (>100-fold) of maternal Tregs with fetal specificity occurs during pregnancy. Here we show postpartum retention of expanded maternal Tregs with fetal specificity requires ongoing stimulation from microchimeric fetal cells retained in mothers after pregnancy. Remarkably, while numerical retention of memory Tregs declined following depletion of fetal microchimeric cells, Tregs with fetal-specificity exhibited similarly accelerated expansion kinetics during secondary pregnancy and provided increased resiliency against pregnancy complications induced by disruptions in fetal tolerance regardless of microchimeric cell depletion. These results highlight the durable protective memory features for maternal Tregs with fetal specificity in mice that parallel human partner-specific protective benefits of prior successful pregnancy against complications in subsequent pregnancies.

Heping Xu - Post-Doctoral Fellow
Division of Immunobiology - Harinder Singh

Visualizing germinal center B cell fate dynamics at single cell transcriptome resolution

During a T-dependent humoral immune response, germinal center (GC) B cells undergo AID-mediated somatic hypermutation (SHM) of immunoglobulin genes, interaction with T follicular helper (Tfh) cells and clonal expansion, eventually resulting in the selection of cells that express high affinity B cell receptors (BCRs) for later memory or plasma cell differentiation. To achieve affinity maturation, GC B cells must rapidly change their cellular states alternating between proliferation and antigen capture and presentation to Tfh cells. We analyzed the molecular programs underlying these states as well as that of transitioning intermediates by single cell RNA -Seq. Profiling of antigen specific GC B cells during the peak of the response, revealed two sharply demarcated genomic states characterized by mitotic or antigen presentation gene expression modules. Importantly, the analysis also uncovered two novel cellular states. One of these states expressed components from both the mitotic and antigen presentation modules and was further distinguished by genes involved in mitochondrial biogenesis and function, implying a unique metabolic demand. Furthermore, compared with the other groups, these cells appeared to manifest the shortest S phase, which is an important feature of GC B cells that have been selected for higher affinity BCRs. Thus we propose that induction of the mitochondrial machinery in a transition state is a fundamental feature of GC B cell dynamics, as cells bearing high affinity BCRs have to utilize it to meet the extreme metabolic requirements of accelerated cell cycles during clonal expansion.

Leah Kottyan - Assistant Professor
Center for Autoimmune Genomics and Etiology

11q13 is an allergic risk-locus that increases EoE risk and increases *LRRC32* expression

Eosinophilic esophagitis (EoE) is a chronic, food-driven, esophageal, inflammatory allergic disease characterized by marked eosinophil accumulation. Noncoding genetic variants at 11q13 have been associated at genome-wide significance in studies of allergic sensitization, asthma, allergic rhinitis, atopic dermatitis, and EoE suggesting that these loci contain variants that participate in the allelic regulation of a molecular pathway that is central to the etiology of allergic disease. We performed a genetic replication and fine-mapping study of the 11q13 locus in a cohort of 1118 subjects with and without EoE and replicated association at 11q13 in an independent cohort and performed a meta-analysis with published results and identified a striking linkage of EoE susceptibility with 11q13. *LRRC32* but not *EMSY* is expressed in an EoE-risk genotype-dependent manner in EoE patient biopsies. IL-13 treatment of esophageal epithelial cells induced 3-fold increased expression of LRRC32 but not EMSY. IL-13 increased H3K27ac marks across the EoE-risk loci. Molecularly, one of the most highly EoE-associated and replicated variants in 11q13 with overlapping H3K27ac marks differentially bound nuclear factors from esophageal epithelial cells. In conclusion, we have identified a potential molecular mechanism for increased EoE risk at 11q13 by differential nuclear factor binding to non-coding genetic variants leading to increased expression of *LRRC32*.

Kun-Po Li - Graduate Student
Division of Immunobiology - David Hildeman

Temporal expression of Bim limits the development of TCR+ double negative thymocytes and CD8αα intestinal intraepithelial lymphocytes.

Complex networks of intestinal intraepithelial lymphocytes (iIELs) function to promote intestinal health. One major subset of iIELs, so-called CD8αα T cells, plays an important role in promoting gut homeostasis, although the apoptotic factors that control their development and homeostasis remain unclear. Here, we found that T cell-specific expression of Bim during early, but not late, thymic development limits the levels of CD8αα T cells in the spleen and iIEL compartments. As IL-15 controls CD8αα cell homeostasis, and antagonizes Bim in other T cells, we examined whether the additional loss of Bim would restore CD8αα T cells in IL-15-/- mice. Strikingly, the additional loss of Bim restored splenic, but not intestinal, CD4-CD8 double-negative (DN) and CD8αα T cells in IL-15-/- mice. IL-15 signaling via Stat5 synergized with TCR signaling in peripheral DN T cells to increase expression of CD8α. Further, adoptive transfer of splenic DN T cells gave rise to CD8αα cells in vivo. Combined, these data show that Bim functions temporally to promote the development of CD8αα precursors in the thymus. Moreover, Bim limits IL-15-driven homeostasis of CD8αα cells in the secondary lymphoid organs; but does not restrict their IL-15-driven maturation that is critical for intestinal homeostasis.

Michael Borchers - Associate Professor
Department of Internal Medicine

Effects of Cigarette Smoking on the Pulmonary Immune System

COPD is now the third leading cause of death and its economic burden is measured by the billions of dollars in the United States. Moreover, COPD exacerbations caused by viral infection are significant contributors to disease progression and morbidity as well as healthcare cost. A prevailing assumption is that CS is a toxicant that broadly inhibits leukocyte function. However, a growing body of literature suggests that this perspective is too narrow and that CS exposure can augment specific immune pathways. NK cells exhibit potent cytotoxic effects towards virally infected or transformed cells and have a considerable capacity to elaborate cytokine/chemokines. Cytokine subsets and a balance of inhibitory and activating surface receptors control NK cell function. Of the activating receptors, NKG2D is best characterized due to its ability to overcome inhibitory signals and induce cytolysis and mediator release. Few, and often contradictory data, exist related to the effects of smoking and NK cell function. We have demonstrated a clear association between NKG2D ligand expression on lung epithelium and COPD in both humans and mice. Moreover, we have shown that NKG2D is necessary and sufficient for the development of COPD pathologies and airflow limitation. Our data also demonstrate that NKG2D is necessary for inflammation and airspace enlargement in COPD-viral exacerbation model and identify a role for a subset of toll-like receptors in the control of NKG2D activation and COPD pathology.

Hitesh Deshmukh - Assistant Professor
Division of Neonatology and Pulmonary Biology

Commensal enteric bacteria induce accumulation of innate lymphoid cells in the lungs and reduces susceptibility to pneumonia.

Neonatal period is characterized by rapid colonization by enteric commensal bacteria. Disruption of commensal bacteria during this developmental window is associated with increased susceptibility of neonates to pneumonia. Here, we report that intestinal commensal bacteria direct the accumulation of RORγt expressing innate lymphoid cells (ILC3) in the neonate lung (ILC3). ILC3 elaborate interleukin (IL)-22 which in turn protect neonatal mice from infection to *Streptococcus pneumonia*. The postnatal accumulation of ILC3 in the lungs is regulated by mucosal dendritic cells, which imprint the expression of chemokine receptor (CCR) 4 on ILC3. CCR4 contributes to ILC3 lung imprinting. Disruption of commensal bacteria impairs the ability of ILC3s to migrate into the lungs not only during homeostasis but also in response to *Streptococcus pneumonia*, thus rendering neonatal mice susceptible to pneumonia. Reconstitution of enteric commensal bacteria restored the expression of CCR4 on the ILC3, restored the ability of ILC3 to migrate into the lungs and improved neonate's resistance to pneumonia.

Courtney Jackson - Graduate Student
Division of Immunobiology - Claire Chougnet

Pro-inflammatory immune response of premature infants exposed to intrauterine inflammation

Neonates, especially those born prematurely are highly susceptible to infections along with an increased frequency of intestinal, respiratory, and neurological inflammatory complications. A leading contributor to premature birth is in utero inflammation; which may localize to the maternal compartment (chorioamnionitis), or become more extensive (funisitis). However, how in utero inflammation exposure impacts neonate's developing immune system remains poorly characterized. We examined the cellular response at the 1st, 2nd and 4th week of life of preterm babies (<28 weeks). Expression of RORC ($p=0.02$) at baseline and IFNγ ($p=0.08$) mRNA after a brief stimulation were increased in the blood of funisitis-exposed infants, compared to unexposed infants. This difference was present at the first week, and persisted over the 4 weeks of analysis. Interestingly, chorioamnionitis exposed neonates were similar to unexposed infants. TBET, GATA3, TNF-α, IL-5, and IL-6 mRNA were not associated with inflammation. FoxP3 mRNA levels were not changed, but there was a persistent increase in the RORC to FoxP3 ratio ($p=0.01$) in funisitis-exposed infants. Because FoxP3 expression may not reflect regulatory T cell (Treg) function, we also analyzed the suppressive capacity of Treg from preterm infants (32-36 weeks). Treg from infants with severe chorioamnionitis (40% funisitis present) were less suppressive of effector T cell proliferation than those from unexposed infants. Severe chorioamnionitis exposure profoundly impacts the neonatal immune system, promoting Th1/Th17-like response while decreasing Treg suppressive function. Such immune disbalance could help neonates fight pathogens, but may contribute to the uncontrolled inflammatory responses that develop in premature infants.

Matthew Alder - Instructor
Division of Critical Care Medicine

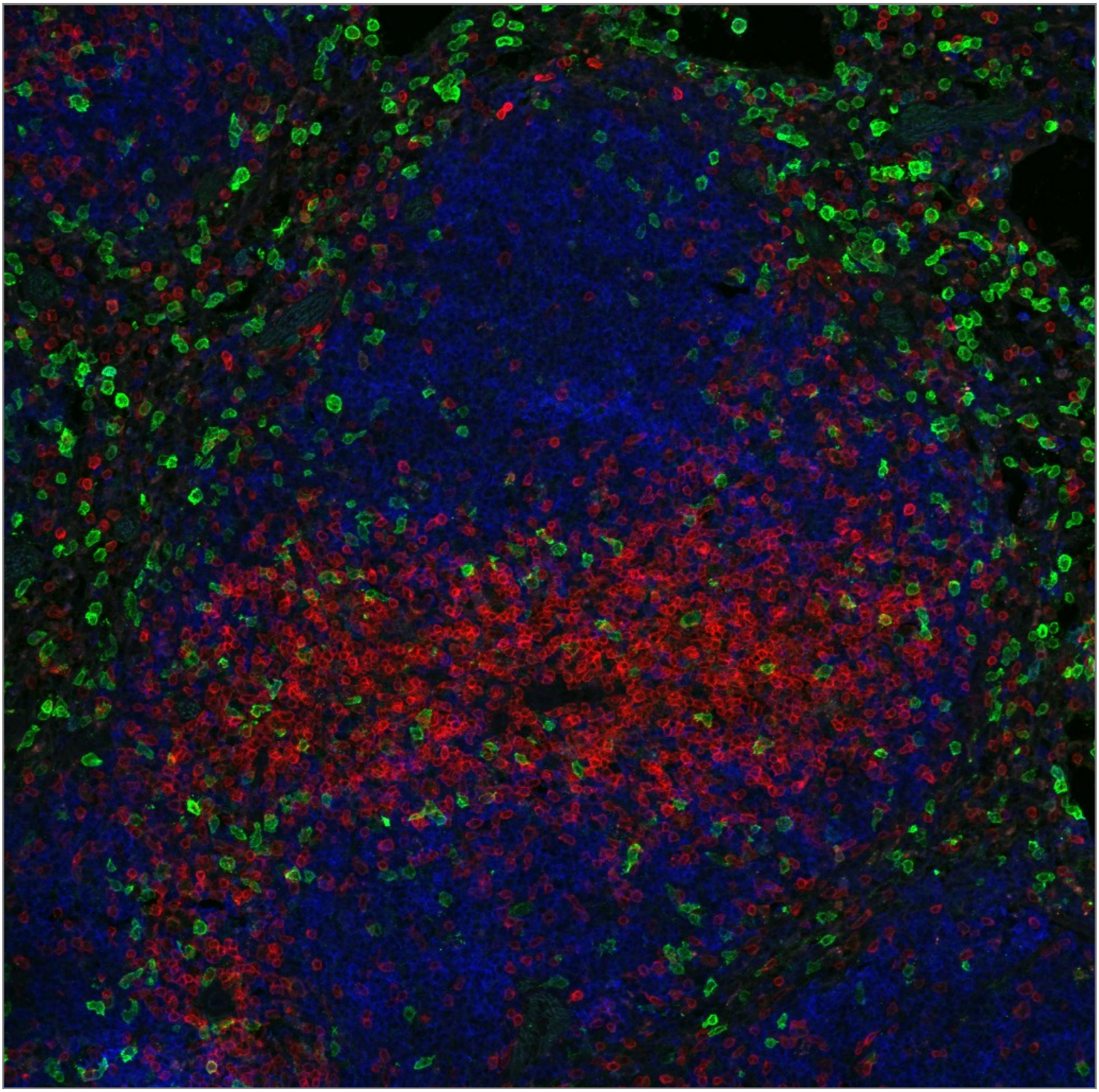
A potential novel role for olfactomedin 4 in sepsis

Sepsis is a major cause of morbidity and mortality and while much of the mechanism of sepsis pathology is understood, novel research may help understand heterogeneity within the syndrome. Through whole genome expression profiling of peripheral blood leukocytes from patients with sepsis we found olfactomedin 4 (OLFM4) as highly up-regulated in septic patients. OLFM4 is expressed in human neutrophils and is one of the few proteins to differentiate neutrophil subsets. Transcripts for OLFM4 were significantly up-regulated in non survivors compared to survivors. Increased protein levels in the serum of septic patients also correlated with complicated course from sepsis. Multiple regression analysis showed increased percent OLFM4 positive neutrophils independently correlates with complicated course in sepsis. Mice also show increased expression of OLFM4 during sepsis and like humans expression is primarily in neutrophils. OLFM4 marks a subpopulation of neutrophils and is up-regulated in patients with sepsis and expression correlates with disease severity. Mice with sepsis demonstrate expression of OLFM4 similar to humans suggesting it may be a potential model to determine the role of OLFM4 in sepsis.

Poster Abstracts

In alphabetical order

Odd numbered abstracts will present 7:30-8:15pm
Even numbered abstracts will present 8:30-9:15pm



Confocal image of murine splenic white pulp showing NK cell (green) localization into this region following viral infection with LCMV-Armstrong.

1 **Maha Almanan - Graduate Student**
Division of Immunobiology - David Hildeman

Type 1 regulatory T cells (Tr1) homeostasis and function in aging

Maha Almanan[1`3], Jana Raynor[1`3], Claire Chougnet[1`3], Surya Amarachintha[2`3], Kris Steinbrecher[2`3] and David Hildeman[1`3]

[1]Division of Immunobiology, [2]Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center. [3]College of Medicine, University of Cincinnati. OH

Type 1 regulatory T cells (Tr1) are a unique population of CD4+ FoxP3− that expresses high levels of IL-10, and have been defined based on their expression of CD49b and LAG-3. Despite the critical roles played by Tr1 cells in controlling T cell responses in autoimmunity and infection, the mechanisms underlying homeostasis of Tr1 cells remain unclear. Here, we investigated the homeostasis, phenotype and function of Tr1 cells with age. We found that Tr1 cells accumulated dramatically with age similar to FoxP3+ Treg. However, Tr1 cells produced more IL-10 per cell compared to FoxP3+ Treg. While aged Tr1 cells expressed significant levels of c-Maf, one of the Tr1 defining transcription factors, Tr1 cells were also heterogeneous in their expression of CD49b and LAG-3 in aged mice. Despite this heterogeneity, LAG-3 identified a population of aged Tr1 cells that potently inhibited T-cell proliferation in a partial IL-10-dependent manner. Strikingly, IL-6 was critical for Tr1 cell accumulation, as aged IL-6 KO mice showed a profound reduction in Tr1 cells. Combined, these data show that IL-6 promotes Tr1 accumulation with age and provide new insight into a novel T cell population that may contribute to age related immune suppression.

NIH grant (AG033057)

2 **Maha Almanan - Graduate Student**
Division of Immunobiology - David Hildeman

CD4+ Foxp3+ Regulatory T cells Control Effector T cell Responses to Murine Cytomegalovirus (MCMV)

Maha Almanan[1`2], Jana Raynor[1`2], Mei Wang[2`3], Claire Chougnet[1`2], Rhonda Cardin[2`3] and David Hildeman[1`2]

[1]Division of Immunobiology, [3]Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center. [2]College of Medicine, University of Cincinnati. OH

Human cytomegalovirus (HCMV) causes a persistent, lifelong infection. The virus persists in a latent state and undergoes intermittent subclinical viral reactivation. Such reactivation drives the accumulation of "inflationary" T cells that suppress viral replication. While T cells are critical to maintain control of infection, the factors that prevent T cells from driving complete viral elimination remain unclear. Here, we investigated the role of regulatory T cells (Treg) in a mouse model of persistent CMV infection using Foxp3-diphtheria toxin receptor (DTR) mice. Foxp3-DTR mice were infected with murine CMV (MCMV) and then Treg were depleted via administration of DT several months later. Treg depletion resulted in a dramatic increase in the numbers of MCMV-specific CD4 and CD8 T cells in the spleen, which were also functional, producing significantly higher levels of IFN-γ and IL-2 compared to non-Treg depleted mice. Strikingly, Treg depletion led to a significant decrease in splenic viral load and profoundly delayed reactivation of latent MCMV from spleen explants in vitro. Unexpectedly, ablation of Treg led to a significant increase in viral load in the salivary gland that was accompanied by augmented local IL-10 production. Combined, these data show that Treg have organ-specific effects on suppression of T cell activation; these effects result in divergent control of MCMV infection in the spleen versus the salivary gland. These data provide new insight into the role of regulatory T cells in controlling the reactivation of latent MCMV infection.

NIH grant (AG033057)

3 Nurit Azouz - Post-Doctoral Fellow
Division of Allergy and Immunology - Marc Rothenberg

Loss of SPINK7 in Esophageal Epithelial Cells Unleashes a Pro-inflammatory Response Characterized by Excessive Cytokine Production and Loss of Barrier Function

Nurit P Azouz1, Demetria M Michael2, Laetitia Furio3, Hovnanian Alain3 and Marc E Rothenberg1

1. Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, 2. Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center. 3.Université Paris Descartes-Sorbonne.

Rationale: The serine peptidase inhibitor kazal-type 7 (SPINK7) is markedly down-regulated in eosinophilic esophagitis (EoE), an inflammatory TH2 type immune disease of the esophagus. We hypothesized that SPINK7 has a key role in the propagation of EoE. Methods: We used an in vitro system of human esophageal epithelial cells that were subjected to air-liquid interface (ALI) to induce squamous cell differentiation. Cells were stably transduced with either non-silencing control or SPINK7 shRNAs. The integrity of the epithelium was examined by barrier function assays complemented by histological and ultrastractural analyses and immune-fluorescence of junctional proteins. Protease activity, transcriptional alterations and identification SPINK7's downstream targets were assessed. Last, cytokines and chemokines secretion was analyzed after SPINK7 gene silencing. Results: we determined that depletion of SPINK7 resulted in transcriptional alterations including down-regulation of other SPINKs and unleashes trypsin-like serine protease activity. In vitro, SPINK7 inhibits the serine protease- kallikrein 5 that is known to be involved in the regulation of the skin barrier. Furthermore, we demonstrated that SPINK7 gene silencing is sufficient for induction of architectural alterations in junctional complexes and a profound loss of the "zipper-like" structures in between the epithelial cells that characterize the healthy esophageal epithelium and is missing in EoE. These alterations followed by impaired barrier function. In addition, loss of SPINK7 induces a series of pro-inflammatory cytokines and Chemokines. Conclusions: we suggest that deficiency of SPINK7 results in uncontrolled proteases activity which is a novel checkpoint for regulating pro-inflammatory esophageal epithelial responses.

4 Kristina Betz - Graduate Student
Division of Gastroenterology, Hepatology and Nutrition - Sean Moore

Undernutrition and Altered Gut Secretory IgA Synergistically Increase Bacterial Burdens in the Mesenteric Lymph Nodes

Kristina J. Betz, Elizabeth A. Maier, Simon P. Hogan, Sean R. Moore

CCHMC Division of Gastroenterology, Hepatology and Nutrition Research CCHMC Division of Allergy & Immunology

Introduction & Background Ubiquitous among children in developing countries, environmental enteropathy (EE) is a sub-clinical condition characterized by gut inflammation, malabsorption and growth faltering. EE's histopathological features include blunted small intestinal villi, crypt hypertrophy, and lamina propria inflammation with increased intestinal permeability, gut-to-blood bacterial translocation, and systemic immune activation. Increased serum IgA and decreased fecal IgA have been previously described in both undernourished children and mice. These dynamics are consistent with decreased expression of the polymeric immunoglobulin receptor (pIgR), the only known receptor to transport dimeric IgA to mucosal surfaces. Specific Experimental Aim: We wanted to determine the contributions of secretory IgA and undernutrition to the development of environmental enteropathy. We hypothesized that a combination of altered secretory IgA in combination with undernutrition in a murine model would be able to recapitulate key histopathological features of human EE. Methods & Design: To evaluate the interaction of undernutrition and secretory IgA in the gut, we randomized dams of DOL10 C57BL/6 or pIgR null mice to either a control diet (CD) or an isocaloric regional basic diet (RBD, low fat and protein). Pups were weaned to their dam's diet at DOL21 and sacrificed at 8 weeks of age. Mice were observed for changes in weight and health. 4 hours prior to sacrifice, mice were orally gavaged with 200ul of 70mg/ml FITC-dextran. Whole small intestine was collected for histology and Ussing chamber studies, mesenteric lymph nodes were collected for aerobic bacterial counts, and serum was collected for FITC detection. Results: All mice on the RBD showed failure to thrive, with no additional weight loss observed in pIgR knockout mice. No group differences were detected in serum FITC-dextran. Additionally, no group differences were detected in transepithelial resistance or short circuit current by Ussing chamber measurements. However, we did find significantly greater aerobic bacterial growth from the mesenteric lymph nodes of the undernourished mice lacking pIgR as compared to the well-nourished pIgR knockout mice ($P<0.05$). Conclusions & Future Directions: Undernutrition and lack of secretory IgA increase bacterial burdens in the mesenteric lymph nodes in the absence of histological changes or standard measures of intestinal barrier function.

Phase II Grand Challenges Explorations Grant from the Bill & Melinda Gates Foundation NIGMS Medical Scientist Training Program T-32 GM063483

5 Taylor Brooks - Graduate Student
Center for Autoimmune Genomics and Etiology - Stephen Waggoner

NK cell crosstalk with myeloid suppressor cells promotes chronic infection

Taylor Rey Brooks, Stephen Noel Waggoner

Center for Autoimmune Genomics and Etiology (CAGE), Cincinnati Children's Hospital Medical Center

Increasing evidence points to natural killer (NK) cell-mediated regulation of adaptive immunity as an important contribution to the pathogenesis of chronic viral infections. We previously demonstrated that NK cells facilitate persistent infection and prevent fatal immunopathology by promoting the functional exhaustion of virus-specific T cells. Immunosuppressive myeloid cells also accumulate during persistent virus infections of mice and humans and have been shown to contribute to immune exhaustion during chronic LCMV infection in mice. Importantly, we found that the frequencies of suppressive CD11b+ myeloid cells in the spleen, bone marrow, and blood were substantially reduced in NK cell-depleted mice relative to control animals during chronic LCMV infection. This change was more evident in the Ly-6Chi/Ly-6G- monocytic subset than the Ly-6Cint/Ly-6G+ neutrophilic subset of suppressive myeloid cells. We hypothesized that this aborted expansion in NK cell-depleted mice was due to decreased levels of inflammatory cytokines necessary for the expansion and survival of myeloid cells. In fact, we observed a significant reduction in the expression levels of TNFα, CCL2, and IL-6 in sera of NK cell-depleted mice relative to non-depleted control animals at multiple time points of persistent LCMV clone 13 infection. Thus, NK cells contribute to the establishment of an inflammatory cytokine milieu as well as the emergence of immunosuppressive myeloid cells during chronic virus infection. This relationship could potentially be targeted in order to enhance immune function in human diseases where NK cell crosstalk has been implicated in disease pathogenesis, including HIV infection and cancer.

This work was supported by an ΑΩΑ Carolyn L. Kuckein Student Research Fellowship (T.R.B.) and grants from The Ellison Medical Foundation, the NIH (DA038017), and Cincinnati Children's Research Foundation (S.N.W.).

6 Laura Brungs - Graduate Student
Center for Autoimmune Genomics and Etiology - Sue Thompson

Fine mapping and functional investigation of IL2RB-associated genetic variants in Juvenile Idiopathic Arthritis (JIA)

Laura A Brungs, Kameron Shams, Matt Weirauch, Leah Kottyan, Halima Moncrieffe, and Susan Thompson

Introduction: Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatic disease affecting approximately 1 in 1,000 children. JIA is a complex genetic trait and encompasses a wide spectrum of clinical heterogeneity. Variants spanning more than 25 loci have been reported as associated with susceptibility to JIA (oligoarticular and polyarticular RF- subtypes) at genome-wide levels of significance ($P<5x10-8$). The mechanism by which these associated variants contribute to disease risk is not known. Aim: To investigate allele-specific differences in transcription factor binding as a mechanism for JIA genetic risk. Methods: Fine mapping studies of the IL2RB locus using data imputed to the 1000 Genome dataset were performed using a cohort of subjects with and without JIA to identify variants likely causal for JIA susceptibility. Bayesian analysis and informatic predictions of transcription factor binding sites provided candidate SNPs for testing. Variation in protein binding from nuclear extract was investigated using electrophoretic mobility shift assays (EMSAs) and DNA affinity purification assays (DAPAs) of those identified variants. Results: An independent genetic effect at the IL2RB locus was found to include 5 SNPs that together explain 95% of the posterior probability. EMSAs and DAPAs confirm differential binding of Jurkat and HeLa nuclear extracts to 2 of 5 risk variants, rs228960 and rs2284033. Risk and non-risk variants of rs228960 are predicted to bind differentially to the Sp family of transcription factors, and ongoing research efforts are in progress to identify specific proteins that bind differentially to risk and non-risk variants of rs228960 and rs2284033.

P30-AR047636 and P01-AR048929

7 Julie Caldwell - Post-Doctoral Fellow
Division of Allergy and Immunology - Marc Rothenberg

CDH26 is an integrin-binding immunomodulator involved in allergic inflammation

Julie M. Caldwell1, Margaret H. Collins2, Katherine A. Kemme1, Joseph Sherrill1, Ting Wen1, Mark Rochman1, Emily M. Stucke1, Lissa Amin1, Haitong Tai1, Philip E. Putnam3, Aleksey Porollo4, J. Pablo Abonia1, Marc E. Rothenberg1

1Division of Allergy and Immunology, 2Division of Pathology, 3Division of Gastroenterology, Hepatology, and Nutrition, 4Division of Rheumatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Eosinophilic gastrointestinal disorders (EGID) encompass a spectrum of diseases defined by abnormal accumulation of eosinophils in particular GI tract segments including the esophagus (eosinophilic esophagitis, EoE) or stomach (eosinophilic gastritis, EG). To understand the molecular pathogenesis of EG, we performed global transcript analysis and identified genes differentially expressed in gastric tissue of EG patients compared to control individuals. CDH26, an unstudied cadherin family member, represented the most highly overexpressed transcript in EG biopsies (20.9-fold compared with healthy controls, $p < 0.01$), and it was the only cadherin differentially regulated in both EG and EoE. IL-13, which was markedly overexpressed in EG patient gastric tissue (375-fold, $p < 0.01$), induced CDH26 expression in GI epithelial cells in vitro. CDH26 mediated calcium-dependent cellular adhesion, exhibited homotypic interaction, and additionally interacted with beta-, alpha-, and p120-catenins. Moreover, CDH26 interacted with integrins α4 and αE. Jurkat T cells, which express α4β1, bound CDH26-hIgG1-Fc in an integrin α4-dependent manner. Activation of CD4+ T cells by anti-CD3 antibodies was inhibited when the cells were cultured in the presence of plate-bound CDH26-hIgG1-Fc, as assessed by expression of cell surface molecules indicative of T cell activation as well as secretion of IL-2 and IL-4. In conclusion, we have identified CDH26 as an inducible cadherin, a receptor for select α-integrins, and as an inhibitor of human T cell activation.

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8 Stacey Cranert- Post-Doctoral Fellow
Center for Autoimmune Genomics and Etiology - Stephen Waggoner

An innate helping hand for B cells on the margin

Stacey A. Cranert1, Michael T. Moran1,2, and Stephen N. Waggoner1,2

1Division and Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center 2Immunology Graduate Program, University of Cincinnati

Natural Killer (NK) cells are innate cytotoxic lymphocytes involved in controlling viral infections and tumor growth. Our lab has demonstrated that NK cells suppress germinal center B cell responses and humoral immunity during acute infection. In contrast, we find that a subset of B cells present in the marginal zone (MZ) of the spleen is sustained by NK cells during chronic infection with the clone 13 strain of lymphocytic choriomeningitis virus (LCMV). Chronic infection caused a transient reduction in the number of MZ B cells that was dependent on CD8 T cells. In the absence of NK cells, loss of MZ B cells during chronic infection was more severe and sustained for a longer period of time than in control mice. Confocal staining revealed a near complete disappearance of the MZ during infection of NK cell-deficient mice. Intriguingly, CD8 T cell depletion alone did not restore the MZ B-cell compartment in NK cell-depleted mice, but combined depletion of both CD4 and CD8 T cells largely prevented the loss. We hypothesize that inflammation or toxicity associated with enhanced T-cell responses during chronic infection in the absence of NK cells causes a purging of MZ B cells. Since MZ B cells are important in immune defense against secondary bacterial infections and are also a major source of the immunoregulatory cytokine, IL-10, reversal of this NK and T cell-mediated phenomenon could prevent tissue pathology and reduce morbidity associated with chronic viral infection.

This project was funded by NIH grant DA038017, New Scholar Award from The Ellison Medical Foundation, and "Research Initiation Funds" and "Research Innovation and Pilot Funds" from the Cincinnati Children's Research Foundation. All flow cytometry data were acquired using equipment maintained by the CCHMC Research Flow Cytometry Core, which is supported in part by NIH grants AR47363, DK78392, and DK90971.

9 Jessica Doll - Graduate Student
Division of Infectious Diseases - Nancy Sawtell

REACTIVATION OF HERPES SIMPLEX VIRUS IN THE BRAINSTEM

Jessica R. Doll, Richard L. Thompson, Nancy M. Sawtell

University of Cincinnati College of Medicine, Department of Molecular Genetics, Biochemistry, and Microbiology- JRD and RLT Cincinnati Children's Hospital Medical Center, Department of Infectious Diseases- JRD and NMS

Herpes Simplex Virus (HSV) infects epithelial cells at the body surface and is then transmitted to innervating sensory neurons. Following orofacial infection with HSV, the virus enters neurons of the trigeminal ganglia (TG), which support both latent infection and periodic reactivation events for the lifetime of the host. HSV also spreads to the central nervous system (CNS) during acute infection and establishes latency in multiple regions of the brain, but reactivation of HSV in the CNS has been technically difficult to approach, so studies to date have largely focused on reactivation in the TG. A correlation has been found between HSV infection and Alzheimer's Disease (AD), however a mechanism whereby HSV reactivation induces pathology consistent with AD has only been postulated. Aggregates of β-amyloid characteristic of AD are believed to originate from damaged brainstem neurons that project to other regions of the CNS, therefore, our goal was to determine the frequency of HSV reactivation and the cell type(s) in which this occurs in the brainstem. We found the following: (i) a high frequency of reactivation in both TG and brainstem, (ii) viral protein expression in TG and brainstem neurons at 24 hours post-hyperthermic stress, and (iii) viral exit from latency in explanted brainstems and TG, showing that reactivation can occur at each site independently. Our findings suggest that HSV reactivation in the brainstem is more common than previously appreciated, warranting further study on the host response to reactivation and the long term effects of periodic reactivation events in the CNS.

NIH R01 AI093614 and NASA NNX13AO47G

10 Dirk Friedrich - University of Lübeck
Department of Infectious Diseases - George Deepe

Histoplasma capsulatum upregulates HIF-1α downstream targets and drives the glycolytic phenotype of human macrophages

Dirk Friedrich[1], Roger Fecher[2, 3], Inga Kaufhold[1], George S. Deepe[2], Jan Rupp[1]

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Histoplasma capsulatum (Hc) is a dimorphic fungus causing severe mycoses in immunocompromised patients. Inhaled spores switch into the pathogenic yeast phase and survive inside macrophages (MΦ) resulting in granuloma formation. Granuloma represent hypoxic environment in mammalian tissue. Under hypoxia, the hypoxia-inducible factor (HIF)-1α is stabilized and regulates cellular metabolism and immune response. Recently, we showed that the surface heat shock protein 60 (Hsp60) of Hc stabilizes HIF-1α in human MΦ. Therefore, we investigated the downstream targets of HIF-1α during Hc infection and the impact of Hc on the host cell metabolism in human MΦ.

Human monocyte-derived MΦ were infected with viable or heat killed Hc (MOI=5:1) and analyzed 24 h post infection (p.i.). We also treated MΦ with recombinant Hsp60 (rHsp60) for 6h under normoxia (21% O_2) or hypoxia (2% O_2) and inhibited Syk kinase with piceatannol. HIF-1α protein levels were assessed by western blot analysis before and after knockdown of HIF-1α via siRNA. We investigated GLUT1 and PDK1 gene expression via qRT-PCR. The metabolic profile of the host cells was analyzed using the Seahorse analyzer XF24.

Infection of human MΦ with Hc ($p<0.05$, $n=3$) stabilized HIF-1α. The inhibition of Syk kinase abrogated the accumulation of HIF-1α protein in rHsp60-treated MΦ. Further, the expression of GLUT1 and PDK1 ($p<0.05$, $n=3$) was upregulated. Knockdown of HIF-1α decreased GLUT1 ($p=0.051$, $n=5$) and PDK1 ($p=0.08$, $n=5$) expression. Treatment with heat killed Hc induced an elevation in the glycolytic activity of MΦ.

In this study, we demonstrated that *Hc* drives the activation of human MΦ by enhancing their glycolytic phenotype via HIF-1α. The impact of HIF-1α on intracellular survival of *Hc* will be addressed in future studies.

11 Daniel Giles - Graduate Student
Division of Immunobiology - Senad Divanovic

Environmental Modulation of the Immune Response Regulates NAFLD Pathogenesis

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Divisions of Immunobiology1, Allergy and Immunology3 and Pathology and Laboratory Medicine4 Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA. Departments of Molecular and Clinical Infectious Diseases2, University of Lübeck, Lübeck, Germany. Molecular Gastroenterology5, University Hospital Schleswig-Holstein, Campus Lübeck, Germany.

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease world-wide. Although the etiology of NAFLD is multifactorial and remains largely enigmatic, it is well-accepted that inflammation is central to NAFLD pathogenesis. However, the inflammatory signaling pathway(s) underlying disease progression remain under-defined. Further, animal NAFLD models fail to fully recapitulate human disease, representing a major knowledge gap—something possibly associated with environmental modulation of mouse physiology. Notably, housing mice within their thermoneutral zone significantly augments metabolism and immune activation. Thus, we hypothesized that thermoneutral housing, in combination with high fat diet (HFD)-stress, exacerbates NAFLD severity, and represents an improved model for interrogating inflammatory pathways central to NAFLD pathogenesis. To test this, C57BL/6 mice were housed in standard or thermoneutral conditions and fed chow or HFD. Thermoneutral housing augmented innate immune cell MHC and co-stimulatory molecule expression, TLR activation and Th17 axis induction. Notably, RNASeq analysis of immune cells revealed alterations in immune pathways central to cytokine production and leukocyte function. Further, HFD feeding combined with thermoneutral housing, compared to standard housing, altered the intestinal microbiome, and exacerbated intestinal permeability, hepatic bacterial translocation, hepatic Th17 infiltration and hepatocellular damage. Notably, gram-negative microbiota depletion, hematopoietic TLR4 deletion and IL-17 axis inactivation resulted in significant protection from NAFLD pathogenesis. In sum, our findings suggest that thermoneutral housing represents a novel NAFLD model that more closely resembles human disease. Further, our findings highlight the relevance of immune-gut-liver axis in NAFLD pathogenesis—something that may provide novel therapeutic targets for prevention of NAFLD.

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12 Michael Horwath - Graduate Student
Department of Infectious Diseases - George Deepe

Hif1α Controls Survival of Macrophages infected with Histoplasma capsulatum

Michael Horwath [1,2], Roger Fecher[1,2], George Deepe[3,4]

[1] Immunobiology Graduate Training Program, Cincinnati Children's Hospital/UCCOM. [2] Medical Scientist Training Program, UCCOM. [5] Division of Infectious Diseases, UCCOM. [6] Cincinnati Veterans Affairs Hospital.

BACKGROUND: *Histoplasma capsulatum* (*Hc*) is an environmental fungal pathogen that causes thousands of hospitalizations each year in the United States. Because *Hc* yeast is adapted for survival after phagocytosis, effective recruitment and activation of myeloid populations is essential for control of the disease. Recently, our laboratory demonstrated that mice deficient in myeloid cell expression of the hypoxia-inducible transcription factor, Hif1α, are highly susceptible to *Hc* infection. Here we investigate the phenotype of Hif1α -deficient macrophages infected with *Hc*, including gene expression, metabolism, and apoptosis.

PURPOSE: Determine the mechanism by which myeloid Hif1α contributes to control of *Histoplasma capsulatum* infection.

APPROACH: We used bone-marrow-derived macrophages from conditional knockout (*LYSM*$^{Cre/Cre}$ *HIF1a*$^{FL/FL}$) and control mice (*HIF1a*$^{FL/FL}$) to investigate gene expression and phenotype *in vitro*. First, we performed RNA-sequencing of *Hc*-infected and uninfected macrophages. This revealed upregulation of gene sets involved in glycolytic shift and apoptosis in infected control, but not HIF1a knockout, macrophages. We then confirmed these phenotypic differences using lactate measurement, Seahorse, and flow cytometry. Finally, we investigated the importance of several differentially regulated genes using small molecule inhibitors and siRNA.

CONCLUSIONS: Hif1α expression in macrophages is necessary for in glycolytic shift and apoptosis following *H. capsulatum* infection. HIF1a's pro-apoptotic effect may be exerted through *Egln3* and *Bnip3* genes, and may be important for clearance of *H. capsulatum* by reducing the reservoir of highly infected macrophages and limiting IL-10 production.

NIGMS Medical Scientist Training Program T-32 GM063483 NIAID R21 AI094971 Veterans Affairs I01 BX000717

13 Tony Jiang - Graduate Student
Division of Infectious Diseases - Sing Sing Way

Commensal enteric bacteria lipopolysaccharide impairs host defense against disseminated Candida albicans fungal infection

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Commensal enteric bacteria maintain systemic immune responsiveness that protects against disseminated or localized infection in extra-intestinal tissues caused by pathogenic microbes. However, since shifts in infection susceptibility after commensal bacteria eradication have primarily been probed using viruses, the broader applicability to other pathogen types remains undefined. In sharp contrast to diminished antiviral immunity, we show commensal bacteria eradication bolsters protection against disseminated *Candida albicans* fungal infection. Enhanced antifungal immunity reflects more robust systemic expansion of $Ly6G^{hi}Ly6C^{int}$ neutrophils, and their mobilization into infected tissues among antibiotic treated compared with commensal bacteria replete control mice. Reciprocally, depletion of neutrophils from expanded levels or intestinal LPS reconstitution overrides the antifungal protective benefits conferred by commensal bacteria eradication. This discordance in antifungal compared with antiviral immunity highlights intrinsic differences in how commensal bacteria control responsiveness for specific immune cell subsets because pathogen-specific $CD8^+$ T cells that protect against viruses were suppressed similarly after C. *albicans* and influenza A virus infection. Thus, positive calibration of antiviral immunity by commensal bacteria is counterbalanced by restrained activation of other immune components that confer antifungal immunity.

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14 Erik Karmele - Post-Doctoral Fellow
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Beyond (natural) killing: a new role for cytolytic machinery of innate lymphoid cells in regulating IgA

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Immunoglobulin A (IgA) is vital for the maintenance of homeostasis between the host and the intestinal microbiome. Antimicrobial IgA is secreted by IgA+ plasma cells that arise from germinal center (GC) reactions within gut-associated lymphoid tissues (GALT). Dysregulation of IgA production can lead to the improper clearance of commensal microbes, allowing for pathogenic strains to flourish and cause disease. Thus, a better understanding of the mechanisms involved in IgA production is critical. Our lab has previously shown that natural killer (NK) cells, a type of innate lymphoid cell (ILC), suppress GC responses and long-lived humoral immunity following systemic viral infection of mice. We now show that depletion of NK1.1-expressing ILCs in uninfected specific-pathogen free (SPF) mice results in enhanced numbers of T follicular helper and GC B cells in GALT. These GC responses against intestinal microbes stimulate the generation of IgA+ plasma cells and secretion of antimicrobial IgA, which were both elevated following ILC depletion. A major function of some ILCs, including NK cells, is the killing of targets via release of cytolytic granules containing perforin and granzymes. In perforin-deficient SPF mice, depletion of ILCs did not result in enhanced GALT GC responses or IgA production. Similar results were observed in beige mice, which have a defect in cytolytic granule release. This novel immunoregulatory role of ILCs within GALT is likely to significantly impact homeostasis of the intestinal microbe community and suggests ILCs as a potential target of interventions that aim to enhance efficacy of mucosal vaccines.

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15 Andrey Kartashov - Application Specialist
Division of Allergy and Immunology - Artem Barski

BioWardrobe: an integrated platform for analysis of epigenomics and transcriptomics data

Andrey V. Kartashov and Artem Barski

High-throughput sequencing has revolutionized biology by enhancing our ability to perform genome-wide studies. However, due to lack of bioinformatics expertise, modern technologies are still beyond the capabilities of many laboratories. Herein, we present the BioWardrobe platform, which allows users to store, visualize and analyze epigenomics and transcriptomics data using a biologist-friendly web interface, without the need for programming expertise. Predefined pipelines allow users to download data, visualize results on a genome browser, calculate RPKMs (reads per kilobase per million) and identify peaks. Advanced capabilities include differential gene expression and binding analysis, and creation of average tag -density profiles and heatmaps. BioWardrobe can be found at http://biowardrobe.com.

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16 Durga Krishnamurthy - Post-Doctoral Fellow
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Suppression of established food allergy with anti- FcεRIα mAb in humanized mice

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Food allergy is mediated predominantly by mast cells, FcεRI and IgE. We recently demonstrated that rapid desensitization with a hamster monoclonal antibody (mAb) to mouse FcεRIα (MAR-1) safely suppresses IgE-mediated anaphylaxis). We have now expanded these studies by evaluating whether the same approach can suppress established disease in a BALB/c mouse model of food allergy. Treatment of egg white-allergic mice with MAR-1 suppressed shock (hypothermia), diarrhea, and mast cell degranulation (MMCP1 secretion) responses to oral gavage with egg white. Although continued suppression of food allergy by repeated MAR-1 injections was eventually limited by development of mouse IgG Abs to hamster IgG, this could be prevented by injecting mice with anti-CD4 mAb at the time of the initial MAR-1 injection. To evaluate the same approach in a humanized mouse model of food allergy, transgenic mice that express human rather than mouse FcεRIα were sensitized intratracheally and intraperitoneally with egg white plus egg yolk. These mice developed diarrhea, but not shock, after repeated administrations of egg white plus egg yolk by oral gavage. Rapid desensitization with hourly doubling i.p. doses of a mouse anti-human FcεRIα mAb, AER-37 (maximum dose, 50 μg), suppressed diarrhea in most treated mice 48 hours later. These mice also developed less severe IgE-mediated shock than mock-desensitized mice when challenged intravenously with anti-mouse IgE mAb. These observations indicate that even partial rapid desensitization with anti-human FcεRIα monoclonal has beneficial effect on established food allergy.

17 Natalia Kunz - University of Lübeck
Department of Surgery - Charles Caldwell

Unexpected anti-inflammatory effects of xenogenic Protein-Nucleic Acid-Complexes (PNACs)

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It is well-established that foreign antigens are highly immunogenic causing complement activation leading to inflammation and necrosis. However, several studies demonstrate a regulatory and even therapeutic potential of certain xenogenic proteins. Our own studies showed that xenogenic Protein-Nucleic Acid-Complexes (PNACs), although containing not only proteins but also foreign DNA and RNA, modulate the immune response towards an anti-inflammatory direction by increasing IL4 expression and promoting wound healing in an autoimmune skin blistering disease model. To characterize the mode of action of this unexpected effect, we studied which cells are ingesting PNACs and if PNACs are modulating their cytokine expression. To test, whether the effect of PNACs is due to their xenogenic origin, we established the production of allogenic murine PNACs. The role of PNACs in vivo was addressed using a model of Leishmania major infection. We found that xenogenic PNACs are preferentially ingested by macrophages. Strikingly, PNACs ingested by zymosan-activated macrophages decrease the expression of pro-inflammatory cytokines by 50% and increase the expression of anti-inflammatory IL10 14-fold. Allogenic murine PNACs showed similar, albeit smaller effects, indicating the anti-inflammatory effect of PNACs is at least partly defined by their specific protein-composition. In vivo, the resulting anti-inflammatory micromilieu favored the polarization of T cells towards a Th2 phenotype as judged by increased IL4 expression in lymph nodes and higher levels of Leishmania major-specific IgG1 in the blood of infected C57BL/6 mice. Apparently, despite being subject of xenogenic rejection protein-Nucleic Acid-complexes have unexpected anti-inflammatory and therapeutic capacities which clearly require further investigations.

18 Celine Lages - Post-Doctoral Fellow
Division of Gastroenterology, Hepatology & Nutrition - Alex Miethke

Hepatic CD4+ responses and initiation of biliary injury in mdr2 knockout mice depend on dendritic cell costimulation

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T lymphocytes are implicated in initiation of cholangiopathies like biliary atresia or primary sclerosing cholangitis (PSC). Dendritic cells (DCs) modulate expansion and activation of T lymphocytes through CD80/86 interaction with CD28. Blockade of CD80-86/CD28 with CTLA4-IgG treatment improve autoimmune diseases. We hypothesize that DC costimulation is critical for T lymphocyte activation during the initiation of bile duct injury in mdr2-/- mice, a model for "toxic bile"-induced PSC. Frequency of hepatic DCs, more specifically myeloid mDC, was increased in mdr2-/- mice at 8 and 14 days of life compared to mdr2+/+ ($p<0.05$). Frequency of DCs expressing CD80 and CD86 was doubled at day 8 of life in mdr2-/-. CTLA4-Ig treatment (20 mcg/g of mdr2-/- male mice every other day between day 7 and 14 of life) was associated with a decrease of hepatocellular injury at day 15 of life (measured by plasma ALT levels: $p=0.02$) and biliary injury (measured by bile duct profiles/portal tract and by cholangiocyte proliferation/bile duct profile: $p<0.02$). Moreover, CTLA4-Ig treatment reduced hepatic CD4+ T cell proportion ($p=0.02$) and maturation (in CTLA4-Ig vs hIgG, respectively: naïve/CD4: 52.14 vs 6.61%, $p=0.001$; effector/CD4: 10.82 vs 60.90%, $p<0.001$). However, the proportion of CD8+ T, NK or NKT cells was not affected. DCs are important for initiation of T cell-mediated hepatobilary injury during the first 2 weeks of life in mdr2-/- mice. In particular, differentiation of hepatic CD4+ lymphocytes during this time depends on DC-mediated costimulation. The effector function of these CD4+ lymphocytes in this model of PSC deserved further investigations.

19 Christopher Link - University of Lübeck
Division of Immunobiology - Fred Finkelman

Treg expansion as a strategy to attenuate sensitization to egg allergens

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Intra-tracheal (i.t.) inoculation of unprimed Balb/c mice with egg white (EW) plus egg yolk plasma (EYP; liquid fraction of egg yolk) induces allergic airway disease and sensitizes mice for development of food allergy in response to subsequent oral gavage (o.g.) with these antigens. The induction of regulatory T cells (Tregs) through IL 2 therapy has recently emerged as potential clinical therapeutic strategy for suppressing allergy. We aim to determine whether expanding the Treg population by administering IL 2/anti-IL 2 complex (IL 2C; prepared by mixing 1 µg rmIL 2 with 5 µg of JES6-1 mAb, which increases IL 2 half-life but blocks IL 2-binding to IL 2Rβ) can limit respiratory and intestinal egg allergy. Balb/c wild-type mice were initially injected i.p. with 200 µL of IL 2C to systemically expand the Treg population. Mice received one additional injection of IL-2C/week throughout the experiments. Mice that were initially treated with IL 2C or saline were sensitized i.t. to EW plus EYP 3x/week for two weeks. Sensitization was verified by non-invasive measurement of airway hyperresponsiveness to methacholine. Sensitized mice were then challenged o.g. with 50 mg of EW plus EYP 3x/week. Rectal temperature and the development of diarrhea were assessed for 1 hour after each o.g. challenge. IL 2C treatment expanded the Treg population, increased in vivo IFN-γ secretion and attenuated airway responsiveness to methacholine. The last effect was partially blocked by treatment with anti-IFN-γ mAb. We are currently evaluating how treatment with IL 2C and/or anti-IFN-γ mAb affects diarrhea and hypothermia responses to oral antigen challenge.

20 Ke Liu - Graduate Student
Center for Autoimmune Genomics and Etiology– John Harley

X chromosome gene Ddx3x is necessary for murine B-cell development

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Ddx3x belongs to the DEAD (Asp-Glu-Ala-Asp) box RNA helicase family and is ubiquitously expressed in various tissues. Previously, Ddx3x has been shown to participate in RNA metabolism, cell cycle control, apoptosis, and tumorigeneses. Ddx3x is often target of viral infections. Our lab previously showed that X chromosome dosage plays a role in female preponderance of systemic lupus erythematosus (SLE), with more X chromosomes there is an increased chance of getting SLE. We hypothesize that ddx3x escaping from X inactivation might contribute to the female preponderance of SLE. SLE is a humoral autoimmune disease in which B cell homeostasis is perturbed, so we are interested to learn the effect of ddx3x on B cell. We obtained ddx3x floxed mice and breed with Vav1-Cre mice in order to create ddx3x-Vav1 conditional knockout mice (Vav1ddx3x). Ddx3x expression was reduced 80% in the bone marrow male Vav1ddx3x mice. Hematopoietic deficiency of ddx3x in male mice is associated with a reduced proportion and absolute number of small pre-B cells, immature B cells and mature B cells in bone marrow. In spleen, the absolute frequency of all B cells was reduced wherein marginal zone B cell numbers were maintained relative to follicular B cells. We demonstrate that ddx3x deficiency affects B cell development resulting in a loss of B cells in bone marrow and periphery which indicate a role in SLE pathogenesis. Our ongoing work is to investigate the role of ddx3x in B cell function and the mechanism underlying loss of B cells.

21 Xiaoming Lu - Graduate Student
Center for Autoimmune Genomics and Etiology - John Harley

Lupus riskvariant increases pSTAT1 binding and decreases ETS1 expression

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Genetic variants at chromosomal region 11q23.3, near the gene ETS1, have been associated with systemic lupus erythematosus (SLE), or lupus, in independent cohorts of Asian ancestry. Several recent studies have implicated ETS1 as a critical driver of immune cell function and differentiation, and mice deficient in ETS1 develop an SLE-like autoimmunity. We performed a fine-mapping study of 14,551 subjects from multi-ancestral cohorts by starting with genotyped variants and imputing to all common variants spanning ETS1. By constructing genetic models via frequentist and Bayesian association methods, we identified 16 variants that are statistically likely to be causal. We functionally assessed each of these variants on the basis of their likelihood of affecting transcription factor binding, miRNA binding, or chromatin state. Of the four variants that we experimentally examined, only rs6590330 differentially binds lysate from B cells. Using mass spectrometry, we found more binding of the transcription factor signal transducer and activator of transcription 1 (STAT1) to DNA near the risk allele of rs6590330 than near the non-risk allele. Immunoblot analysis and chromatin immunoprecipitation of pSTAT1 in B cells heterozygous for rs6590330 confirmed that the risk allele increased binding to the active form of STAT1. Analysis with expression quantitative trait loci indicated that the risk allele of rs6590330 is associated with decreased ETS1 expression in Han Chinese, but not other ancestral cohorts. We propose a model in which the risk allele of rs6590330 is associated with decreased ETS1 expression and increases SLE risk by enhancing the binding of pSTAT1.

22 Katelyn Melgar - Graduate Student
Division of Experimental Hematology - Daniel Starczynowski

Novel small molecule FLT3 inhibitors for the treatment of FLT3-ITD AML

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FMS-like Tyrosine Kinase 3 (FLT3) is a receptor tyrosine kinase that promotes growth and survival of hematopoietic stem and progenitor cells. Mutations in the gene encoding FLT3 are found in 20-30% of acute myeloid leukemias (AML), with approximately 25% of all AMLs containing an internal tandem duplication (ITD) in the juxtamembranal region. FLT3-ITD AML is associated with poor prognosis, with a 5 year survival rate of approximately 15% as compared to 40% for wild-type FLT3 AML. As such, FLT3 inhibitors have been developed to treat FLT-ITD AML. Although the inhibitors show considerable efficacy in vitro and in animal models, clinical trials using FLT3 inhibitors as single agents have been underwhelming. Through chemical and structure-activity relationship studies, we have identified a novel class of FLT3 tyrosine kinase inhibitors. After initial biochemical and functional analysis, NCGC-2327 emerged as our lead compound, and further optimized with improved solubility, stability, and permeability properties suitable for in vivo utility (NCGC-1481). Both compounds have excellent selectivity against the kinase activity of FLT3-ITD and FLT-ITD D835Y at a subnanomolar concentration (IC50 <5.08 x 10-10 M). AC220, NCGC-2327, and NCGC-1481 treatment of primary human FLT3-ITD-mutant AML cells show EC50 at subnanomolar concentrations (0.5 nM, 0.4 nM, and 0.1 nM respectively) as determined by CellTiter Glo proliferation assays. To assess the ability of the compounds to induce apoptosis, we treated FLT3-ITD-mutant AML cells with AC220, NCGC-2327, NCGC-1481, or Crenolanib (a FLT3 inhibitor that can inhibit FLT3-ITD-D835Y) for 72 hours. NCGC-1481-treated cells showed the greatest levels of apoptosis (AnnexinV+) as compared to Crenolanib, AC220, and NCGC-2327 ($P < 0.01$). Consistent with the viability assays, NCGC-1481 treatment showed the greatest inhibition of leukemic progenitor function in methylcellulose. Relapse and resistance is a primary concern for patients treated with AC220, therefore we investigated leukemic subclonal resistance in vitro after treatment with Crenolanib, AC220, NCGC-2327, or NCGC-1481. FLT3-ITD-mutant AML cells were treated for 72 hours, washed and then allowed to recover in the absence of the compounds for one week. Subclonal recovery was assessed by measuring cell viability (AnnexinV+) and leukemic progenitor function (methylcellulose) for up to 1 week post treatment. NCGC-2327 and NCGC-1481 delayed, and in some instances prevented, subclonal recovery as compared to AC220 or Crenolanib treatment. NCGC-2327 and NCGC-1481 show comparable potency to current FLT3 inhibitors (i.e., AC220 and Crenolanib) in regards to inhibition of FLT3 signaling, proliferation, and induction of apoptosis in FLT3-ITD-mutant AML. However, NCGC-2327 and NCGC-1481 are exquisitely effective at preventing subclonal recovery of FLT3-ITD-mutant AML as compared to both AC220 and Crenolanib. Taken together, these findings suggest our novel FLT3 inhibitors show promise for the treatment of FLT3-ITD positive AML, and particularly for patients that have intrinsic and/or acquired resistance to FLT3 tyrosine kinase inhibitors.

23 Daniel Miller - Research Assistant
Center for Autoimmune Genomics and Etiology - Matthew Weirauch

Allele-specific binding of a viral protein to autoimmune disease-associated genetic variants

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Pathogenesis of disease involves complex interplay of both genetic and environmental risk factors. These risk factors have been well characterized for a variety of diseases, but the etiologies of many autoimmune diseases remain idiopathic. Certain environmental agents that increase disease susceptibility can be especially important in the context of specific genetic risk factors. Specifically, Epstein Barr virus (EBV) has suggestive ties to many autoimmune diseases and EBV infection is nearly ubiquitous in adults. The molecular mechanisms underlying these associations, however, remain unclear. Here, we demonstrate for the first time that differential binding of a viral protein to a disease-associated genetic variant can result in altered levels of host gene expression. We show that three genetic variants, associated with multiple sclerosis, systemic lupus erythematosus, and juvenile idiopathic arthritis, respectively, affect the binding of the EBV-encoded transcription factor (TF) Zta. Since Zta is a viral protein, and thus is potentially more amenable to safe manipulation, our findings open up a wide range of therapeutic possibilities for the many autoimmune diseases where EBV is thought to play a role.

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24 Cora Miracle - Undergraduate Student
Division of Allergy and Immunology - Marc Rothenberg

Dynamic Binding of IL-33 to Chromatin in Living Cells

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The fundamental property of nuclear proteins is their ability to constantly move with in the nucleus. The mobility is dependent on chromatin binding and measured by the rate of recovery of fluorescent bound proteins following photo-bleaching. In this study we focused on the chromatin binding properties of IL-33, focusing on the mobility of IL-33 in the nucleus. We concluded that IL-33 is highly mobile depending on its chromatin binding.

25 Michael Moran - Graduate Student
Center for Autoimmune Genomics - Stephen Waggoner

Moving in for the kill: natural killer cell migration in regulation of humoral immunity

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Natural killer (NK) cells are cytotoxic innate lymphocytes that promote immune defense against pathogens and tumors, and act as potent regulators of adaptive immunity. Notably, NK cells inhibit antiviral humoral immunity by restricting the magnitude of follicular helper T-cell (TFH) and germinal center (GC) B-cell responses in a perforin-dependent manner. This suppression occurs at an early stage of infection that coincides with anatomic distribution of NK cells within secondary lymphoid organs. Specifically, infection with lymphocytic choriomeningitis virus (LCMV) triggers transient NK cell redistribution from the vascularized red pulp into the white pulp of the spleen. Many of these NK cells localize at the border between T and B cell zones where cognate interactions between TFH and B cells facilitates development of GCs. Some NK cells also penetrate deep into the B cell follicle. We have investigated this phenomenon by utilizing an intravascular staining method for analysis by flow cytometry, as well as through confocal microscopy. In addition, we have observed a novel subset of NK cells expressing the B cell follicle homing receptor, CXCR5, which arises in the spleen at the same time point of LCMV that NK cells appear near B cell follicles. We hypothesize that this subset of NK cells utilizes CXCR5 to localize near TFH and B cells participating in the early steps of GC formation, allowing for cell-contact and perforin-dependent killing of one or more of these subsets as a means to repress the GC and subsequent development of protective antiviral humoral immune responses.

This project was funded by NIH grant DA038017, New Scholar Award from The Ellison Medical Foundation, and "Research Initiation Funds" and "Research Innovation and Pilot Funds" from the Cincinnati Children's Research Foundation. All flow cytometry data were acquired using equipment maintained by the CCHMC Research Flow Cytometry Core, which is supported in part by NIH grants AR47363, DK78392, and DK90971.

26 Nazanin Navabi - Post-Doctoral Fellow
Division of Immunobiology - Theresa Alenghat

An essential role for epithelial cell histone deacetylase 3 in intestinal host defense

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Intestinal microbial pathogens are a leading cause of diarrhea and mortality, particularly among children, however the molecular mechanisms regulating host susceptibility to infectious agents in the intestine is not well understood. Intestinal epithelial cell (IEC) expression of histone deacelylase 3 (HDAC3) is necessary to maintain healthy intestinal homeostasis in the presence of microbiota-derived signals. Therefore, to test whether IEC-intrinsic HDAC3 regulates responses to an intestinal pathogen, we employed *Citrobacter rodentium*, a natural enteric mouse pathogen that causes colitis similar to attaching and effacing *Escherichia coli* infection in humans. Mice with IEC-specific deletion of HDAC3 ($HDAC3^{\Delta IEC}$ mice) exhibit increased peak bacterial burdens and prolonged C. *rodentium*-induced intestinal pathology following infection. Similarly, targeted deletion of HDAC3 in IECs following C. *rodentium* exposure causes increased susceptibility to infection. C. *rodentium* induces IFNg production and clearance is dependent, in part, on IFNg expression. Interestingly, C. *rodentium* infected $HDAC3^{\Delta IEC}$ mice exhibit decreased induction of IFNg relative to control mice in the colonic tissue. Preliminary data indicate that decreased IFNg expression may largely reflect defective activation of T cells in the colonic lamina propria of $HDAC3^{\Delta IEC}$ mice. In addition, $HDAC3^{\Delta IEC}$ mice produce a thicker colonic mucus layer, which may exacerbate delayed clearance of C. *rodentium*. These results suggest that HDAC3 in IECs mediates epithelial-intrinsic host responses to intestinal pathogens as well as epithelial crosstalk with mucosal IFNg-producing T cells.

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27 Zubin Patel - Graduate Student

Center for Autoimmune Genomics and Etiology - John Harley & Leah Kottyan

Identification of SLE-associated risk variants in the STAT1-STAT4 locus and their effect on differential Transcription Factor Binding

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Systemic Lupus Erythematosus (SLE or lupus) is a chronic autoimmune disease with debilitating inflammation that affects multiple organ systems. The STAT1-STAT4 locus is one of the first and most highly replicated genetic loci associated with SLE risk. In this study, we aimed to identify all the SLE-associated common variants at this locus most likely to be causal and to further identify the biological mechanism mediating the increased disease risk. We genotyped 328 SNPs spanning the STAT1-STAT4 locus in 13,581 subjects representing four ancestral groups. We performed imputation and applied frequentist and Bayesian statistical analyses to identify the individual variants statistically most likely to causally increase lupus risk. We further used a separate larger African-American study to generate an Ancestry Informed Credible Set (AICS) of four variants. We computationally predicted differential transcription factor (TF) binding of AICS variants and identified the AT-hook family of TFs as a strong candidate for three of the four AICS variants. After identifying AT-hook family member HMGA1 as binding to rs11889341 through DNA Affinity Precipitation Assay (DAPA) followed by mass spectrometry, we confirmed binding of HMGA1 to two of four AICS variants with genotype-dependent binding by DAPA and Electrophoretic Mobility Shift Assay. In summary, we used large genetic datasets to identify a set of variants that are most likely to be causal for the STAT1-STAT4 association with increased lupus risk and identified a potential disease-risk mechanism in which HMGA1 differentially binds three genetic variants in a lupus-risk haplotype.

28 Bhesh Paudyal - University of Lübeck

Division of Immunobiology - Julio Aliberti

Small Lipid Mediator metabolism in tuberculosis: Targets for host directed therapy

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Small lipid mediators (SLM) are arachidonic acid (AA), eisosapentaeinoic acid (EPA) and docosahexaenoic acid (DHA) metabolites generated through various enzymes including phospholipases (PL), cycloxygenases (COX), lipoxygenases (LO). SLMs act in an autocrine and paracrine manner by binding to specific G-protein coupled receptors (GPCRs). Certain SLMs such as prostaglandins (PG) and leukotrienes (LT) have pro-inflammatory and neutrophil attracting properties, whereas others such as lipoxins (Lx) and resolvins (Rv) are anti-inflammatory as well as pro-resolving, respectively. The functions of SLM in the pathogenesis during *Mycobacterium tuberculosis* (Mtb) infection are not well understood.

Using ELISA and mass spectrometry (MS) based lipidomics, we have analyzed SLM production in lung homogenates from Mtb infected C57BL/6 (B6) and C3HeB/FeJ (C3H) mice. Specific inhibitors for SLM synthesis pathways such as the anti-inflammatory 5-lipoxygenase (LO) inhibitor, zileuton, have been used to specifically study the functions of individual SLM synthesis pathways. (Immuno-) histochemistry was employed to characterize numbers and types of immune cells as well as expression of eicosanoid receptors in the infection site.

Mtb infection led to a significantly increase in the pulmonary production of pro-inflammatory SLM in both strains when compared to uninfected mice. SLM levels remained elevated throughout infection indicating chronic lung inflammation. This notion is further supported by recruitment of inflammatory cells, i.e. eicosanoid receptor positive macrophages and neutrophils, to the infection site during the chronic phase. Oral administration of zileuton from day 1 post infection on, altered inflammatory responses, SLM profiles and disease outcome in Mtb infected C3H mice. Hence, zileuton can be a good candidate for host directed therapy (HDT) adjunct to antibiotic treatment of Mtb infection.

In summary, global analysis of SLM profiles allows identification of putative targets for immune modulating HDT strategies accompanying classical anti-Mtb therapy.

29 Sarah Potter - Post-Doctoral Fellow
Division of Reproductive Sciences - Tony DeFalco

Role of Testicular Macrophages in Germline Stem Cell Development

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Unchecked proliferation of primordial germ cells (PGCs), the germline progenitor population that gives rise to adult spermatogonial stem cells (SSC), is thought to contribute to the most common form of cancer affecting adolescent and young adult males, testicular germ cell tumors (TGCTs). The fetal somatic testis microenvironment (the PGC "niche") controls PGC proliferation, and alteration of this niche plays an important role in TGCT development. Currently, the PGC niche, including cell types and critical genes, in the context of TGCT progression has not been well defined. We show that macrophages with immunosuppressive characteristics are present in large numbers in the mouse testis during mid-gestational development (when TGCTs arise); our previous analyses also revealed that macrophages drive vascularization and the formation of testis architecture. Additionally, we demonstrated that testicular macrophages phagocytose germ cells found outside the PGC niche. Together, these data suggest that macrophages not only promote fetal organogenesis, but also influence PGC localization. In adults, we observed that there are two functionally distinct macrophage populations within the testes, with differential gene expression according to stage and cell type. We also described the ability of macrophages to influence SSCs, through regulating their differentiation and/or proliferation. These studies reveal the importance of macrophages in controlling spermatogenesis. Overall, these data suggest that macrophage–PGC interactions are required during normal development and macrophages regulate the niche microenvironment during adult maintenance, thus opening up a new area of investigation into how immune cells establish and maintain stem cell dynamics and potential.

Cancer Free Kids (PI: Sarah Potter) and March of Dimes (PI: Tony DeFalco)

30 Jeremy Riddell - Graduate Student
Center for Autoimmune Genomics and Etiology - Matthew Weirauch

Computational Prediction of Transcription Factor Composite Motifs

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Computational prediction of functional transcription factor (TF) binding sites in cis-regulatory regions is of critical importance for the development of comprehensive gene regulatory models. Position weight matrices (PWMs) have proven to be a robust method for encapsulating TF binding specificities, yet most PWM-derived motifs possess a low information content leading to an overabundance of predicted binding sites. Composite motifs (CMs) representing multimeric cooperative element (CE) binding sites for two or more TFs provide a larger, more informative footprint. We hypothesize that CEs favoring discreet stereospecific TF binding configurations with respect to their relative order, orientation, and spacing serve as markers for cis-regulatory regions. We therefore developed the Combinatorics of Stereospecific Motif Orientation and Spacing (COSMOS) algorithm to identify enriched CMs present in input DNA sequences. When we applied COSMOS to an H3K27ac time-course ChIP-seq dataset from LPS-stimulated B cells, the top two CM predictions matched the ETS-FOX CE (EFCE, $p<1e-44$) and the ETS-IRF CE (EICE, $p<1e-42$). The EFCE has been identified as an enhancer-specific fate driver for endothelial cell types and possibly others, while the EICE has been previously shown to play an important role during B cell differentiation. We also found differential enrichment for many CMs at varying timepoints, implying functionally distinct roles for these binding sites. By applying COSMOS to existing ChIP-seq datasets for additional cell types and antibody targets, we will extend the number of known CEs as well as improve understanding of their role in driving gene network reprogramming and CE-dependent cell fate decisions.

Systems Biology and Physiology

31 Cesar Rueda - Post-Doctoral Fellow
Division of Immunobiology - Claire Chougnet

HDL mediated survival of Human Regulatory T cells.

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High-density lipoprotein-cholesterol (HDL) and its major protein constituent Apolipoprotein A-I (ApoA-I) is broadly considered as an anti-inflammatory mediator. ApoA-I has been shown in murine models to increase the proportion of regulatory T-cells (Treg) in lymph node after subcutaneous injection. However, the underlying mechanisms remain unclear. We hypothesized that HDL promotes survival of human Treg. Herein, we showed that exposure to HDL and ApoA-I, but not LDL, significantly increased the absolute number of Treg and reduced the percentage of Annexin-V+/7AAD+ Treg. In contrast, HDL did not alter naïve or memory CD4+ T-cells. Mechanistically, we found that Treg bound, uptake and stored high amounts of HDL compared to the other CD4+ T-cell subsets. HDL increased Treg mitochondrial activity parameters, such as basal respiration, maximal respiration and spare respiratory capacity. Addition of etomoxir (ETX), an inhibitor of fatty acids (FA) oxidation, blocked the HDL-mediated increase of Treg survival and its effect on mitochondrial activity. Taken together, our studies demonstrate that Treg have singular metabolic requirements that differ from naïve and memory CD4+ T-cells. Treg can internalize HDL from their microenvironment as a source of energy to activate FA β-oxidation. These results also suggest a new immunomodulatory mechanism effect of HDL and ApoA-I, through enhanced Treg survival.

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32 Jeffrey Rymer - Graduate Student
Division of Allergy and Immunology - Marc Rothenberg

Subcellular Localization of Calpain-14 in Human Esophageal Epithelial Cells

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Rationale: Eosinophilic esophagitis (EoE) is an allergic inflammatory disease of the esophagus. An esophageal specific, intracellular protease called calpain-14 (CAPN14) was shown to be associated with EoE both genetically and by increased expression in esophageal biopsies from EoE patients. Yet, its function and contribution to disease remains to be elucidated. In this study, we investigated subcellular localization of CAPN14 in human esophageal epithelial cells. Methods: Immunofluorescence and biochemical fractionation were performed on phorbol myristate acetate (PMA) and ionomycin treated immortalized human esophageal epithelial cells (EPC2) stably overexpressing CAPN14 grown in submerged culture. Fractionation was also performed on untransduced EPC2 cells grown at the air-liquid interface (ALI) and on primary esophageal epithelial cells grown in submerged culture. Results: CAPN14 was detectable in whole cell lysates from transduced, but not untransduced, EPC2 cells. Fractionation revealed ~70% of CAPN14 to be in the cytoplasm, with ~10% detectable in the membrane and ~20% in the nucleus in undifferentiated EPC2 cells grown in submerged culture. However, following differentiation into a stratified squamous epithelium, endogenous CAPN14 was mainly localized in the nucleus. Immunofluorescent staining of EPC2 cells activated with PMA and ionomycin showed changes in CAPN14 localization from cytoplasm to the nucleus and then to the plasma membrane after 30 and 180 minutes, respectively. Conclusions: CAPN14 is localized to the cytoplasm, membrane, and nucleus in human esophageal epithelial cells. Following cellular activation (PMA/ionomycin), CAPN14 shows a dynamic distribution, most notable by its presence in the nucleus, consistent with a key cellular function, yet to be described.

33 Ankur Saini - Post-Doctoral Fellow
Division of Immunobiology - Harinder Singh

NICE: a novel genomic regulatory DNA element that directs cooperative assembly of NFAT and IRF4 complexes during lymphocyte activation

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Composite DNA regulatory elements are a special set of genomic sequences as they direct the cooperative binding of two or more transcription factors in stereo-specific configurations. The transcription factor IRF4, which regulates lymphocyte activation and differentiation, is rather unusual in that it has been shown to bind two distinct classes of composite DNA elements termed EICE and AICE. The former element promotes cooperative binding with Ets family members PU.1 or Spi-B whereas the latter with AP-1 family members BATF and JunB. Given that IRF4 has been shown to functionally cooperate with NFAT family transcription factors in activated B and T lymphocytes we used a computational strategy to search for NFAT:IRF4 composite elements within genomic regulatory regions that are marked with the activating histone modification H3K27Ac. We have identified a novel NFAT-IRF4 (NICE) composite element, which promotes the cooperative assembly of recombinant NFATc2 and IRF4. Importantly, protein-DNA complexes containing NFATc1 or c2 and IRF4 that are induced by antigen receptor signaling are detected in nuclear extracts of activated B and T lymphocytes. We are currently determining if genes that contain regulatory regions with the NICE motif are co-targeted as well as co-regulated by NFAT family members and IRF4. This involves ChIP-Seq experiments along with RNA-Seq analysis of activated lymphocytes lacking IRF4 or NFAT family members. It is likely that these newly discovered transcription factor complexes regulate key aspects of lymphocyte activation, proliferation and differentiation.

34 Natallia Salei - University of Lübeck
Division of Immunobiology - Edith Janssen

Apoptotic cells enhance the survival of *Leishmania major* in neutrophil granulocytes

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Neutrophils are the first leukocytes that migrate to the site of infection, encounter, engulf, and kill pathogenic microorganisms. However, certain pathogens such as *Leishmania major* (*L. major*) can survive within neutrophils. At cutaneous sites of *L. major* infection freshly recruited neutrophils encounter apoptotic cells. Since neutrophils can engulf apoptotic cells, we studied how phagocytosis of apoptotic cells by human neutrophils affect the course of *L. major* infection in vitro.

We observed that human neutrophils infected with *L. major* in vitro showed enhanced capacity to engulf apoptotic cells. This was associated with increased expression of receptors involved in phagocytosis (CR1, CR3) on neutrophil surface. Next, to investigate a role of apoptotic cell phagocytosis in *L. major* survival, human neutrophils were infected with the parasites and incubated 18 h in the presence or absence of apoptotic cells. By using the limiting dilution assay, we found that significantly more parasites survived in neutrophils exposed to apoptotic cells.

Our results indicate that *Leishmania* parasites facilitate the engulfment of apoptotic cells to enhance their own survival within neutrophils. Our ongoing experiments are aimed at testing whether apoptotic cells promote the survival of other intracellular pathogens (*Anaplasma phagocytophilum*, *Chlamidia pneumoniae*) in neutrophils.

35 Unni Samavedamn - Post-Doctoral Fellow
Division of Immunobiology - Fred Finkelman

Saturated fatty acids promote pro-Th2 cytokine expression by activation of unfolded protein response (UPR) and ER stress

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Saturated triglycerides and fatty acids stimulate endoplasmic reticulum (ER) stress and an unfolded protein response (UPR), while unsaturated trigycerides and fatty acids do not have these effects. Recently, we have found that the same dichotomy holds for the ability of saturated vs. unsaturated triglycerides to promote the induction of food allergy and to stimulate increased epithelial cell expression of 3 pro-Th2 cytokines, TSLP, IL-25 and IL-33, which stimulate an allergic (Th2) cytokine response. These observations led us to hypothesize that the high concentrations of saturated triglycerides in two common food allergens, cow's milk and chicken eggs, promote food allergy by inducing a UPR response in epithelial cells, which, in turn, causes these cells to express pro-Th2 cytokines. Two sets of experiments were performed to test this hypothesis. First, we evaluated whether saturated medium chain triglycerides, egg yolk plasma (the liquid, triglyceride fraction of eggs) and cow's milk cream can stimulate UPR and pro-Th2 gene expression, as well as increased permeability, in transformed human intestinal epithelial CACO2 cells. Each of these stimuli, but not protein-rich, lipid-poor egg white, increased expression of the UPR-associated PERK, BiP, CHOP, XBP1 and/or XBP1s genes after 6 hours, and increased epithelial permeability and expression of all 3 pro-Th2 cytokine genes after 24 hours. Further, the increases in pro-Th2 cytokine expression were blocked by each of 3 UPR inhibitors: metformin, 4PBA and TUCDA and the egg yolk plasma-induced increase in epithelial permeability was blocked by the only UPR inhibitor tested (metformin). Secondly, monoclonal antibodies to IL-25, IL-33 and TSLP each individually blocked induction of food allergy to egg white by a combination of egg white and medium chain triglycerides. These observations support our hypothesis and provide an explanation for the strong allergenicity of cow's milk and chicken eggs.

36 Anna Sliz - Graduate Student
Division of Immunobiology - Kasper Hoebe

Gab3: A Novel Regulator of Natural Killer Cell Function and Development

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Natural killer cells are innate lymphocytes that play an important role in control of viral infections and elimination of tumor cells. While the mechanism of NK cell killing by perforin and granzyme is well characterized, much of the regulatory signaling that controls NK cell activation and development is poorly defined. Our laboratory conducted a forward genetic approach using N-ethyl-N-nitrosourea mutagenesis to identify non-redundant genes required for NK cell function and development. We identified an ENU germline mouse (A961) that exhibited impaired missing self-recognition and produced significantly less IFNγ. Linkage analysis and WES of the A961 genome revealed the causative mutation to be a C ⇒T missense mutation in Gab3 located on the X-chromosome. Gab3 is a scaffold protein that belongs to the Grb2-associated binding family including Gab1 and Gab2 and is highly expressed in NK cells, CD8 T cells and macrophages. Members of this family have been implicated in PI3K signaling through interactions with p85α and SHP2. Importantly a recent GWAS study suggests specific SNPs in Gab3 to be associated with type I diabetes development. Interestingly, experiments in our laboratory revealed A961 mice are partially protected from streptozotocin-induced type I diabetes, confirming the important role of gab3 in type I diabetes. Using confocal microscopy, we were able to show that Gab3 localizes to the plasma membrane and Rab5+ vesicles. These data indicate that Gab3 is a novel critical signaling component required for NK cell function and loss of Gab3 function is associated with reduced type I diabetes disease development.

37 KC Sullivan - Graduate Student
Division of Immunobiology - Andrew Herr

Purification of the High Affinity IgE Receptor FcERI Ectodomain for Biophysical Analysis of Anti-human FcERI Antibody and IgE Binding

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The high affinity IgE receptor, FcERI, is an essential mediator of Type I hypersensitivity reactions, including allergic rhinitis, asthma, and systemic anaphylaxis. Given the central role of FcERI in many immediate hypersensitivity reactions, there is interest in developing anti-FcERI antibodies for therapeutic use. Two murine monoclonal antibodies, AER-37 (CRA-1) and 15.1, target the alpha subunit of human FcERI and have demonstrated differential interaction profiles. The 15.1 mAb competes with IgE for the Fc receptor, while mAb AER-37 is non-blocking and can bind to the FcERI:IgE complex at 4 C. However, in vivo, pretreatment with AER-37 induces FcERI internalization, but also leaves a sub-population of FcERI on the cell surface (as shown by 15.1 staining) that cannot interact with IgE. One hypothesis is that AER-37 binding induces an allosteric conformational change in FcERI that precludes IgE binding at 37 C. In order to quantitatively characterize the interactions of 15.1, AER-37, and IgE with FcERI, we purified the FcERI ectodomain and characterized the receptor in the context of these antibodies. We expressed the fusion protein Fcγ-FcERI in mammalian HEK293T cells, followed by cleavage and purification of the ectodomain. The FcERI ectodomain was further characterized by analytical ultracentrifugation, and ELISA data corroborated the expected binding specificities and competition of these mAbs for FcERI. Future studies will aim to characterize the kinetics and binding affinities of IgE, 15.1, and AER-37 to FcERI as a function of temperature and to determine whether AER-37 binding induces conformational changes in FcERI.

38 Diana Taft - Post-Doctoral Fellow
Center for Autoimmune Genomics and Etiology - John Harley

ASSOCIATION OF CHORIOAMNIONITIS WITH ABERRANT NEONATAL GUT COLONIZATION AND ADVERSE CLINICAL OUTCOMES

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Objective: Chorioamnionitis (inflammation of the placenta and fetal membranes) and abnormal gastrointestinal colonization are associated with an increased risk of sepsis and death in preterm infants. We sought to determine if chorioamnionitis is associated with an altered infant fecal microbiome indicating abnormal GI colonization, and to determine if these microbiome alterations are associated with adverse outcomes. Methods: Infants born at gestational age less than 28 weeks were enrolled from 3 level III NICUs in Cincinnati and Birmingham. Stool samples were collected from infants through day of life 7 and subjected to DNA extraction and 16S gene sequencing. Chorioamnionitis was diagnosed based on placental histology. Late onset sepsis and death were analyzed in relation to fecal microbiota associated with chorioamnionitis. Results: A total of 106 infants met inclusion criteria. Infants were categorized into three groups: Infants without chorioamnionitis (NC, n=48), infants with acute chorioamnionitis without funisitis (AC, n=32) and infants with acute chorioamnionitis and funisitis (ACF, n=26). The fecal samples of ACF infants had higher relative abundance of family Mycoplasmataceae (phylum Tenericutes), genus Prevotella (phylum Bacteroidetes) and genus Sneathia (phylum Fusobacteria). The incidence of sepsis or death was greater in AC and ACF infants. Presence of specific clades - genus Sneathia or family Mycoplasmataceae - in fecal samples was associated with risk of sepsis or death. Conclusion: Preterm AC and ACF infants have a different fecal microbiota in the first week of life and increased sepsis/death, compared to NC infants. Intestinal dysbiosis associated with chorioamnionitis may predispose to adverse neonatal outcomes.

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39 Jared Travers - Graduate Student
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IL-33 is Selectively Expressed by Esophageal Epithelial Progenitor Cells during Allergic Inflammation

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Background: Recent studies on the pathogenesis of allergic disorders have focused on the involvement of innate cytokines produced by epithelial cells that promote the development of Th2 cell immunity. Herein, we focused on the involvement of the innate cytokine IL-33 in eosinophilic esophagitis (EoE). We aimed to test the hypothesis that IL-33 is increased in EoE. Methods: Quantitative real-time PCR (qRT-PCR), immunohistochemistry (IHC) and immunofluorescence (IF) were performed on esophageal biopsies of patients with inactive and active EoE or control individuals. Results: We noted a profound alteration in IL-33 protein expression in the diseased state as only patients with active disease had detectable epithelial expression of IL-33; expression was limited to the nuclei of the cellular layer in direct contact with the basement membrane between papillae. Consistent with this, IL-33 mRNA was increased (2-fold; $p = 0.045$) in active EoE biopsies compared to control biopsies. IL-33+ cells expressed E-cadherin, keratin 5 and keratin 14, and the epithelial progenitor marker p75NTR. They did not express the proliferation marker Ki-67. Additionally, a subset of primary esophageal epithelial cells grown in vitro expressed IL-33 but not Ki-67. Conclusions: IL-33 is selectively present only during active EoE disease in the most basal layer in a quiescent progenitor population.

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40 Simone Vanoni - Post-Doctoral Fellow
Division of Allergy and Immunology - Simon Hogan

Dysregulation of SLC9A3 and acid-base transport in Pediatric Eosinophilic Esophagitis

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Eosinophilic esophagitis (EoE) is a recently emerging chronic inflammatory disease of the esophagus and is mediated by dietary food antigens and clinically characterized by upper gastrointestinal (GI) symptoms including dysphagia and food impaction. In an effort to expand the molecular signature of EoE, we recently performed RNA sequencing on esophageal biopsies from healthy controls and patients with active EoE and compared the differential EoE gene signature from RNA-Seq to the EoE transcriptome identified by standard microarray approach. While there was substantial overlap of upregulated genes the most highly induced gene unique to the RNA-Seq profile was solute carrier family 9, subfamily A, member 3 (SLC9A3; NHE3, sodium-hydrogen exchanger member 3), which was induced 33-fold in EoE. This finding was intriguing to us as previous bioinformatics analyses showed a statistical enrichment in ion transport pathways (SLC family), including acid-base ion transport (carbonic anhydrase [CA] II pathway), as part of the EoE transcriptome by standard microarray. RNA-Seq analyses of esophageal biopsy samples from patients with EoE with active disease identified altered expression of ion transport genes; gene ontogeny network analyses revealed that a notable proportion of these ion transport genes (SLC9A3, SLC26A4, CAII) are integrally involved in the acid-base transport circuit of the esophagus. Notably, we observed a positive Spearman correlation between the level of inflammation (eosinophils/hpf) and expression level of SLC9A3 and CAII (FPKM), indicating a relationship between gene expression and EoE severity ($r2 = 0.51$, * $p < 0.005$ and $r2 = 0.42$, $p < 0.01$). Analyzing electrical properties (basal potential difference (PD), short circuit current (Isc), transepithelial resistance (RT)) of esophageal biopsy samples from pediatric normal controls (NL) and eosinophilic esophagitis (EoE) patients revealed a low PD (-8.9 ± 2.2 vs -3.0 ± 1.4 mV; mean ± SEM, $p < 0.05$), high Isc (5.0 ± 1.1 vs 9.2 ± 1.2 μA/cm2; mean ± SEM, $p < 0.05$) with high RT (477.1 ± 34.1 vs 950.5 ± 110.6 □.cm2; mean ± SEM, $p < 0.01$) in EoE vs. NL biopsies, suggesting increased active transport (cation absorption) and increased resistance in EoE. Acute pulse exposure of biopsies to a weak acid (NH4Cl) stimulated an increase in Isc that was further enhanced in EoE (NL 11.9 ± 4.5 vs 17.7 ± 2.1 □Isc (μA/cm2) NL vs EoE biopsy, $p < 0.05$). Notably, the recovery response was inhibited by SLC9 blockade (EIPA) and was not sensitive to the anionic transport inhibitor DNDS, suggesting SLC9-specific dependency. Collectively these data indicate dysregulation of SLC9A3 function in esophageal epithelial cells in EoE.

41 Filipa Varela - Graduate Student
Division of Immunobiology - David Hildeman

Vaccination against tuberculosis is enhanced by regulatory T cell depletion

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Despite the big advances in the Tuberculosis (Tb) field, this disease is still one of the most important bacterial infections worldwide, causing a substantial amount of morbidity. TheA major challenge facing the fieldof this disease is thatarrives from the fact that, even with an appropriate immunity, the body is unable to eradicate the bacteria leading to a latent stage with high risk of reactivation. The uniquecurrently licensed vaccine, *M. bovis* Bacillus Calmette- Guérin (BCG), has shown to be ineffective in adults. Thus, more effective prophylactic therapies are urgently needed. New Tb- vaccines are now ongoing clinical trials, including the multistage H56 .
Previous work has suggested shown an increase of that regulatoryFoxp3- expressing cells in Tb- infected lung T cells contribute to the inability to eliminate Tb, suggesting that Treg may impede anti-Tb immunity. Here, we evaluated the role of regulatory T cells (Treg) during vaccination against Tb using Foxp3- diphtheria toxin receptor (DTR) mice as model.
To do this, Therefore, Foxp3-DTR mice were vaccinated with H56/CAF01 and Treg were depleted every 3 days via DT administration for a time period of protective immunity development.
Treg depletion resulted in a dramatic increase of activation markers in CD4 T cells, as well as differentiation of naïve T cells into effector memory T cellsa significant loss of naïve T cells. The number of ESAT-6- specific CD4 T cells was also increased in the spleen and lymph nodes, and these cells which produced higher levels of IL-2 and TNF-α compared to non- Treg depleted mice. Unexpectedly, ablation of Treg led to an increase of IL-10 production by $Lag3^{pos}$ Tr1 cells. Unfortunately, prolonged Treg depletion led to a severe systemic immune activation and associated morbidity. Followed by continuous T reg depletion comes also the development of a severe scurfy- phenotype, associated to proliferation of granulocytes and monocytes.
Together, these data show a promisingthat Treg depletion could enhance of H56/CAF01 vaccine responses. protective effect against Tb by Treg depletion. However, future studies will determine whether punctual depletions will be tested to evaluate whether represent the same protective trend, but averting the profound side- effects. could promote anti-vaccine responses without systemic immune activation. These results provide new insights into Tb vaccination.

42 David Wu - Post-Doctoral Fellow
Division of Allergy and Immunology - Simon Hogan

MicroRNA-375-KLF5 regulation of intestinal epithelial CFTR function

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We have recently demonstrated in vitro and in vivo a role for the IL-13/Type II IL-4 receptor (Il4R□ and IL13R□1) and STAT-6 in the up regulation of intestinal epithelial Cystic Fibrosis Transmembrane Conductance Regulator (ATP-Binding Cassette Sub-Family C, Member 7) Cftr-dependent Cl- conductance. In an attempt to identify the molecular pathways involved in this process, we performed miRNA microarray and qRT-PCR analyses of IL-13-stimulated intestinal epithelial cells and revealed IL-13-mediated downregulation of four miRNA's, miR-375, miR-212, miR-181a-2 and miR-145. To determine the involvement of miRNA-375 to IL-13-induced Cftr-Cl- secretory activity, we examined forskolin-induced Isc and Cl- secretion in Caco2bbe cells following overexpression of miR-375 (lentivirus expressing PreMir-375) and antagonism of miRNA-375 (antagomir). Overexpression of miRNA-375 (40-fold induction) significantly abrogated IL-13-induced Cftr mRNA and protein expression and Cl- secretion (CFTR mRNA Fold-induction: 50.220±28.204 vs 6.559±2.882; Forskolin responses; 85.474±17.075 vs 53.090±9.602 (ΔμA/cm2); mean ± SD; n=6; preMIR control vs preMIR 375 respectively; $p<0.01$). Conversely, antagonism of miR-375 amplified IL-13-induced Cftr mRNA and protein expression and CFTR-dependent Cl- secretion (CFTR mRNA Fold-induction: 3.323±0.377 vs 8.137±1.407; Forskolin responses: 96.414±6.613 vs114.707±5.778 (ΔμA/cm2); mean±SD; n=6; antagomir-control vs antagomir-375 respectively; $p<0.01$). Analyses of the 3' untranslated region of the CFTR mRNA using TargetScan (version 5.0) revealed that miR-375 was not predicted to interact with CFTR. Performing MiR-375 pulldown studies we revealed that miR-375 interacts with the transcription factor Kruppel like factor-5 (Klf5). Indeed functional in vitro and ex vivo studies employing shRNA-KLF-5 technology revealed that miR-375 negative regulation of Cftr mRNA and protein expression and Cl- secretion was dependent on the negative regulation of the Klf5. Chromatin immunoprecipitation (ChIP) assays revealed Klf5 binding to the Cftr promoter. Further, intestinal epithelial specific-overexpression of KLF5 (Villincre KLF5Tg) enhanced CFTR-dependent Cl- secretion in small intestine of mice. Our findings indicate that the epithelial miR-375 is a key regulator of Cftr-dependent Cl- secretion and gut homeostasis and mucosal immunity. These observations demonstrate a central role for the IL-13/ IL-13R□1 pathway via miR-375/KLF-5 axis in the regulation of intestinal epithelial cell CFTR Cl- secretion.

43 Alexander Yarawsky - Graduate Student
Division of Immunobiology - Andrew Herr

Polyproline II Helix Formation by an Intrinsically Disordered Region of the Staphylococcus epidermidis Accumulation Associated Protein

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Staphylococcus epidermidis is responsible for a large percentage of healthcare-associated infections due to its remarkable ability to form biofilms on virtually any indwelling medical device. The Accumulation Associated Protein (Aap) is a key factor in biofilm formation through Zn2+-mediated adhesion of S. epidermidis cells. The Zn2+-binding superdomain of Aap is followed by a proline/glycine-rich region (PGR) composed of a repetitive AEPGKP sequence and an LPXTG cell wall anchor motif. We hypothesize the PGR region projects Aap away from the cell wall, allowing it to fulfill its role in biofilm formation. The polyproline II (PPII) secondary structure of the PGR region has been characterized by far-UV circular dichroism (CD). A strong temperature dependence was observed in which the negative signal near 200nm became more negative at low temperatures, a trend often observed with intrinsically disordered proteins (IDP) and polyproline II helices. Chemical denaturants had little effect, and trifluoroethanol failed to induce alpha-helical structure. As expected for a PPII helix, screening electrostatics did not affect the CD spectrum. Analytical ultracentrifugation (AUC) was used to examine the highly elongated nature of this protein and its temperature dependence. Dynamic light scattering and modelling provide additional information regarding the degree of elongation of the PGR region. The PGR region of Aap likely functions as an elongated stalk, supporting the ability of Aap to form biofilms. This investigation will provide insight into the structure and function of other low-complexity regions of cell wall-anchored proteins.

NIH R01 GM094363

44 Chang Zeng - Graduate Student
Division of Allergy and Immunology - Simon Hogan

IL-13-induced Dilated Intracellular Space (DIS) formation in esophageal epithelial cells is dependent on SLC9A3 function

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**Department of Pharmacology and Cell Biophysics, College of Medicine, University of Cincinnati, OH*

Histopathological manifestations of Eosinophilic esophagitis (EoE) include intraepithelial eosinophilic inflammation and alterations of the esophageal epithelium such as basal layer hyperplasia and dilated intercellular spaces (DIS), which are thought to drive the substantial esophageal dysfunction of EoE. We have recently employed standard microarray and RNA sequencing (RNA-Seq) analyses to identify differential transcript signatures in esophageal specimens from patients with active EoE compared to healthy controls. Comparing the differential EoE gene signature from RNA-Seq to the EoE transcriptome identified by standard microarray approach, we identified solute carrier family 9, subfamily A, member 3 (SLC9A3; NHE3, sodium-hydrogen exchanger member 3) as the most highly induced gene unique to the RNA-Seq profile, which was induced 33-fold in EoE. Expression level of SLC9A3 (FPKM) positively correlated with the level of inflammation (eosinophils/hpf) and DIS formation indicating a relationship between gene expression, DIS formation and EoE severity ($r2 = 0.51$, $p < 0.005$; $r2 = 0.47$, $p < 0.05$ respectively). To determine the relationship between SLC9A3 and histopathological features of EoE, we employed the immortalized esophageal epithelial progenitor cell line (EPC2) in ALI culture (EPC2-ALI). RNA-seq, qRT-PCR and western blot analyses revealed that IL-13 treatment of EPC2-ALI cultures induced SLC9A3 mRNA (17-fold induction compared to unstimulated) and protein expression. The increased expression was associated with esophageal dysfunction including decreased RT (ohms.cm2 784.1 ± 46.3 vs. 306.5 ± 120.68; n = 3 cultures; mean ± SD; $p < 0.05$) and increased Isc (μA/cm2 1.8 ± 0.2 vs. 2.5 ± 0.`; n = 3 cultures; mean ± SD; $p < 0.05$), specifically associated with increased SLC9A3 inhibitor EIPA-sensitive Isc (% total inward Na+ Isc EIPA-sensitive: 15.0 vs. 37.0%; $p < 0.05$) and acid secretion (pH-STAT 0.7 ± 0.1 vs. 2.3 ± 0.3 nmoles.min-1 NaOH; n = 3; mean ± SEM; $p < 0.05$) and DIS-formation. Collectively, we conclude that SLC9A3 contributes to dysregulated esophageal ion transport and DIS formation in EoE.

45 **Erin Zoller - Post-Doctoral Fellow**
Center for Autoimmune Genomics and Etiology - John Harley

Using CRISPR/Cas9 to probe disease etiology in the post-genomic era

Erin Zoller, PhD 1 Kathryn Gilbert 2 Alyssa Wolfinger 2 Laura Brungs 1 Leah Kottyan, PhD 1 John Harley, MD, PhD 1, 3

1. Center for Autoimmune Genomics and Etiology, CCHMC 2. College of Engineering and Applied Science, University of Cincinnati 3. VA Medical Center, Cincinnati, Ohio

The genomic era has revealed an abundance of genetic polymorphisms statistically associated with disease development, but the causal relationships between many of these variants and pathogenesis, as well as the biological mechanisms linking them, remain to be established. The CRISPR/Cas9 genome editing approach allows for specific targeting of a locus of interest, such as a polymorphism, to introduce any number of sequence changes within live cells. We hypothesize that the CRISPR/Cas9 system can be used to causally link genetic variants to disease-associated cellular phenotypes. Our strategy is to create cell lines that are genetically identical save for the alleles of our variant of interest. We will then test for differential phenotypes between the cell lines. To achieve this we will induce genomic editing through the use of locus-specific CRISPR guide RNAs (gRNAs) and the Cas9 nuclease. We have designed multiple gRNAs, and established techniques to test the targeting and cutting efficiency of gRNAs in vitro. We have also carefully considered and experimented with the various methods of introducing CRISPR/Cas9 components into cell lines. Importantly, we have developed screening workflows to easily assess post-editing allele variety and overall editing efficiency in our cell lines. We find that there is a wide range of post-repair sequence insertions or deletions. Our data thus far have highlighted a number of successes as well as pit-falls in using the CRISPR/Cas9 system. These insights and considerations will allow us to make our desired cell lines and are of value to others interested in using genome editing.

CCHMC GAP Funding, "Causal Variants and SLE and Related Autoimmune Diseases."

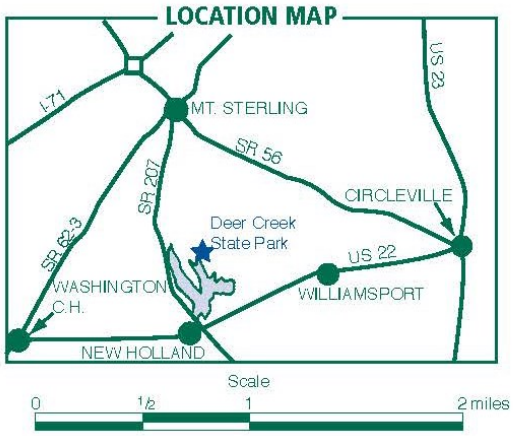
Deer Creek State Park

20635 State Park Road 20
Mt. Sterling, Ohio 43143

(740) 869-3124 - Park Office
(740) 869-3508 - Camp Office
(740) 869-3088 - Golf Course
(740) 869-3728 - Lake Condition

Deer Creek Resort Lodge
22300 State Park Road 20
Mt. Sterling, Ohio 43143

(740) 869-2020 - Lodge Front Desk
1-800-282-7275 - Lodge/Cottage Reservations



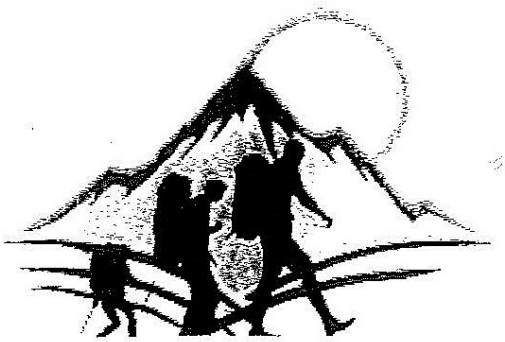
LEGEND

- Park Office
- Picnic Area
- Shelterhouse
- Restroom or Latrine
- Amphitheater and Nature Center
- Basketball
- Beach
- Boat Launch Ramp
- Boat Swim Area
- Camping
- Hiking Trail
- Miniature Golf
- Park & Wildlife Area Boundary
- Playground
- Public Phone
- Snowmobile/Bridle Trail
- Volleyball
- Wheelchair Accessible

HIKING TRAILS

- A Meadow
- B Rolling Hills
- C Marsh Walk
- D Ridge
- E Adena Ridge
- F Van Horn
- G Waterloo

Rev. 9/13



Deer Creek State Park
Hiking Trails

A **Hawkview Meadow Trail:** A 1 mile loop trail runs through an old oak woodlot that opens into a gentle rolling meadow. Bordered By Clarks Run Creek. Mostly wooded, some hills.
Starts approximately 50 yards past the camp office. Marked with yellow blazes.

B **Rolling Hills Trail:** This is a 2.5 mile one way trail that leads you through scenic wooded rolling hills and ravines. The trail borders the lake shoreline between the lodge and the campground. It offers the hiker some good views of the lake and surrounding woods. This is a one way trail, so hikers need to be ready to hike the 2.5 miles back to the start.
Starts in the lodge parking lot to the left of the tennis courts or in the campground near Wash-House 4. Marks with yellow blazes.

C **Ghost Tree Swamp:** A 1 mile loop trail traverses the shoreline and winds through hilly woodlands which included both a pine and oak wood lot.
Starts at the East boat ramp parking lot. Marked with yellow blazes.

D **Ridge Trail:** This 1.5 mile loop trail runs through mostly wooded terrain with some shoreline hills. A nature observation blind allows the hiker to observe an area where wildlife may be enjoying corn or a salt block.
Starts in the lodge parking lot near the baseball field. Marked with yellow blazes.

E **Adena Ridge Trail:** This ¾ mile loop trail borders lake shoreline the entire route. It boasts some excellent views of both the lake and beach area. Mostly wooded, partially uphill, downhill slopes.
Starts to the right of Cabin 25. Marked with yellow blazes.

F **Rich VanHorn Nature Trail:** A 1 mile loop trail in the woods started on the East side of the lodge. It passes by a small pond and traverses the lake shore. Ends to the left of the tennis courts in the lodge parking lot. Has signs to assist Nature Identification. Marked with red blazes.

G **Waterloo Trail:** a ¼ mile trail in length. It winds through the cabin area and offers a short after dinner walk for the cabin guests not wishing to go far.
Starts and ends near the first circle of cabins and Cabin Road. Marked with red blazes.

Activities at the park

http://www.deercreekstateparklodge.com/deer-creek-activities/

DEER CREEK
LODGE AND CONFERENCE CENTER

Boating:

- Paddle boat rentals
- Kayaking rentals

Other On-Site Activities:

- Hiking trails (see previous page for descriptions)
- Indoor pool
- Hot Tub
- Bicycle rentals
- Kayak rentals
- 9-hole miniature golf
- Game room and billiards
- Board games
- DVD rentals

Nearby:

- Tanger Outlet Mall
- Slate Run Living Historical Farm
- Columbus Zoo

Golf:

350 acre 18-hole championship golf course and pro-shop are located near the park entrance. Practice facilities are also available for use.

- Basketball courts
- Lighted tennis courts
- Sand volleyball
- Baseball diamond
- Group fire pit areas
- Fitness center
- Children's playground
- Horseshoe pits
- Shuffleboard court


Reds