Immunology Graduate Group
27th Annual Retreat

October 17 – 19, 2014
The Grand Hotel
Cape May, NJ

University of Pennsylvania

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Immunology Graduate Group | Retreat Program

FRIDAY, OCTOBER 17, 2014

NOTES:

- Hotel check-in begins at 3:00 PM. If possible, please leave your luggage in your vehicle until check-in at the end of Session II.
- All sessions and breaks will be held in the Penthouse Ballroom (5th floor). All meals will be held in the Grand Ballroom Complex (2nd floor).

11:00 AM **Registration**

12:00 PM **Lunch**

1:20 PM **Welcome**

Paula Oliver, Ph.D., Retreat Co-Chair

1:30 PM **Session I: Host Defense (Chair – Alan Copenhaver)**

Gretchen Harms Pritchard (S1B)
"Diverse roles for T-bet in the effector responses required for resistance to infection"

EnJun Yang (S1C)
"Augmentation of diacylglycerol signaling enhances NK cell function without affecting Ly49 receptor acquisition by NK cells during development"

Nelson Glennie (S1D)
"Skin-resident memory CD4 T cells enhance protection against Leishmania major infection"

Lehn Weaver (S1E)
"The role of myeloid progenitor cells in TLR9-driven inflammation"

Alan Copenhaver (S1A)
"Complementary immune roles for uninfected and infected cells during Legionella pneumophila infection"

3:10 PM **Break**

FRIDAY, OCTOBER 17, 2014 (continued)

3:30 PM **Session II: Immunoregulation (Chair – Claire O'Leary)**

Theresa Leichner (S2B)

"Factors released in local responses in the skin can systemically increase regulatory T cell numbers and protect against type 1 diabetes"

Martin Naradikian (S2C)

"IL-4 and IL-21 reciprocally regulate T-bet expression in the context of TLR9-stimulated B cells"

Julia Rood (S2D)

"Blockade of Interleukin-33 signaling prevents death in the murine model of familial Hemophagocytic Lymphohistiocytosis"

Gaia Muallem (S2E)

"The cytokine IL-27 limits acute and chronic manifestations of lung disease in a mouse model of post-viral asthma"

Claire O'Leary (S2A)

"Ndfip1 and Ndfip2 activate catalytic E3 ubiquitin ligases to prevent aberrant T cell activation and Th2 differentiation"

5:10 PM **Break**

5:30 PM **Dinner (and hotel check-in)**

7:00 PM **Faculty Talks**

Jorge Henao-Mejia, M.D., Ph.D.

"Regulation of the host-microbial interface by the inflammasomes"

Assistant Professor of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, University of Pennsylvania Institute for Immunology, Perelman School of Medicine

Michael Povelones, Ph.D.

"The mosquito immune system limits malaria parasite development"

Assistant Professor, Pathobiology, University of Pennsylvania School of Veterinary Medicine

Nicholas Restifo, M.D.

"Memory CD8+ T cells induce precocious differentiation of naïve T cells through quorum sensing-like behavior"

Senior Investigator, Surgery Branch, Center for Cancer Research, National Cancer Institute

9:00 PM **Social**

SATURDAY, OCTOBER 18, 2014

8:00 AM **Breakfast**

9:00 AM **Hill Update**

Shaun O'Brien, Ph.D.
Postdoctoral Fellow, Children's Hospital of Philadelphia, AAI Public Policy Fellow

9:10 AM **Session III: Translational (Chair – Susanne Linderman)**

Shaun O'Brien (S3A)
"Inhibition of the transcription factor Ikaros augments the tumoricidal capacity of CD8+ T cells expressing Chimeric Antigen Receptors"
Susanne Linderman (S3E)
"Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013-2014 influenza season"
Michael Cho (S3B)
"Cross-reactivity of VH1-46 antibodies to desmoglein 3 and rotavirus VP6 May explain their persistence in Pemphigus Vulgaris"
Emily Roberts (S3C)
"Preliminary Evaluation of CD8+ T Cells in Early Acute SIV Infection"
Ian Lamborn (S3D)
"Novel immunodysregulation disorder associated with a gain-of-function mutation in GNAI2"

10:50 AM **Break**

11:00 AM **Keynote: John O'Shea, M.D.**

"Genomic switches and lymphocytes searching for their identity"

Scientific Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases; Senior Investigator, Molecular Immunology and Inflammation Branch, NIAMS

12:30 PM **Lunch**

1:30 PM **Career Session: The Future of Biomedical Research in Drug Development**

Scott Berger, Ph.D.

Manger, Discovery Biology, GlaxoSmithKline

Lisa Kozlowski, Ph.D.

Associate Dean for Postdoctoral Affairs & Recruitment, Jefferson Graduate School of Biomedical Sciences, Thomas Jefferson University

John Monroe, Ph.D.

Senior Director, Immunology, Genentech

2:30 PM **Free Time**

SATURDAY, OCTOBER 18, 2014 (continued)

4:30 PM **Poster Session and Happy Hour**

6:00 PM **Dinner**

7:30 PM **Awards and Closing Remarks**
David Allman, Ph.D., IGG Chair

8:30 PM **Social**

SUNDAY, OCTOBER 19, 2014

8:00 AM **Breakfast**

NOTE: All attendees must check out of the hotel by 11:00 AM.

S1A. Complementary immune roles for uninfected and infected cells during Legionella pneumophila infection

Alan M. Copenhaver[1], Cierra N. Casson[1], Hieu Nguyen[1], Thomas Fung[1], Matthew Duda[1], Craig Roy[2], and Sunny Shin[1]

[1] *Department of Microbiology, University of Pennsylvania Perelman School of Medicine*
[2] *Microbial Pathogenesis, Yale University School of Medicine*

The innate immune system responds to virulent pathogens, yet many pathogens manipulate host signaling pathways which should limit immune activation. To understand how the immune system overcomes pathogenic manipulation, we study the intracellular bacterium *Legionella pneumophila*, the cause of the severe pneumonia Legionnaire's disease. Legionella utilizes a type IV secretion system to inject proteins into the cytosol of infected cells. Several of these bacterial effector proteins inactivate host cell factors involved in protein translation. Despite the ability of *L. pneumophila* to block host protein translation, inflammatory cytokines are still made during infection. It is unclear how infected cells can mount a cytokine response when host protein synthesis is blocked. Our studies demonstrate that infected cells do not produce many cytokines, such as IL-6 and TNF, critical for controlling *L. pneumophila* infection. Instead, uninfected, bystander cells produce these cytokines. Infected host cells do produce IL-1 *de novo*. These data suggest that infected cells have mechanisms to overcome protein synthesis inhibition to produce IL-1 and that uninfected, bystander cells are important contributors to the immune response during infection with Legionella. This mechanism of immune activation has broad significance as many other bacterial pathogens manipulate host cell processes, including immune cell signaling.

S1B. Diverse roles for T-bet in the effector responses required for resistance to infection

Gretchen Harms Pritchard[1], Aisling O'Hara Hall[1], Steven L. Reiner[2], Christopher A. Hunter[1]

[1] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA 19104*
[2] *Departments of Microbiology & Immunology and Pediatrics, College of Physicians & Surgeons of Columbia University, New York NY 10032*

The transcription factor T-bet has been most prominently linked to natural killer (NK) and T cell production of interferon-γ (IFN-γ), a cytokine required for the control of a diverse array of intracellular pathogens. Indeed, in mice challenged with the parasite *Toxoplasma gondii*, NK and T cell responses are characterized by marked increases of T-bet expression. Unexpectedly, T-bet$^{-/-}$ mice infected with *T. gondii* develop a strong IFN-γ response that controls parasite replication at the challenge site, but display high parasite burdens at secondary sites colonized by *T. gondii* and succumb to infection. T-bet was not required for the generation of parasite-specific T cells, yet the absence of T-bet resulted in lower T cell expression of CD11a, Ly6C, KLRG-1, and CXCR3 and fewer parasite-specific T cells at peripheral sites of infection. Together, these data highlight T-bet independent pathways to IFN-γ production, and reveal a novel role for this transcription factor in coordinating the regional T cell responses necessary to control infection.

S1C. Augmentation of diacylglycerol signaling enhances NK cell function without affecting Ly49 receptor acquisition by NK cells during development

EnJun Yang[1], Amanda Schimdt[1], Taku Kambayashi[1]

[1] *Department of Pathology and Laboratory Medicine Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*

Signaling through immnotyrosine-based activation motif (ITAM)-bearing receptors (immunoreceptors) plays an important role in anti-tumor and anti-pathogen responses by NK cells. One key specific signaling event downstream of immunoreceptors is PLCg-mediated cleavage of PIP_2 into inositol trisphosphate and diacylglycerol (DAG). Although PLCg has been shown to be critical for NK cell function and for Ly49 receptor acquisition during NK cell development, role of DAG in NK cell function is unknown. To investigate the impact of DAG signaling on NK cells, we studied NK cell function and development in mice lacking diacylglycerol kinase (DGK). DGKs are enzymes that catabolize DAG, and thus, DGK deficiency leads to the accumulation of DAG in activated cells. In this study, we focused on DGKζ, which is highly expressed in lymphocytes.

Compared to WT NK cells, immunoreceptor-activated DGKζ-deficient NK cells displayed an ~2-fold increase in IFNg production and degranulation. The increase in NK cell function by DGKζ deficiency occurred in a cell-intrinsic and development-independent manner as similar effects were observed in mixed bone marrow chimeras and in mature NK cells inducibly deleted of DGKζ. Retroviral reconstitution with WT and mutant forms of DGKζ demonstrated that the kinase function of DGKζ was necessary for attenuation of NK cell function. Immunoreceptor-activated DGKζ-deficient NK cells displayed increased ERK phosphorylation, a key signaling molecule activated downstream of DAG. In contrast to PLCg2-deficient mice, NK cells from DGKζ-deficient deficient mice displayed no cell-intrinsic differences in Ly49 receptor expression compared to WT NK cells. Together, these data suggest that DAG signals play an important role in NK cell function but not in immunoreceptor-mediated acquisition of Ly49 receptors during development.
Our data presented here are in contrast to NK cells from mice lacking other negative regulators of signaling such as SHP-1 and SHIP, which are hyporesponsive and display increased Ly49 receptor expression. Despite the hyperresponsive NK cell phenotype, DGKζ-deficient mice have no apparent pathology, Moreover, on an H-2^b background, Ly49C$^+$ NK cells (self-MHC-binding) were hyperresponsive compared to Ly49C$^-$ NK cells even in the absence of DGKζ, suggesting that these NK cells are appropriately tuned toward self-targets *in vivo*. Thus, DGKζ might be an attractive target to enhance NK cell function without altering self tolerance or development of NK cells.

S1D. Skin-resident memory CD4 T cells enhance protection against Leishmania major infection

Nelson D. Glennie[1], Venkat A. Yeramilli[1], Daniel P. Beiting[1], Susan W. Volk[1], Casey T. Weaver[2], Phillip Scott[1]

[1] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania*
[2] *Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama*

The intracellular parasite *Leishmania* causes significant disease burden around the world. Resolution of a primary infection leads to a highly refractory state against subsequent *Leishmania* infections, but unfortunately this protection has yet to be recapitulated in a human vaccine. While circulating central and effector memory T cells are known to play a crucial role in the protective immune response, the importance of memory T cells present in peripheral tissues remains poorly

defined. We have identified a population of skin-resident *Leishmania*-specific memory CD4 T cells. These CD4 T cells produce IFNγ in response to restimulation, and remain resident in the skin when transplanted by skin graft onto naive mice. Gene expression profiling and adoptive transfer experiments demonstrate that these skin-resident cells act as sentinels during *Leishmania* challenge by rapidly recruiting circulating cells to the skin, resulting in a decreased parasite burden. These findings indicate that protective immunity to *Leishmania* and thus the success of a vaccine may depend not only on generating circulating memory T cells, but also on generating a memory T cell population in the tissue.

S1E. The role of myeloid progenitor cells in TLR9-driven inflammation

Lehn K. Weaver[1] and Edward M. Behrens[1]

[1] *The Children's Hospital of Philadelphia, The Division of Pediatric Rheumatology*

Cytokine storm is characterized by hypercytokinemia, unremitting fevers, cytopenias, splenomegaly, hepatitis, coagulopathy, multisystem organ failure, and death in its most severe form. However, startlingly little is known about the risk factors, pathogenesis, or molecular mechanisms that perpetuate sustained systemic inflammation in cytokine storm syndromes. Our recent data implicates repeated adjuvant stimulation using the Toll-like Receptor 9 (TLR9) agonist CpG as a model of cytokine storm. In this model, repeated stimulation through TLR9 leads to an interleukin (IL)-12-driven, interferon-g-dependent feed-forward inflammatory response culminating in systemic immunopathology. These results contradict the well-known immunoregulatory phenomenon of TLR tolerance, whereby the initial activation of TLRs results in impaired proinflammatory responses to subsequent TLR stimuli. We now show that repeated injections of CpG in C57BL/6 mice results in a dose-dependent increase in cytokine production, cellular tissue infiltration, and immunopathology. Interestingly, this systemic inflammatory response correlates with continued *ex vivo* TLR9 responsiveness of splenic, liver, and bone marrow leukocytes after isolation from a CpG inflammatory environment, although these cells undergo TLR9 tolerance normally *ex vivo*. Identification of the immunopathogenic IL-12-producing cell as a $Ly6C^{+}CCR2^{+}$ inflammatory monocyte implicates these cells and the chemokine, CCL-2, as important contributors to systemic inflammation during cytokine storm. Finally, the continuous generation of new CpG-responsive inflammatory monocytes from myeloid precursors may provide a continuous source of TLR9 responsive cells and enhance systemic inflammatory responses during TLR9-mediated cytokine storm. These data support a novel mechanism whereby persistent adjuvant is sufficient to drive uncontrolled cytokine storm and immunopathology by bypassing TLR9 tolerance *in vivo*.

S2A. Ndfip1 and Ndfip2 activate catalytic E3 ubiquitin ligases to prevent aberrant T cell activation and Th2 differentiation

O'Leary CE[1], Riling C[1], Deng G[2], Spruce L[2], Seeholzer S[2], Oliver PM[1,2]

[1] *Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA* [2] *Department of Pathology, Children's Hospital of Philadelphia, Philadelphia PA*

Ubiquitylation tunes signaling pathways in stimulated T cells to regulate activation and function. Catalytic E3 ubiquitin ligases, like Itch, have known roles in these processes. We have found that Nedd4-family interacting protein 1 (Ndfip1) and Ndfip2 promote ubiquitin charging of Itch, a requisite step for Itch catalytic activity. *In vitro*, Ndfip1 and Ndfip2 have overlapping function. *In vivo*, Ndfip1 negatively regulates T cell activation and Th2 polarization. The *in vivo* role of Ndfip2 is unknown. To investigate this, we generated Ndfip2-/- mice. We found that, unlike Ndfip1, Ndfip2 is not a

prominent negative regulator of T cell activation or Th2 polarization. However, loss of Ndfip2 exacerbates the inflammatory Ndfip1-/- phenotype, suggesting that, like Ndfip1, Ndfip2 dampens inflammatory processes. Our data indicate a T cell intrinsic role for Ndfips in limiting T cell activation and function, as Ndfip1/Ndfip2 DKO CD4+ T cells in mixed fetal liver chimeras are more activated and produce more cytokine than WT CD4+ T cells in the same host. These results suggest that together Ndfip1 and Ndfip2 limit T cell activation, proliferation and Th2 cytokine production. To understand what aberrantly ubiquitylated proteins might contribute to this phenotype, we have taken a quantitative proteomic approach using Stable Isotope Labeling of Amino Acids in Cell Culture (SILAC) in combination with enrichment of ubiquitylated proteins using a Tandem Ubiquitin Binding Entity (TUBE). This proteomic approach has yielded multiple putative substrates of Ndfip-E3 ligase complexes that will be the focus of future experiments.

S2B. Factors released in local responses in the skin can systemically increase regulatory T cell numbers and protect against type 1 diabetes

Theresa Leichner[1], Atsushi Satake, Brian Kim, Mark Siracusa, Ali Naji, David Artis, and Taku Kambayashi

[1] *Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania*

Regulatory T cells (Tregs) are a subset of CD4+ T cells with suppressive function critical to limiting autoimmunity. Increasing Treg numbers has been shown to be beneficial for the treatment of inflammatory disorders, however the mechanisms that control the expansion of Tregs are not fully understood. Here, we provide evidence that the skin can exert systemic effects on Treg numbers. Topical, but not intraperitoneal, administration of the Vitamin D3 analog MC903 resulted in a doubling of Treg percentages throughout treated mice. The increase in Tregs was dependent on thymic stromal lymphopoietin (TSLP) release from MC903-treated skin; TSLP was increased in the serum of MC903-treated mice and MC903 treatment of TSLP receptor (TSLP-R) knock out (KO) mice abrogated Treg increases. Adoptive transfer of TSLP-R Tregs demonstrated that TSLP-R expression on Tregs was not required for their proliferation after MC903 treatment. *In vitro* cultures revealed that DC stimulation of Treg proliferation occured in the absence of IL-2 if given TSLP, and that TSLP synergized with IL-2 to drive further Treg expansion, suggesting that TSLP may increase Treg numbers by affecting the DC/Treg interaction. To test whether MC903-treated skin affects the progression of an autoimmune disorder, non-obese diabetic (NOD) mice were treated topically with MC903 or vehicle. Treatment with MC903 significantly lowered the incidence of diabetes from 100% to 40%. Together these data describe the ability of local events in the skin to affect systemic Treg numbers, which could serve as a strategy to induce systemic immunomodulation in the treatment of autoimmune diseases.

S2C. IL-4 and IL-21 reciprocally regulate T-bet expression in the context of TLR9-stimulated B cells

Martin S. Naradikian[1], Rosanne Spolski[3], Warren J. Leonard[3], E. John Wherry[4], Christopher A. Hunter[5], Ann Marshak-Rothstein[2], and Michael P. Cancro[1]

[1] *Department of Pathology, University of Pennsylvania, Philadelphia, PA, 19104*
[2] *Department of Medicine, University of Massachusetts School of Medicine, Worcester, MA 01605*
[3] *Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute (NHLBI), US National Institutes of Health, Bethesda, Maryland 20892, USA*
[4] *Department of Microbiology and Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*
[5] *Department of Pathobiology, University of Pennsylvania, Philadelphia, PA 19104, USA*

A sizable literature has characterized the transcription factor T-Box Expressed in T cells (T-bet) in the T lineage. Since T-bet expression in B cells has been linked with class switching to IgG_{2c}, propensity for humoral autoimmunity, and the formation of age-associated B cells (ABCs), the extrinsic signals sufficient for the induction of T-bet in the B lineage are of particular interest for vaccination and therapeutic reasons. Here we show that concomitant engagement of TLR7 or TLR9 and the IL-21 receptor induces *Tbx21* expression in B cells. In this context, IL-21 upregulates T-bet and CD11c, closely matching the phenotype of ABCs. Coculture experiments indicate IL-21 and TLR9 agonists function in a cell intrinsic manner and induce a phenotype distinct from IFN-γ. Moreover, IL-4 antagonizes IL-21-induced T-bet and CD11c expression in a STAT6 dependent manner. We further recapitulate this novel regulation of T-bet *in vivo* by immunizing *Il-4*$^{-/-}$ mice with streptavidin-CpG complexes (STREP9). Streptavidin specific antibodies from STREP9 immunized *Il-4*$^{-/-}$ mice were primarily of the IgG_{2c} isotype compared to IgG_1 from WT counterparts. Lastly, we show that aged mice accrue antibodies of the IgG_{2c} isotype consistent with reports showing that T follicular helper cells from aged mice produce as much IL-21 but less IL-4 compared to adult mice. Future studies will investigate the role of this previously unappreciated pathway in the production of class switched DNA autoantibodies and the emergence of ABCs in aged mice.

S2D. Blockade of Interleukin-33 Signaling Prevents Death in the Murine Model of Familial Hemophagocytic Lymphohistiocytosis

Julia Rood[1], Portia Kreiger[2], Erietta Stelekati[1], E. John Wherry[1], and Edward Behrens[1,3]

[1] *Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA*
[2] *Department of Pathology, Alfred I. duPont Hospital for Children, Wilmington, DE*
[3] *Division of Rheumatology, The Children's Hospital of Philadelphia, Philadelphia, PA.*

Hemophagocytic syndromes, such as macrophage activation syndrome and familial hemophagocytic lymphohistiocytosis (FHL), represent important causes of mortality in pediatric rheumatology. Studies of a mouse model of FHL, in which lymphocytic choriomeningitis virus (LCMV) infection of perforin knockout (PKO) mice triggers the disease, have demonstrated that FHL is driven by an excess of LCMV-specific $CD8^+$ T cells and their overproduction of interferon-γ (IFNγ). While this overactive T cell response is thought to arise from excess antigen stimulation through the TCR, data from our lab suggest that non-TCR signaling pathways may additionally contribute, as mice deficient in both perforin and MyD88 are protected from FHL. We have identified interleukin-33 (IL-33), a MyD88-dependent cytokine released by damaged tissue, as playing an important role in driving FHL. LCMV-infected PKO mice receiving IL-33 receptor-blocking antibody (IL-33RB) show markedly improved survival compared to isotype-treated controls ($p=0.0005$). IL-33R blockade reduced weight loss ($p<0.0001$), hepatic parenchymal damage ($p=0.0014$), and serum levels of IFNγ ($p=0.0005$). Additionally, IL-33RB mice have both lower frequencies and lower

IFNγ MFI of IFNγ^{+} CD8^{+} T cells (p=0.03 and p=0.02, respectively). Despite the reduced inflammation in IL-33RB mice, they maintain equivalent titers of LCMV in the spleen compared to controls. These data demonstrate that disruption of IL-33 signaling improves morbidity and mortality in a mouse model of FHL without exacerbating the underlying viral infection. Our results identify signaling via the tissue damage-associated cytokine IL-33 as an additional pathway contributing to disease and suggest blockade of this pathway as a viable treatment strategy for FHL.

S2E. The Cytokine IL-27 Limits Acute and Chronic Manifestations of Lung Disease in a Mouse Model of Post Viral Asthma

Gaia Muallem[1,2] Sagie Wagage[1] Carolina B Lopez[1] and Christopher Hunter[1]

[1] *Department of Pathobiology, University of Pennsylvania, Philadelphia, PA*
[2] *Department of Nephrology, University of Pennsylvania, Philadelphia, PA*

IL-27 is a heterodimeric cytokine composed of the subunits EBi3 and IL-27p28 that is involved in limiting the intensity and duration of T cell responses. One important function of IL-27 is modulation of the Th17 response during infection. In this study, we evaluate the role of IL-27 on the acute and chronic effects of upper respiratory infection. Sendai virus (SeV) is a murine parainfluenza virus that replicates at a high efficiency in the mouse lung. In this infection, acute injury is followed by a delayed chronic airway disease with similarities to human post-viral asthma and characterized by goblet cell hyperplasia and increased mucus production. SeV infection is associated with high levels of type I interferons which are potent inducers of IL-27. Here, IL-27 is shown to limit the Th17 response to infection with SeV. Acutely, increased expression of IL-17 in infected IL-27KO mice leads to enhanced neutrophil recruitment to the site of infection. Chronically, IL-27 deficiency exacerbates the postviral phenotype seen in Sendai infection. IL-27KO mice, despite having cleared the virus, have evidence of increased markers of Th2 inflammation, including Muc5a, Fizz1, and MMP12, when compared to wild-type. This work identifies a role for IL-27 in the amelioration of acute and chronic effects of SeV in the mouse lung with potential therapeutic implications for post-viral inflammatory lung disease.

S3A. Inhibition of the transcription factor Ikaros augments the tumoricidal capacity of CD8+ T cells expressing Chimeric Antigen Receptors

Shaun O'Brien[1,2], Kheng Newick[1], Rajan M Thomas[2], Cody E. Cotner[1], Veena Kapoor[1], Paul Kennedy[1], Albert Lo[5], Ellen Puré[3,4,5], Carl H June[2,4], Liang-Chuan S. Wang[1*‡], Steven M Albelda[1], Andrew D Wells[2*‡].

[1] *Thoracic Oncology Research Laboratory*
[2] *Department of Pathology and Laboratory Medicine*
[3] *Cell and Molecular Biology Graduate Group*
[4] *Abramson Family Cancer Research Institute, Perelman School of Medicine*
[5] *Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.*

Recent success of adoptive transfer of T cells expressing Chimeric Antigen Receptors (CARs) in patients with blood-borne malignancies has resulted in growing enthusiasm to treat solid tumors. Effective tumor immunotherapy of solid tumors requires overcoming the immunosuppressive tumor microenvironment and intrinsic T cell negative regulators. One such regulator is Ikaros, a key chromatin-remodeling factor that restrains naïve CD8+ T cell differentiation and prevents immunopathology. Intriguingly, naïve CD8+ T cells with reduced levels of Ikaros are able to differentiate in the absence of CD4 help or exogenous cytokines and have enhanced anti-tumor activity *in vivo.* We hypothesized that CAR T cells with reduced Ikaros activity would augment tumor

immunotherapy of solid tumors. We demonstrate that anti-mesothelin targeted CAR T cells on a Ikaros-deficient background have enhanced lytic function *in vitro* and this is associated with increased IFN-γ, TNF-α, Granzyme B and CD107α activity. This enhanced effector function is not due to proximal TCR signaling defects and appear related to Ikaros' regulation of the cytokine gene loci. *In vivo,* Ikaros-deficient CAR T cells have enhanced anti-tumor activity compared to WT CAR T cells when used to treat established mesothelin-expressing tumors and achieve this through increased persistence in the immunosuppressive tumor microenvironment and increased levels of IFN-γ. These findings have clinical revelance, as Ikaros inhibition can be achieved through the use of the immunomodulatory drug Lenalidomide, which targets Ikaros for ubiquitination. Our preliminary data indicates that Lenalidomide mediated inhibition of Ikaros results in enhanced cytokine production in human PBMCs and tumor infiltrating lymphocytes (TILs) and could provide a novel method to restore anti-tumor function of TILS and adoptively transferred CAR T cells.

S3B. Cross-reactivity of VH1-46 Antibodies to Desmoglein 3 and Rotavirus VP6 May Explain Their Persistence in Pemphigus Vulgaris

Cho MJ[1], Hammers CM[1], Sapparapu G[2], Crowe JE[2], Payne AS[1].

[1] *University of Pennsylvania School of Medicine*
[2] *Vanderbilt Medical Center, United States*

Pemphigus vulgaris (PV) is a potentially fatal blistering disease caused by autoantibodies (autoAbs) to desmoglein 3 (Dsg3). To better understand how Dsg3 autoAbs develop, we have performed high throughput B cell receptor (BCR) cloning from six PV patients, and have identified VH1-46 gene usage in the anti-Dsg3 repertoire among all PV patients studied to date. Common gene usage indicates common mechanisms for developing autoimmunity, even among unrelated patients. Interestingly, shared VH1-46 gene usage also occurs in the Ab response to the rotavirus capsid protein VP6, and few to no somatic mutations are required for VH1-46 antibodies to bind Dsg3 or VP6. We investigated whether Dsg3-reactive VH1-46 Abs are cross-reactive to VP6 and/or clonally related to VP6-reactive Abs within the same patient, which may help explain the tolerance of autoreactive clones in the Ab repertoire. Panning an IgM library from a PV patient against Dsg3 revealed that 4/8 heavy chains used VH1-46. We cross-panned this same library against both Dsg3 and VP6 and isolated 7 unique VH1-46 IgM heavy chain sequences, 3 of which were also found in the Dsg3-only panning. Confirmatory ELISA studies demonstrate that 3 VH1-46 and 1 VH3-64 clones are cross-reactive to VP6 and Dsg3. Functional studies are ongoing to determine whether Dsg3-reactive Abs can inhibit VP6- dependent rotavirus transcription. Our data suggest that a VH1-46 Ab response to rotavirus may be an initiating event that develops into an autoimmune response to Dsg3 in certain individuals.

S3C. Preliminary Evaluation of CD8+ T Cells in Early Acute SIV Infection

Emily R. Roberts[1,2], Korey R. Demers[1,2], Guido Silvestri[3], Michael R. Betts[1]

[1] *Department of Microbiology, University of Pennsylvania, Philadelphia, PA*
[2] *Graduate Biomedical Graduate Studies, Immunology and Cellular and Molecular Biology-Microbiology, Virology, and Parasitology at the University of Pennsylvania, Philadelphia, PA*
[3] *Divison of Microbiology and Immunology, Emory Vaccine Center, Yerkes National Primate Research Center, Atlanta, Georgia, United States of America*

Human Immunodeficiency Virus (HIV) infection is characterized by rapid and persistent immune activation. Chronic immune activation has been described in both HIV+ patients as well as in

Rhesus macaques (RM) infected with Simian Immunodeficiency Virus (SIV), thus validating the use of RM SIV model for studying HIV pathogenesis. More recent data from our lab describes a rapid progression of peripheral blood CD8+ T cells towards a memory, non-naive, phenotype during early acute phase HIV infection that is accompanied by HLA-DR expression in the absence of T-bet expression. To validate the RM SIV infection as a model of acute phase HIV immunopathogenesis, we looked to confirm these findings in SIV infected RM. CD8+ T cells from SIV infected RM, from pre-infection through 6 weeks post infection time points, were evaluated by flow cytometry for expression of markers of T cell activation, and cycling, as well as T-bet and effector molecules.

We found that peripheral blood CD8+ T cells in SIV infected RM do not display the same shift to memory phenotype, nor the same magnitude of activation by HLA-DR, as detected in acute phase HIV infection. However, activation by CD69 and CD38 describe some kinetics in CD8+ T cells during acute infection. Additionally, CD8+ memory cells in some acute SIV infection display activation in the absence of Granzyme B expression, and to a lesser extent T-bet, unlike during acute HIV infection.

We have identified differential activation levels, and expression of factors associated with CD8+ T cell effector functions during acute phase SIV as compared to acute phase HIV infection. Thus, this may highlight some unparalleled immunopathology between the RM SIV model and HIV infection during the acute phase. Moreover, it should be evaluated more fully what affects these acute phase immune activation differences may have on the use of SIV in RM to study HIV pathogenesis in humans.

S3D. Novel Immunodysregulation disorder associated with a gain-of-function mutation in GNAI2

Ian T. Lamborn[1,2], Yu Zhang[1], Huie Jing[1], Alexey Koval[3], Judith N. Mandl[4], Heardley M. Murdock[1], Joshua McElwee[5], Helen F. Matthews[6], Uimook Choi[7], Sherry M. Koontz[7], Harry L. Malech[7], Ronald N. Germain[4], Kirk M. Druey[8], Vladimir L. Katanaev[3], Peter J. Mustillo[8], Michael J. Lenardo[6], and Helen Su[1]

[1] *Human Immunological Disease Unit, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, Bethesda MD 20892*
[2] *Immunology Graduate Group, University of Pennsylvania, Philadelphia 19104*
[3] *Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland CH-1005*
[4] *Lymphocyte Biology Section, Laboratory of Systems Biology, National Institute of Allergy and Infectious Diseases, Bethesda MD 20892*
[5] *Merck Research Laboratories, Merck & Co., Boston, MA 02115*
[6] *Molecular Development of the Immune System Section, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda MD 20892*
[7] *Genetic Immunotherapy Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, Bethesda MD 20892*
[8] *Molecular Signal Transduction Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda MD 20892*
[9] *Department of Allergy and Immunology, Nationalwide Children's Hosptial, Columbus OH 43205*

Primary immunodeficiencies frequently co-present with severe autoimmunity; however, the molecules essential for both immunoprotection and self-tolerance are incompletely understood. We therefore studied a patient with life-threatening autoimmune cytopenias and psoriatic arthritis who also had recurrent mucocutaneous bacterial and viral infections. Additional features include myelocytosis, fused vertebrae, and growth retardation. We identified a *de novo* gain-of-function missense mutation in the heterotrimeric GTPase Gαi2 (GNAI2), which is the primary GTPase

mediating hematopoietic chemotaxis and serves essential roles in inflammation, hematopoiesis, bone formation, and metabolism. Patient T cells showed impaired chemokine induced Ca^{2+} flux and chemotaxis to SDF-1α and SLC. Overexpressing the mutant protein *in vitro* dominantly impaired chemokine induced Ca^{2+} flux and chemotaxis in healthy T cells, and biased toward myeloid lineage commitment in hematopoietic stem cells. *Ex vivo*, patient T cells show overproduction of IL-17 and IL-4 by CD4+ and CD8+ T cells respectively. To better understand the mechanism of this disease, experiments are underway to determine if the mutant protein intrinsically biases T cell differentiation or impairs regulatory T cell function. Thus, our work describes the genetic and cellular basis of a novel immunodysregulation disorder defined by defective chemokine signaling and pleiotropic defects in immunity, self-tolerance, and development.

S3E. Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013-2014 influenza season

Susanne L. Linderman[1,2], Benjamin S. Chambers[1,3], Seth J. Zost[1,3], Kaela Parkhouse[1], Yang Li[1,3], Christin Herrmann[1,3], Ali H. Ellebedy[4,5], Donald M. Carter[6], Sarah F. Andrews[7], Nai-Ying Zheng[7], Min Huang[7], Yunping Huang[7], Donna Strauss[8], Beth H. Shaz[8], Richard L. Hodinka[9], Gustavo Reyes-Teran[10], Ted M. Ross[6], Patrick C. Wilson[7], Rafi Ahmed[4,5], Jesse D. Bloom[11], Scott E. Hensley[1,2,3,]

[1] *Wistar Institute, Philadelphia, PA 19104, USA*
[2] *Institute for Immunology, Perelman School of Medicine, The University of Pennsylvania, Philadelphia, PA 19104, USA*
[3] *Department of Microbiology, Perelman School of Medicine, The University of Pennsylvania, Philadelphia, PA 19104, USA*
[4] *Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA 30322, USA*
[5] *Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA*
[6] *Vaccine and Gene Therapy Institute of Florida, Port St. Lucie, FL 34987*
[7] *Department of Medicine, The University of Chicago, Chicago, IL 60637, USA*
[8] *New York Blood Center, New York, NY 10065, USA*
[9] *Clinical Virology Laboratory, Children's Hospital of Philadelphia, and Laboratory Medicine, Perelman School of Medicine, The University of Pennsylvania, Philadelphia, PA 19104*
[10] *Center for Research in Infectious Diseases, National Institute of Respiratory Diseases, Mexico City, Mexico*
[11] *Division of Basic Sciences and Computational Biology Program, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA*

Influenza viruses typically cause the most severe disease in children and elderly individuals. However, H1N1 viruses disproportionately affected middle-aged adults during the 2013-2014 influenza season. Although H1N1 viruses recently acquired several mutations in the hemagglutinin (HA) glycoprotein, classical serological tests utilized by surveillance laboratories indicate that these mutations do not change antigenic properties of the virus. Here, we show that one of these mutations is located in a region of HA targeted by antibodies elicited in many middle-aged adults. We find that over 42% of individuals born between 1965 and 1979 possess antibodies that recognize this region of HA. Our findings offer a possible antigenic explanation of why middle-aged adults were highly susceptible to H1N1 viruses during the 2013-2014 influenza season. Our data further suggest that a drifted H1N1 strain should be included in future influenza vaccines to potentially reduce morbidity and mortality in this age group.

P1. Functional education of monocytes during infection occurs in the bone marrow

Michael H. Askenase[1,2], John R. Grainger[2,3], Allyson Byrd[2,3], and Yasmine Belkaid[1,2,3]

[1] *Immunology Graduate Group, University of Pennsylvania, Philadelphia, PA*
[2] *Immunity at Barrier Sites Initiative, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda MD*
[3] *Mucosal Immunology Section, Laboratory of Parasitic Diseases, NIAID, NIH Bethesda, MD*

Infection induces rapid recruitment of circulating inflammatory cells to inflamed tissue, and this influx of cells necessitates regulatory mechanisms to limit inflammation. We recently reported a previously unappreciated dual activity of Ly6C^{hi} monocytes in controlling pathogen expansion while limiting immunopathology in response to commensal microbes during acute mucosal infection. These cells ensure host survival via production of the regulatory mediator Prostaglandin E2 (PGE_2). We hypothesized that systemic signals emanating from mucosal tissue early during infection induce these unique regulatory features in monocytes prior to tissue recruitment.

To address this question, we infected mice with the intestinal parasite *Toxoplasma gondii*. Early post-infection when the parasite is limited to the intestine and its draining lymph, we found that Ly6C^{hi} monocytes and their precursors in the bone marrow and blood dramatically altered their gene expression profile and acquired a previously undescribed MHC II^{hi} Cx3CR1^{lo} phenotype. Monocytes from *T. gondii* infected mice produced significantly more PGE_2 and IL-10 than those from naïve mice when stimulated. The observed changes to monocytes were dependent on IFN-γ, and administration of IFN-γ was sufficient to impart monocytes with regulatory function *in vivo*. Notably, innate lymphoid cells in the bone marrow produced IFN-γ early during infection, and depletion of these cells impaired changes to monocyte phenotype. These findings indicate that during *T. gondii* infection, Ly6C^{hi} monocytes acquire critical regulatory functions in response to early IFN-γ signaling. This highlights the importance of systemic signals in educating myeloid development during infection and directing the function of these cells upon tissue recruitment.

P2. Globin genes compete dynamically for enhancer activity in single cells

Caroline R. Bartman[1], Chris Hsiung[1], Jeremy Rupon[1], Arjun Raj[2], and Gerd A. Blobel[1]

[1] *Department of Pediatrics, Children's Hospital of Pennsylvania*
[2] *Department of Bioengineering, University of Pennsylvania*

Cell identity is defined by the expression of cell-type-specific genes, including transcription factors (e.g. T-bet in T_H1 cells) and effector molecules (e.g. IFN-γ), that are under the control of gene-specific distal enhancers. Enhancers are thought to activate transcription by physically contacting their target gene promoters. Whether enhancers stimulate transcription of all alleles in a population at any given time or whether enhancer-gene contacts are dynamic, occurring only at a fraction of alleles that would result in variegated or pulsating gene expression, has not been carefully studied.

We examined enhancer activity by combining a system that employs forced enhancer-promoter chromatin looping with single molecule RNA FISH experiments. This newly developed model system enables quantitative examination of gene expression (in this case of the beta-type globin genes) in response to controlled alterations of enhancer function in primary human erythroid cells.

Forced juxtaposition of the globin enhancer and the fetal globin promoter in adult erythroid cells

increases the number of cells expressing only fetal globin while reducing the number of cells expressing only the adult globin gene. This suggests that there is a tradeoff between fetal and adult globin expression in single cells. Surprisingly, the proportion of cells transcribing both fetal globin and adult globin remains largely constant upon fetal globin induction. Moreover, a fraction of alleles cotranscribes fetal and adult globin genes simultaneously from the same allele. This observation suggests that the globin enhancer may be able to rapidly switch between target genes within the measured time window. Future directions include confirming the enhancer dependence of gene cotranscription, defining enhancer elements that facilitate transcription initiation and gene switching, and examining the kinetics and memory of enhancer dependent gene activation.

P3. Development of a CyTOF panel to analyze lymphocyte subset complexity

Bertram Bengsch[*1], Ramin Herati[*1], Emily Roberts[*1], Daniel Traum[2], Suzanne Ho[2], Jang-June Park[2], Michael R. Betts[*1], Kyong-Mi Chang[*2], E. John Wherry[*1]

[*] equal contribution
[1] *Institute for Immunology, Dpt. Microbiology, University of Philadelphia*
[2] *Philadelphia VAMC , Philadelphia, PA , USA*

Measuring cellular properties by FACS is currently practically limited to <18 probes, mainly due to difficulties with fluorescent probe availability, spillover compensation and instrument restrictions. The development of mass cytometry based on the differential detection of time-of flight of rare metal isotopes by CyTOF allows the use of metal-tagged probes that overcome this limitation. The first aim of this group effort between the IFI and the VA is the establishment of a lymphocyte phenotyping panel suitable for the simultaneous analysis of relevant human T cell, B cell and NK cell subsets.

Rare metal isotopes with high purity were loaded to polymers and conjugated to purified antibodies targeting relevant lymphocyte antigens. Antibody titrations were performed to determine optimal staining conditions. Cells were determined by Iridium intercalation and ion cloud event length. Mass cytometry data was analyzed by FlowJo software and compared to flow cytometry results. Visualization of high-dimensional single-cell data was performed using SPADE (Spanning Tree Progression of Density Normalized Events) that organizes cells into hierarchies of related phenotypes.

We achieved the simultaneous detection of 27-30 lymphocyte antigens on single-cells allowing a comprehensive phenotyping of human T cell, B cell and NK cell subsets. In a next step, we will target the simultaneous detection of 36-38 mass parameters by end of 2014 and the development of a multi-cytokine and intracellular signaling panel.

P4. TET2 regulates CD8$^+$ T cell differentiation

Shannon A. Carty[*], Mercy Gohil[*], Gary A. Koretzky[§] and Martha S. Jordan[†]

[*] Department of Medicine Perlman School of Medicine, University of Pennsylvania, Philadelphia, PA
[§] Department of Medicine, Weill Cornell Medical College, New York, NY
[†] Department of Pathology and Laboratory Medicine, Perlman School of Medicine, University of Pennsylvania, Philadelphia, PA

Regulation of DNA methylation is critical for proper T cell differentiation and function. Antigen-specific CD8$^+$ T cells undergo global remodeling of DNA methylation following viral infection,

suggesting that DNA methylation may direct antigen-specific T cell responses. TET2 is a member of the Ten-Eleven-Translocation (TET) family, which converts 5-methylcytosine (5mC) in DNA to 5-hydroxymethylcytosine (5hmC) and subsequent intermediates ultimately leading to DNA demethylation. How TET2 regulates T cell differentiation and function is unknown. Here we demonstrate that TET2 expression is regulated by TCR signaling in primary T cells. Using a novel flow cytometric assay to measure 5hmC levels on a single cell basis, we find that TCR signaling also regulates TET activity as evidenced by a rapid increase in global 5hmC levels after TCR stimulation. Preliminary data suggest TET2 contributes to this increase as 5hmC levels are lower in T cells lacking TET2. To determine the role of TET2 in T cell responses, we generated mice deficient for TET2 in the T cell compartment ($TET2^{fl/fl}CD4Cre^{+}$ mice). These mice develop grossly normal thymic and peripheral T cell subsets. Following infection with LCMV-Armstrong, viral specific $CD8^{+}$ T cells from $TET2^{fl/fl}CD4Cre^{+}$ expand normally and display normal effector T cells responses; however TET2 deficient T cells exhibit significantly enhanced memory $CD8^{+}$ T cell differentiation compared to control mice. These data demonstrate that TET2 plays a critical role in directing $CD8^{+}$ T cell differentiation and function. Studies to identify specific TET2 target genes that are important in the development of $CD8^{+}$ T cell memory are ongoing.

P5. Human caspase-4 is a functional homolog of mouse caspase-11 and mediates inflammasome activation against bacterial pathogens

Cierra N. Casson[1], Frances O. Taschuk[1], Janet Yu[1], Valeria R. Ruiz[1], Anjana Yadav[2], Alan M. Copenhaver[1], Hieu T. Nguyen[1], Ronald G. Collman[2], and Sunny Shin[1]

[1] *Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA*
[2] *Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA*

Inflammasome activation is important for defense against bacterial pathogens because it induces cell death and regulates the secretion of IL-1 family cytokines. The canonical inflammasome activates caspase-1 to mediate cell death and cytokine release. In murine cells, caspase-11 also contributes to inflammasome activation. For *Legionella pneumophila* and other Gram-negative bacteria, caspase-11 controls IL-1α release and cell death and contributes to IL-1β secretion. However, humans do not encode caspase-11. Instead, humans encode caspase-4 and caspase-5, two closely related homologs of caspase-11. Therefore, we aimed to determine the inflammasome components in human cells that respond to bacterial pathogens, using *L. pneumophila,* the causative agent of the severe pneumonia known as Legionnaires' disease in humans, as a model. We find that both immortalized human monocytes and primary human monocyte-derived macrophages activate the inflammasome in a manner dependent on the type IV secretion system, a virulence factor the bacterium uses to establish its intracellular niche. While IL-1β secretion requires caspase-1 catalytic activity, IL-1α release and cell death are independent of caspase-1 activity. We find that caspase-4 contributes to IL-1α release in response to pathogenic *L. pneumophila* and other Gram-negative bacterial pathogens that use specialized secretion systems to access the host cell cytosol. Our findings demonstrate that human caspase-4 functions similarly to mouse caspase-11 to facilitate inflammasome activation in human macrophages and initiate host defense against bacterial pathogens.

P6. Transcriptional Profiling of Macrophages Injected, but Not Infected, by Toxoplasma gondii

Lucas A. Dawson[1], Daniel P. Beiting[1], Jung Oh[2], David A. Christian[1], David S. Roos[2], Anita A. Koshy[3], Christopher A. Hunter[1]

[1] *Department of Pathobiology, School of Veterinary Medicine, University if Pennsylvania*
[2] *Department of Biology, University of Pennsylvania*
[3] *Department of Neurology, University of Arizona*

Toxoplasma gondii is able to infect every warm blooded vertebrate, and establishes a chronic infection despite host immune pressure. The mechanisms that T. gondii uses to evade host immune responses are not well understood. One factor potentially important for T. gondii immune evasion is ROP16, a kinase that is injected into host cells' cytosol. ROP16 activates host STAT6 leading to increased transcription of STAT6 target genes. To track host cells that interact with ROP16, a T. gondii strain was created to express mCherry and inject Cre along with virulence factors into host cells(SeCreEt strains). When used with Ai6 mice, the fluorochrome ZsGreen1 is only expressed in cells injected with parasite virulence factors. This system led to the identification of cell populations into which T. gondii injects virulence factors, but does not infect(uninfected-injected,U-I). To determine if T. gondii induces STAT6 mediated transcriptional changes in U-I cells, this reporter system was used to perform in vitro infections of bone marrow-derived macrophages(BMDM) followed by microarray analysis. This revealed that transcriptional changes in U-I BMDM are similar to those in infected BMDM, and STAT6 dependent genes are induced similarly in U-I cells and infected cells. These findings show that T. gondii virulence factors act not only on infected cells but can act on U-I cells, and suggests that U-I cell populations may be involved in T. gondii immune evasion.

P7. IL-27 and inhibitory receptor expression

Jonathan DeLong[1], Aisling O'Hara Hall[1,2], Gaia Muallem[1], Qun Fang[1], Chris Hunter[1]

[1] *Dept. of Pathology, University of Pennsylvania*
[2] *Rockefeller University*

A variety of immunoregulatory mechanisms limit pathology from aberrant immune responses. One of these mechanisms is suppression by ligation of inhibitory receptors, which is able to inhibit both cells expressing the receptor and cells expressing the cognate ligands. For example, LAG-3 expressed by Tregs inhibits maturation of dendritic cells and PD-L1 expressed by T cells inhibits differentiation of naive T cells to the Th17 lineage. Ligation of Ly6C with antibodies inhibits CD4 T cell proliferation and IL-2 secretion. While inhibitory receptors have been shown to be key in the immune response, their regulation is not fully understood. IL-27 was demonstrated in 2012 to induce expression of PD-L1 on CD4+ T cells. The present study demonstrates that IL-27 also induces LAG-3 and Ly6C and that it does so on naïve CD4+ T cells, regulatory T cells and CD8+ T cells. In the absence of IL-27, the $CD4^+$ T cell response to *Toxoplasma gondii* becomes lethally pathogenic, and it is possible that PD-L1, LAG-3 and Ly6C play a role in controlling this $CD4^+$ T cell response. Indeed, the expression of PD-L1 during toxoplasmosis is lower in IL-27 deficient mice. Ongoing studies are designed to determine the significance of this in controlling infection-induced pathology.

P8. The RNA-binding protein HuR has critical roles in B lymphocyte development and function

†¥DeMicco, A., ‡*Naradikian, M., ‡Cancro, M.P., †Bassing, C.H.

† *Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia*
¥ *Cell and Molecular Biology Graduate Group, University of Pennsylvania*
‡ *Department of Pathology and Laboratory Medicine, University of Pennsylvania*
* *Immunology Graduate Group, University of Pennsylvania*

Post-transcriptional regulation of gene expression is critical for the survival, differentiation, and function of B and T lymphocytes. In other cell types, the RNA-binding protein HuR (Elavl1) modulates a wide variety of target genes, including many involved in lymphocyte development and function. To investigate roles of HuR in B cells, we generated mice conditionally deleted for HuR beginning in the earliest stages of B cell development using Cre recombinase driven by the Mb1 promoter. These mice have slightly increased numbers of pro-B cells, but reduced numbers of pre-B, transitional, and mature B cells. HuR-deficient pro- and pre-B cells have no proliferative defect or increased apoptosis, suggesting that differentiation signals may be aberrant or lacking. Despite developmental abnormalities, HuR-deficient mature B cells accumulate to 50% of wild type numbers, allowing us to investigate roles of HuR in B cell function. HuR is required for normal proliferation during *in vitro* stimulation, but is dispensable for immunoglobulin class switch recombination. HuR-deficient B cells also display aberrant antibody secretion *in vitro*. *In vivo,* HuR mutant mice have very low serum antibody titers and greatly reduced numbers of germinal center B cells and plasma cells. Upon immunization with NP-OVA, HuR-deficient mice show profound blocks in the generation of germinal centers and NP-specific antibodies. We are mining published PAR-CLIP and gene expression datasets to identify specific HuR targets involved in B cell function. Together, these data show that HuR is essential for normal adaptive immunity in mice by promoting B cell development and activation.

P9. Expression of Nedd4-family interacting protein-1 in T_{reg} cells prevents autoimmune disease

Guoping Deng, Claire O'Leary, Emily Moser and Paula Oliver

Regulatory T cells (T_{regs}) are a subpopulation of T cells that suppress immune function to prevent autoimmune disease and prevent collateral damage during infection. Understanding how T_{regs} acquire and execute their suppressive function is relevant for the study of disease pathogenesis and for modulating activity for therapeutic effect. The ubiquitin system is known to regulate the differentiation and function of conventional T (T_{conv}) cells. Our lab has shown that the E3 ubiquitin ligase Itch, and its co-activator Ndfip1, are required for indicible T_{reg} (iT_{reg}) differentiation. Our new data supports that Ndfip1 also regulates the function of T_{regs} following differentiation.

We generated mice lacking Ndfip1 only in T_{regs} (*Ndfip1$^{fl/fl}$Foxp3-YFP^{Cre}, aka Ndfip1$^{reg\text{-}null}$*). By 12 weeks of age, *Ndfip1$^{reg\text{-}null}$* mice developed inflammation at mucosal barrier surfaces, namely skin and lung. Additionally, spleens and lymph nodes of *Ndfip1$^{reg\text{-}null}$* were enlarged and serum antibody levels were elevated. Consistent with this, the frequency of activated T_{conv} cells in the spleens lungs of the mice were increased and these cells were making cytokines characteristic of T_H1, T_H2 and T_H17 cell subsets. Importantly, Ndfip1 was not required for T_{regs} to persist, nor was it required for T_{regs} to gain access into peripheral tissues, as the frequency of T_{regs} in the spleens and lungs were significantly increased in *Ndfip1$^{reg\text{-}null}$ animals*. Together, these data suggest that Ndfip1 is dispensable for T_{reg} survival but may be required for T_{reg} function. Current efforts are focused on whether and how Ndfip1 regulates the suppressive qualities of T_{reg} cells.

P10. IgA, IgG1 and IgG4 harbor clonally distinct desmoglein 3 autoreactive repertoires in pemphigus vulgaris

Christoph T. Ellebrecht[1], Eric M. Mukherjee[1], Eun J. Choi[1], Aimee S. Payne[1]

[1] *Dermatology, University of Pennsylvania, Philadelphia, PA, United States*

Pemphigus vulgaris (PV) is a potentially fatal autoimmune disease of the skin and mucous membranes characterized by autoantibodies (autoAbs) to desmoglein (Dsg) 3. During active disease, the majority of PV autoAbs belong to the IgG4 and less so the IgG1 subclass. However, >50% of patients have serum anti-Dsg3 IgA, the role of which in PV has not been characterized. To better understand the clonal relationships among isotype-specific autoreactive B cells in PV, we cloned anti-Dsg3 IgA1, IgA2, IgG1, and IgG4 repertoires from a mucocutaneously affected PV patient by antibody phage display. We screened >8x10 combinatorial B cell clones per antibody subclass and characterized 55, 20, 40 and 88 clones from the IgA1, IgA2, IgG1, and IgG4 libraries after Dsg3 selection, which identified 17, 5, 15, and 29 unique anti-Dsg3 heavy chain sequences comprising 5, 4, 4, and 7 CDR3 clonal families, respectively. One clonal family was shared between IgA1 and IgA2. None were shared between IgA1/2, IgG1 and IgG4. Also, by CDR3-specific RT-PCR, we found no evidence of the IgA clones in the IgG4 repertoire. No anti-Dsg3 IgA were found in an unaffected individual. Interestingly, an expanded IgA1 family was identified that used the VH3-15 gene segment, the predominant VH gene used in the natural and vaccine induced response to *Haemophilus influenzae* type B capsular polysaccharide (HIB PRP). The VH3-15 anti-Dsg3 mAb specifically crossreacted to HIB PRP, but not other self-antigens such as RNA polymerase II or BP180 glycoprotein. This crossreactivity was encoded by the heavy chain and was lost after reversion of somatic mutations to the germline VDJ sequence. Additionally, a VH3-15 anti-HIB PRP IgA1 clone isolated by heterohybridoma from an adult after HIB infection showed crossreactivity with Dsg3 by ELISA and immunofluorescence. In summary,we find that anti-Dsg3 IgA, IgG1 and IgG4 repertoires in PV are clonally unrelated and that anti-Dsg3 IgA can harbor pathogen cross-reactivity. Our results suggest independent evolution of anti-Dsg3 isotype-specific B cell lineages in PV.

P11. Pre-B cells suppress RAG expression in response to DNA damage

Megan Fisher[1], Craig Bassing[1]

[1] *Immunology Graduate Group, University of Pennsylvania; Division of Cancer Pathobiology, Children's Hospital of Philadelphia*

B and T cells create unique antigen receptors by V(D)J recombination, a process of DNA rearrangement in which RAG proteins make DNA double-strand breaks (DSBs) at specific gene segments. This process must be carefully regulated to prevent translocations and other aberrant events. We have found that in pre-B cells, both DSBs induced exogenously by ionizing radiation (IR) and RAG-induced DSBs lead to decreased expression of the RAG recombinase. Expression of Gadd45α, which promotes *Rag1* and *Rag2* transcription in pre-B cells, also decreases in the presence of DSBs. Therefore we hypothesized that DSBs suppress RAG expression primarily by inhibiting *Rag1* and *Rag2* transcription. To measure *Rag* transcription, we used Click-iT nascent RNA capture, measuring the accumulation of *Rag1* or *Rag2* mRNAs labeled with a modified uracil over time, in cells either exposed to IR or unexposed. We found that transcription of *Rag1* and *Rag2* decreases dramatically after IR, while similar experiments showed only a minor effect of IR on *Rag* mRNA stability. Additionally, we have found the regulation of *Rag1* and *Rag2* mRNA expression by DSBs depends on the DNA-damage response protein ATM. However, preliminary experiments suggest that an additional ATM-independent mechanism may exist to regulate RAG2 protein expression independently of mRNA levels. Together, these data indicate that pre-B cells

have evolved multiple mechanisms regulating the RAG recombinase in the presence of DSBs. In ongoing studies, we seek to identify intermediaries in these pathways and determine their contribution to preserving genomic stability in developing lymphocytes.

P12. Activating receptor signaling is required for natural killer cells to acquire Ly49 receptors during development

Jacquelyn Freund[1], Rebecca M. May[1], and Taku Kambayashi[1]

[1]Department of Pathology and Laboratory Medicine Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Natural killer (NK) cells function by balancing intracellular signals from germline-encoded activating and inhibitory receptors that are expressed on the NK cell surface. In mice, a large portion of NK cell receptors fall under the Ly49 receptor family. Ly49 receptors are necessary for missing-self recognition and play an important role in virus and tumor recognition, complementary to CD8 T cells. Ly49 receptor expression on NK cells is very diverse, with certain NK cells expressing multiple receptors and others expressing none. The diversity of expression determines target cell specificity and aids in self-tolerance, but the mechanism behind what regulates Ly49 receptor acquisition is undetermined. Here, we investigated what Ly49 receptor acquisition during NK cell development. We found that the adaptor molecule SH2 domain-containing leukocyte protein of 76kD (Slp-76) is critical for Ly49 receptor expression on NK cells. Slp-76 is a required adaptor protein for NK cell activating receptor signaling. Antibody-mediated crosslinking of Slp-76-dependent activating receptors *in vitro* leads to Ly49 receptor acquisition. Furthermore, data suggests that loss of Slp-76 in NK cells leads to defects early bone marrow development Ly49 receptor transcription. These results, and others, suggest that activating receptor signaling in NK cells, potentially through non-Ly49 activating receptors, drive the expression of Ly49 receptors during development.

P13. Lymphoid tissue-resident commensal bacteria colonize dendritic cells and induce local Th17 and group 3 innate lymphoid cell responses

Thomas C. Fung[1,2,3,4], Matthew R. Hepworth[3,4], Dmytro Kobuley[2], Kelvin Wang[2], David Artis[3,4,5], Gregory F. Sonnenberg[3,4,5]

[1] *Immunology Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*
[2] *Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*
[3] *Department of Medicine, Gastroenterology Division, Weill Cornell Medical College, New York, NY*
[4] *Jill Roberts Institute for Research in Inflammatory Bowel Disease, Weill Cornell Medical College, New York, NY*
[5] *Department of Microbiology and Immunology, Weill Cornell Medical College, New York, NY*

The mammalian intestine is colonized with trillions of commensal bacteria that are essential for normal host physiology. However, segregation of commensal bacteria and host immune cells is required to limit chronic inflammation that is associated with the development of inflammatory bowel disease and colon cancer. In contrast, recent reports have shown that defined populations of commensal bacteria can reside within healthy mammalian lymphoid tissues, yet how commensal bacteria can persist within lymphoid tissues and the functional consequences of this colonization for the host remain poorly understood. Here we demonstrate that *Achromobacter*, a lymphoid tissue-

resident commensal bacterium, can live within murine dendritic cells and induce the cytokines IL-1β and IL-23 *in vitro*. *In vivo*, *Achromobacter* colonizes the Peyer's patches and mesenteric lymph node of germ-free and conventional mice, and selectively induces T helper 17 and group 3 innate lymphoid cell responses in these tissues. These data demonstrate a previously unrecognized role for lymphoid tissue-resident commensal bacteria in regulating mammalian immune cell responses. Ongoing studies are interrogating the function of this unique host-commensal bacteria interaction during health and inflammation.

P14. Localization of ELAVL1 correlates with Sendai Virus defective viral genome replication and activation of innate immunity

Jennifer T. Grier[1], Sabrina Tsang[1], Wenzhi Song[1], Carolina B. López[1]

[1] *University of Pennsylvania School of Veterinary Medicine, Department of Pathobiology*

During rapidly replicating RNA virus infections, the viral polymerase generates shortened defective viral genomes (DVGs). These DVGs induce a potent antiviral immune response through the RIG-I-like receptor (RLR) family of viral RNA sensors. Given the similarity of RNA sequences of the viral genome and the DVGs, it remains unclear why only DVGs trigger the immune response during natural infection. We hypothesize that additional host RNA binding proteins (RBPs) preferentially bind to DVGs and mediate their interactions with the RLRs. An understanding of host factors that mediate the DVG-triggered antiviral immunity could be employed to bolster protective immunity following vaccination against viruses such as Measles or Respiratory Syncytial Virus, which naturally produce DVGs. Sendai Virus (SeV), is a negative sense ssRNA virus that serves as a model to study mechanisms of virus-host interactions in the presence or absence of DVGs. Immunoprecipitation of SeV nucleoprotein (NP), the viral-encoded RBP, identified a host RBP, ELAVL1, which only precipitated when DVGs were present. During SeV infection, it was observed by immunofluorescence that ELAVL1 translocated from the nucleus into the cytoplasm, the site of SeV replication, and at late time points, strongly co-localized with SeV NP. The timing of ELAVL1 re-localization closely matched induction of the innate immune response as well as a shift in the ratio of (+) to (-) stranded DVGs towards the immunostimulatory (+) sense. Thus, ELAVL1 appears to interact with SeV NP and/or DVGs during viral replication, and may play a role in the host antiviral response to SeV infection.

P15. Non-Canonical Functions of the RAG1 Protein Stimulate Igl^{+} B Cell Development by Promoting Igl Locus Accessibility and Transduce Pro-Survival Signals in Pre B cells

Horowitz, J.E., and Bassing, C.H.

Functions of the RAG1/ RAG2 (RAG) endonuclease in V(D)J recombination have been studied primarily using truncated "core" enzymes—the minimal forms required for generating DNA double strand breaks (DSBs). Humans and mice expressing truncated "core", but not full-length Rag1 (Rag1$^{C/C}$ mice), have reduced numbers of progenitor lymphocytes associated with decreased D-to-J and V-to-DJ recombination at IgH and TCRb loci. However, roles for non-core RAG1 regions in promoting V-to-J rearrangements at Igk and Igl loci in developing pre B cells have not been elucidated. To determine whether non-core RAG1 regulates Igk/Igl recombination, we analyzed late B cell development in Rag1$^{C/C}$ mice. We find that Rag1$^{C/C}$ mice exhibit slight impairment in Igk^{+} B cell development but profound defects in Igl^{+} B cell development associated with reduced Vl-to-Jl recombination. Development of Igl^{+} B cells depends on RAG accessibility to the Igl locus and activation of signals that induce expression of pro-survival genes. Accordingly, we find that Rag1 protein promotes accessibility of Igl gene segments for recombination. Further, RAG DSBs in pre B

cells induce pro-survival factors including *Pim2*, which promotes Igl^+ B cell development. We find that $Rag1^{C/C}$ pre B cells do not upregulate *Pim2* following RAG DSBs and that expression of pro-survival BCL2 protein rescues Igl^+ cell development in $Rag1^{C/C}$ mice. Collectively, these data demonstrate novel requirements for the RAG endonuclease in establishing a normal immune repertoire by promoting accessibility of Igl gene segments prior to recombination and by upregulating pro-survival *Pim2* during RAG DSBs.

P16. T-bet expression in B cells during HIV infection

James J. Knox[1], Michael R. Betts[1]

[1] *Department of Microbiology, Perelman School of Medicine, University of Pennsylvania*

While the antibody response fails to control HIV replication in most infected individuals, macaque B cell depletion studies suggest that B cells can contribute to control of SIV replication. Additionally, HIV-specific antibodies exert significant pressure on the virus and affect viral fitness. Despite evidence of antibody-mediated viral control and the recent interest in generating vaccine-induced broadly neutralizing antibodies, the mechanisms promoting an effective anti-HIV B cell response are poorly understood. Murine studies suggest that B cells expressing the transcription factor T-bet are a critical component of antiviral responses, but the role of human $T\text{-}bet^+$ B cells during HIV infection has not been examined. In this study, we characterized T-bet expression and cellular activation levels in various peripheral blood B cell subsets in two HIV^+ cohorts: the acutely infected RV217 cohort and the chronically infected SCOPE cohort. We demonstrate a peak in the frequency of T-bet expression in multiple memory B cell subsets during early acute infection and an association between T-bet expression and cellular activation. Elevated T-bet levels are maintained into chronic infection but partially resolve upon reduction of viremia, suggesting that chronic immune activation may sustain the $T\text{-}bet^+$ B cell populations. We are actively investigating the functional consequences of excessive B cell T-bet expression during chronic HIV infection.

P17. Ndfip1-mediated ubiquitin networks intrinsically limit proinflammatory cytokine production in activated T cells

Awo Akosua Kesewa Layman and Paula Oliver, PhD

The immune response is a specific, potent, mechanism for dealing with pathogens that threaten the integrity of the body. However, it is imperative that this response be activated only when needed, and also be turned off after the pathogen has been controlled or eliminated, in order to limit unintended destruction of normal tissue. Ubiquitination and subsequent degradation of essential effector proteins is an important way of terminating an immune response. Three classes of enzymes: E1 ubiquitin activating enzymes, E2 ubiquitin conjugating enzymes, and E3 ubiquitin ligases, work sequentially to ubiquitinate selected substrates. Ndfip1 (Nedd4-family interacting protein 1) is a protein that activates the catalytic function of several related members of one E3 ubiquitin ligase family. Previous work from our lab showed that Ndfip1 is expressed in T cells and mice lacking Ndfip1 have increased numbers of T_H17 cells. We hypothesized that Ndfip1 has a T cell-intrinsic role in limiting T_H17 cell differentiation. Using a mixed chimera model, as well as a T cell-specific knockdown of Ndfip1, we show that T cells that lack Ndfip1 are much more likely to be activated and to produce not just IL-17A, but also GM-CSF and IFNg cytokines as well. Not surprisingly, mice that lack Ndfip1 are more susceptible to colon pathology in a DSS colitis model, compared to their Ndfip1-sufficient counterparts. Ndfip1 thus plays a biologically relevant role in limiting effector cytokine production, as well as associated inflammatory pathology. Our recent data suggests that Ndfip1 may work with Itch E3 ligase to regulate T cell activation and proinflammatory cytokine production *in vivo*. Further research is ongoing to dissect the specific components of

Ndfip1-mediated ubiquitin complexes and pathways by which Ndfip1 limits proinflammatory cytokine production by activated T cells.

P18. Development of a Canine Model of Anti-Tumor CAR Therapy

M. Kazim Panjwani[1], Rebecca Rosenthal[1], and Nicola Mason[2]

[1] *Immunology Graduate Group, University of Pennsylvania*
[2] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania*

Adoptive immunotherapy using T cells genetically re-directed with a CD19-specific Chimeric Antigen Receptor (CAR) has shown remarkable efficacy in patients with leukemia. However, this approach is less effective in patients with significant nodal involvement presumably due to the immunosuppressive tumor microenvironment. To explore this further we are developing CAR therapy for dogs with spontaneous lymphoma. To effectively re-direct canine T cells, it has been necessary to develop and optimize a lentiviral transduction protocol. We have developed two methods for activating and expanding canine T cells and achieved stable transduction of 8-40% canine T cells using lentiviral GFP, and are in the process of transducing canine T cells with CARs. Three canine [c]CAR constructs were assembled containing the signaling domain of cCD3ζ alone or in combination with canine CD28 or 4-1BB, linked to a target-specific single chain variable fragment (scFv). Primary canine T cells were transduced with lentiviruses containing each of the 3 cCAR constructs targeting human CD19, and co-cultured with K562s bearing human CD19 (hKt19s) or control K562 cells. T cells modified with human CD19-targeting cCAR constructs proliferated in response to hKt19 cells and killed these antigen-bearing cells whereas no response to K562 cells was observed. These findings demonstrate successful antigen-dependent activation and cytotoxicity mediated through cCARs. Additionally, a scFv targeting the canine B cell antigen CD21 has been developed in our lab, and canine CD21-targeting T cells reduced primary B cell numbers in co-culture. We believe that lymphomas that develop in immune competent dogs more faithfully recapitulate the tumor microenvironment that impedes CART function and that development of this model will enable evaluation of stategies to augment CART efficacy within solid tumors.

P19. Impact of PD-1 blockade on sustainability of exhausted CD8+ T cell responses

Kristen E. Pauken[1], Pamela M. Odorizzi[1], Christina Twyman[1], Jonathan B. Johnnidis[1], Jason M. Schenkel[2], Andrew Rech[1], Robert H. Vonderheide[1], Andy Minn[1], E. John Wherry[1]

[1] *University of Pennsylvania, Philadelphia, PA*
[2] *University of Minnesota, Minneapolis, MN*

Cytotoxic CD8+ T cells have the capacity to eliminate infected and/or malignant cells. However, the persistent stimulation during chronic viral infections and cancer can lead to a state of dysfunction termed exhaustion. Exhausted CD8+ T cells lose the ability to efficiently perform effector functions and fail to acquire normal memory T cell properties such as antigen-independent persistence. One hallmark of exhausted T cells is over-expression of the inhibitory receptor PD-1, and, importantly, blockade of PD-1 or its ligand PD-L1 can restore functionality to exhausted CD8+ T cells. PD-1 pathway blocking reagents are expected to revolutionize the treatment of cancer. Despite this promise, central questions remain about sustainability and durability of reinvigoration of exhausted CD8 T cells. Here, we found in a mouse model of chronic infection, the boost in anti-viral CD8+ T cell functions waned long-term following cessation of treatment with anti-PD-L1. However, anti-PD-L1 promoted the ability of exhausted CD8+ T cells to persist in the complete absence of antigen,

suggesting that PD-1 blockade may be capable of restoring properties of antigen-independent renewal. We also found that in a mouse model of melanoma, the stage of exhaustion at the time of treatment could predict the magnitude of the response following blockade, providing potential biomarkers for PD-1 pathway intervention. These data suggest that while the boost in revitalized effector functions following PD-1 pathway blockade may be transient, this treatment may enhance other properties of CD8+ T cells that could promote durable anti-viral and anti-tumor immune responses.

P20. Regulation of inflammation and programmed cell death by the ubiquitin-editing enzyme A20

Lance Peterson[1,2], Naomi Philip[1,2], Averil Ma[3], Igor Brodsky[1,2]

[1] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania*
[2] *Institute for Immunology, Perelman School of Medicine, University of Pennsylvania*
[3] *Department of Medicine, School of Medicine, University of California at San Francisco*

Innate immune responses to microbes and proinflammatory cytokines require regulatory controls for limiting inflammation and immunopathology. The ubiquitin-editing enzyme A20 is induced in innate immune cells upon activation and plays a key role in limiting inflammatory responses *in vitro* and *in vivo*. Using A20-deficient macrophage cultures, we show that A20 limits cell death in response to the bacterial pathogen *Yersina pseudotuberculosis*. Furthermore, pyroptosis and programmed necrosis, forms of proinflammatory cell death, are enhanced in A20-deficient macrophages, resulting in the increased release of cell death associated cytokines and danger signals. These findings suggest an additional role of A20 in the regulation of multiple programmed cell death pathways elicited in macrophages. Identification of cellular targets of A20 that mediate its pro-survival and anti-inflammatory functions, as well as their altered interactions in programmed cell death pathways, will likely improve the understanding of how inflammation and cell death converge to influence immune responses.

P21. Caspase-8 regulates innate immune responses to *Yersinia*

Naomi H. Philip[1,2], Christopher P. Dillon[3], Annelise Snyder[1], Patrick Fitzgerald[3], Meghan Wynosky-Dolfi[1], Erin E. Zwack[1], Baofeng Hu[1], Andrew Oberst, Douglas R. Green[3], Igor E. Brodsky[1,2#]

[1] *University of Pennsylvania School of Veterinary Medicine, Department of Pathobiology, Philadelphia, PA*
[2] *University of Pennsylvania Perelman School of Medicine, Institute for Immunology, Philadelphia, PA*
[3] *St. Jude Children's Research Hospital, Department of Immunology, Memphis, TN*
[4] *University of Washington, Department of Immunology, Seattle, WA*

Programmed cell death is an evolutionarily conserved response to infection that can promote host defense or microbial virulence. Pathogens manipulate various immune signaling pathways through the activity of virulence factors that access the host cell cytosol. Cell death is a major consequence of infection with pathogenic *Yersinia* species and requires the *Yersinia* virulence factor YopJ, a potent inhibitor of NF-κB and MAPK signaling. However, the pathways that regulate cell death in response to *Yersinia* infection and the precise mechanisms by which cell death mediates protective immunity are not well understood. We find a novel requirement for caspase-8 and receptor interacting protein kinase 3 (RIPK3) in *Yersinia*-induced cell death. Mice deficient in caspase-8 and RIPK3 in their hematopoietic compartment were highly susceptible to wild type *Yersinia* infection

and were unable to sustain innate cytokine production. However, infection with YopJ-deficient *Yersinia* partially restored cytokine production and resulted in better control of bacterial burdens. These data suggest that in the absence of NF-κB blockade, *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ chimeric mice are capable of protective inflammatory responses. We hypothesize that activation of caspase-8 and cell death pathways during *Yersinia* infection induces specific pro-inflammatory signals that shape innate and adaptive responses to promote microbial clearance.

P22. Hemophagocytic lymphohistiocytosis caused by a dominant negative mutation in STXBP2 that inhibits SNARE-mediated membrane fusion

Waldo A. Spessott [1], Maria L. Sanmillan [1], Margaret E. McCormick [1], Nishant Patel [2], Joyce Villanueva [3], Kejian Zhang [4], Kim E. Nichols [2*] and Claudio G. Giraudo [1*]

[1] *Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia & University of Pennsylvania, Philadelphia, PA- USA*
[2] *Department of Pediatrics, Division of Oncology, The Children's Hospital of Philadelphia & University of Pennsylvania, Philadelphia, PA- USA*
[3] *Division of Bone Marrow Transplant and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Department of Pediatrics University of Cincinnati College of Medicine, Cincinnati, OH-USA*
[4] *Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Department of Pediatrics University of Cincinnati College of Medicine, Cincinnati, OH-USA*

Primary immunodeficiencies (PIDs) characterized by impaired cytotoxic T lymphosyte (CTL) and natural killer (NK) cell cytotoxicity most commonly manifest as Familial Hemophagocytic Lymphohistiocytosis (F-HLH) or Griscelli Syndrome Type 2 (GS), an often-fatal group of disorders caused by biallelic inactivating germline mutations in genes such as *PRF1*, *UNC13D*, *STX11* and *STXBP2*, or *RAB27A*, respectively. Although monoallelic mutations have been identified in certain HLH or GS2 patients, the clinical significance and molecular mechanisms by which these mutations influence CTL and NK cell function remain poorly understood. Here, we characterize a novel monoallelic F-HLH-associated mutation in *STXPB2*, the gene encoding Syntaxin-Binding Protein-2, a member of the SEC/MUNC18 family. Unlike previously described *STXBP2* mutations, STXBP2^{R65Q} retains its ability to interact with and stabilize Syntaxin-11. However, endogenous expression of STXBP2^{R65Q} in patient-derived lymphocytes, and forced expression in control CTL and NK cells, significantly diminishes cytotoxic activity. Mechanistic studies reveal that the R65Q mutation hinders membrane fusion by arresting the late steps of SNARE complex assembly. These results reveal for the first time a direct role for SEC/MUNC18 proteins in promoting SNARE-complex assembly *in vivo* and suggest that STXBP2^{R65Q} operates in a dominant-negative fashion to impair lytic granule fusion and contribute to the pathogenesis of F-HLH.

P23. CD8 T cells responses to chronic viral infections regulated by microRNAs

Erietta Stelekati[1], Donald T. Gracias[2], Jennifer L. Hope[2], Daniel Beiting[3], Mohammed-Alkhatim Ali[1], Peter D. Katsikis[2], and E. John Wherry[1]

[1] *Department of Microbiology and Institute for Immunology, University of Pennsylvania Perelman School Medicine*
[2] *Department of Microbiology and Immunology, Drexel University College of Medicine*
[3] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania*

Persistent pathogenic viral infections are a major cause of morbidity and mortality worldwide. CD8 T

cells are crucial mediators of antiviral immunity and memory CD8 T cells provide protection upon secondary challenge. However, during persistent infections, chronic antigen stimulation results in dysfunctional, "exhausted" T cells that cannot provide protection. Previous studies have defined the transcriptional signatures of T cells at different stages of acute and chronic infections, indicating distinct molecular networks of exhaustion and memory. However, the involvement of non-coding RNAs in regulating CD8 T cell responses to chronic viral infections remains largely unknown. Here, we investigated the microRNA profiles of CD8 T cells responding to acute or chronic LCMV infection. We identified distinct microRNA signatures of naïve, effector, memory and exhausted CD8 T cells. Further functional studies identified two microRNAs, miR-155 and miR-21, that control differentiation of antigen-specific CD8 T cells during chronic viral infections. Targeting individual microRNAs to regulate differentiation of CD8 T cells during chronic viral infections may have important therapeutic implications for treating chronic viral infections.

P24. Naturally Occurring Defective Viral Genomes Stimulate Strong Antiviral Immune Responses to Respiratory Syncytial Virus in Humans

Yan Sun[1], Micah Gilbert[1], Deepika Jain[1], Karla Tapia[1], Cynthia J. Koziol-White[2], Reynold A. Panettieri Jr.[2], Richard L. Hodinka[3], and Carolina B. López[1]

[1] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 380 South University Avenue, Philadelphia, Pennsylvania 19104, USA*
[2] *Department of Medicine, University of Pennsylvania, 125 South 31st Street, Philadelphia, Pennsylvania 19104, USA*
[3] *Clinical Virology Laboratory, Children's Hospital of Philadelphia, 34th Street and Civic Center Blvd, Philadelphia, Pennsylvania 19104, USA*

Human respiratory syncytial virus (RSV) causes illness in children and high-risk adults and is a primary cause of hospitalization due to asthma. Better understanding of the interactions between the virus and the host is needed to develop strategies to better serve patients. Our laboratory recently showed that defective viral genomes (DVGs) generated during virus replication are the primary danger signals for the triggering of the immune response during infection with Sendai and influenza virus in mice. We hypothesized that RSV DVGs stimulate the antiviral response and influence virus virulence in humans. To test this hypothesis, we generated virus stocks with a high content of DVGs (HD) or DVG-free virus (LD). HD not only strongly stimulated the expression of antiviral genes, but also potently inhibited virus replication *in vitro* and *ex vivo*. Supplementation of RSV LD infection with purified defective viral particles (pDPs) confirmed that DVGs drive the antiviral response. *In vivo*, mice infected with HD developed a strong and rapid antiviral response and showed reduced pathology. To determine whether DVGs are present in human patients, we analyzed pediatric respiratory secretions from RSV-infected children admitted to the CHOP. DVGs were present in more than 45% (20/41) of the specimens. Excitingly, DVG-positive samples showed a robust antiviral response, similar to what we observed in mice and in human lung tissue *ex vivo*. Altogether, our data demonstrate that RSV DVGs are critical triggers of the host antiviral response in humans and that DVGs may be a good target for preventive and therapeutic treatments.

P25. Mapping the immunostimulatory activity of a Sendai virus defective viral genome

Jie Xu[1], Xiomara Mercado-López[1], Yan Sun, Carolina B. López[1]

[1] *Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, 19104, United States*

Defective viral genomes (DVGs) generated during Sendai virus infection are the primary triggers of the host antiviral response. DVGs induce the expression type-I interferons (IFN) and other cytokines upon binding through the intracellular viral sensors RIG-I and MDA5. The molecular mechanism behind the superior immunostimulatory activity of DVGs is unknown. To identify RNA motifs that provide potent immunostimulatory activity to DVGs, we generated a series of deletion mutants of a prototype DVG derived from Sendai virus. *In vitro* transcribed RNA from these mutants were tested for their ability to induce type I IFNs upon transfection. *In silico* single strand RNA modeling of the mutants folding identified an AU-enriched stem loop domain formed by nucleotides 70-114 of the DVG that is essential for type I IFN induction. Consistent with this prediction, we demonstrate that mutants lacking this region lose their stimulatory activity, while mutants that kept intact this region preserved it Thus, a minimal RNA motif at the 5' but not the 3' complimentary sequence is critical for maximal DVG activity and oligonucleotides including such region may represent novel alternatives to be harnessed as potent adjuvants for vaccination.

P26. Fas expression in memory CD8+ T cell subsets augments cellular differentiation and effector function

Tori N. Yamamoto[1,2], Anthony J. Leonardi[2], Hui Liu[3], Ena Wang[3,4], Luca Gattinoni[5], Richard M. Siegel[6], Nicholas P. Restifo[1], and Christopher A. Klebanoff[1,7]

[1] *Center for Cancer Research, NCI/NIH, Bethesda, MD 20892, USA*
[2] *Immunology Graduate Group, University of Pennsylvania, Philadelphia, PA 19104, USA*
[3] *Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, NIH, Bethesda, MD 20892, USA*
[4] *Sidra Medical and Research Centre, Doha, Qatar*
[5] *Experimental Transplantation and Immunology Branch, NCI/NIH, Bethesda, MD 20892, USA*
[6] *National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD 20892, USA*
[7] *Clinical Investigator Development Program, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD 20892, USA*

Memory $CD8^+$ T cells (T_{Mem}) have the capacity to provide lifelong protection against intracellular pathogens and cancer. Despite phenotypic and functional heterogeneity among T_{Mem}, the expression of Fas, a tumor necrosis family receptor (TNFR) superfamily member conventionally known as a death receptor, is held in common among all T_{Mem} subsets across multiple species. As Fas has also been shown to mediate other effects besides death signaling, we therefore set out to elucidate the role of Fas signaling in defined T_{Mem} subsets, including T stem cell memory (T_{SCM}), T central memory (T_{CM}), and T effector memory (T_{EM}). We found that augmenting Fas signaling in stimulated T_{SCM} using an oligomerized form of its ligand FasL (lz-FasL) resulted in augmented cellular differentiation and a corresponding loss in IL-2 secretion capacity. Conversely, deprivation of Fas signaling in T_{CM} using a blocking, non-agonistic antibody to FasL (αFasL) retarded cellular differentiation. Gene expression analysis demonstrated that T_{Mem} expanded with αFasL expressed greater levels of memory-associated transcription factors relative to IgG-treated controls. Moreover, preventing Fas-signaling significantly altered the metabolic state of activated T_{Mem}, most notably by limiting the acquisition of glycolytic metabolism. When used *in vivo*, T_{Mem} expanded with αFasL showed greater on-target immunity compared to IgG controls. These studies demonstrate that Fas

signaling promotes not only cell death but also T_{Mem} differentiation towards a more effector-like state, a finding which has implications for the design and execution of T cell-based immunotherapies in patients with cancer or infectious disease.

P27. Role of inflammasome cytokines IL-1 and IL-18 during neurotropic mouse hepatitis virus infection

Zachary B Zalinger[1], Susan R Weiss[1]

[1] *University of Pennsylvania, Department of Microbiology*

The inflammasome is a cytosolic multiprotein complex that mediates secretion of pro-inflammatory cytokines IL-1 and IL-18. These cytokines have been found to play a role in restriction of a wide range of pathogens, and to contribute to the pathogenesis of many autoimmune conditions. IL-18 signaling has a known role in promoting production of interferon gamma from T and natural killer cells, while IL-1 polarizes Th17 responses and recruits neutrophils to sites of infection. However, work with influenza A and West Nile virus have shown that IL-1 signaling can also be necessary to promote strong anti-viral T cell responses via activation of dendritic cells. This effect was found to be specific to the site of infection. To further elucidate the roles of these cytokines we have infected mice lacking the IL-1 or IL-18 receptor with a dual neuro- and hepatotropic strain of mouse hepatitis virus (MHV). IL-18 receptor deficient mice succumb to normally non-lethal doses of MHV while IL-1 receptor deficient animals are somewhat protected from lethal infection, suggesting the cytokines play opposite roles to each other. The T cell response is similar in all genotypes, suggesting the cytokines act on innate populations of cells. We are currently investigating the behavior of the neutrophil and natural killer cell populations in inflammasome cytokine deficient mice. This work should elucidate the effect of inflammasome signaling on innate immune responses in differing sites of infections.