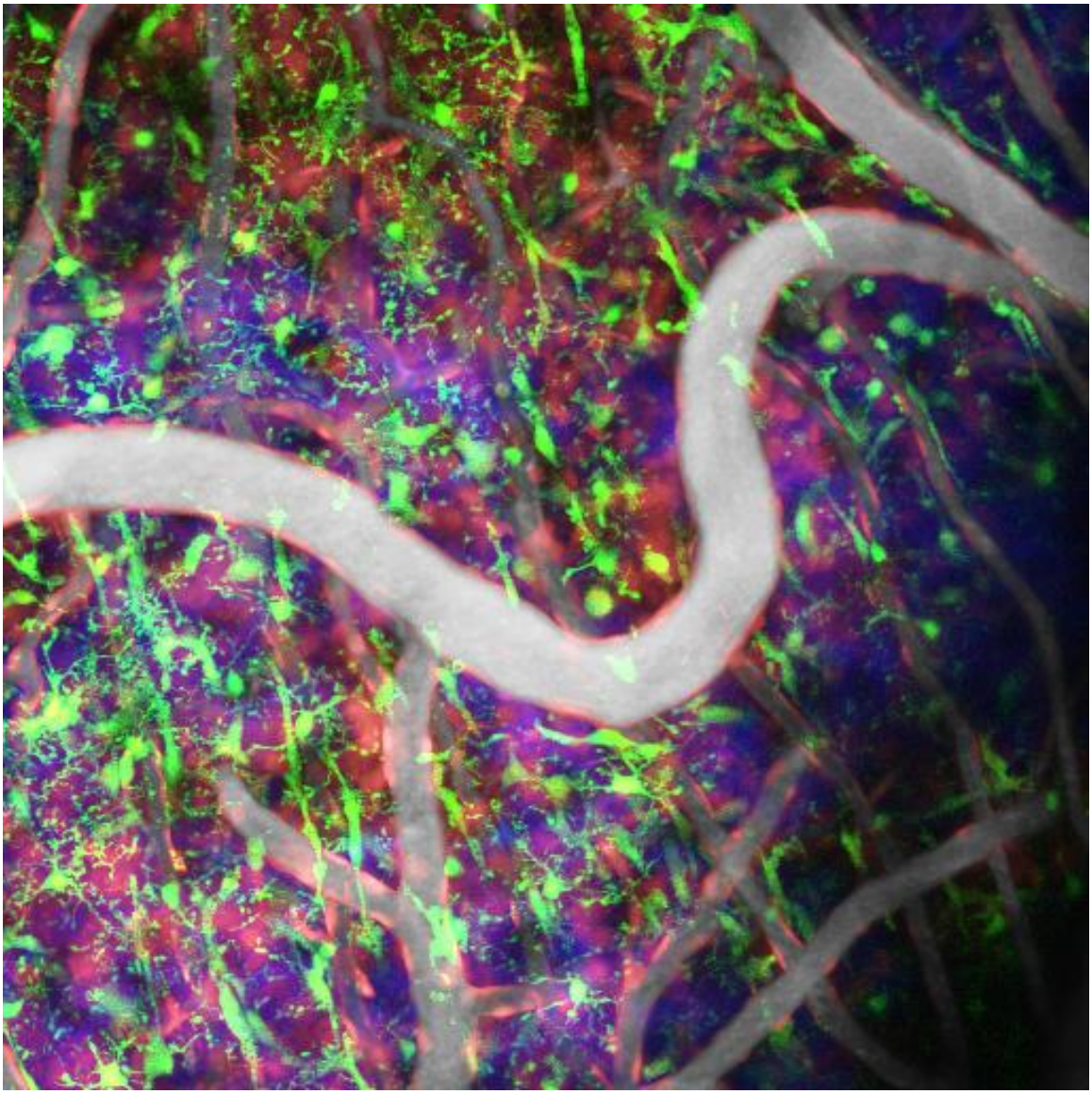
26th IMMUNOLOGY GRADUATE GROUP RETREAT
October 18-20, 2013
Cape May, NJ



The Immunology Graduate Group gratefully acknowledges the financial support of all our contributors for the 26th Annual Retreat:

Grant Support

- T32 CA009140 Immunobiology of Normal and Neoplastic Lymphocytes
- T32 AI 055428 Immune System Development and Regulation

Institutes, Centers, Departments, and Divisions

- Abramson Family Cancer Research Institute
- Department of Microbiology
- Department of Pathobiology
- Department of Pathology, The Children's Hospital of Philadelphia
- Department of Pathology and Laboratory Medicine
- Department of Pediatrics, The Children's Hospital of Philadelphia
- Institute for Immunology
- Joseph Stokes, Jr. Research Institute
- VMD-PhD Program

Corporate Sponsorship

- BD Biosciences
- MedImmune

Cover Photo

Maximum projection of the meninges/brain parenchyma of a skull thinned CX3CR1-GFP/DSRed cross.

Blue (Second harmonics) = Skull
Green (GFP) = CX3CR1 (Meningeal macrophages and microglial cells)
Red (DSRed) = all cells

Photo courtesy of Christoph Konradt, laboratory of Christopher Hunter (August 2013)

26th Annual Immunology Retreat
Friday to Sunday, October 18-20, 2013
The Grand Hotel
1045 Beach Avenue, Cape May, NJ 08204

FRIDAY, OCTOBER 18, 2013

NOTE: You will not be able to check into your hotel room until after 3 pm. We recommend that you leave your luggage in your vehicle until check-in at the end of Session II.

All sessions and breaks to be held in the Penthouse Ballroom, 5th floor. All meals will be held in the Grand Ballroom Complex, 2nd floor.

11:00-12:00 PM	**Retreat registration and program pick-up, Grand Ballroom Atrium**
12:00-1:20	**Lunch: Deli Buffet**
1:20-1:30	**Welcome:** Avinash Bhandoola, M.B., B.S., Ph.D., IGG Chair
1:30-2:50	**Session I: Immune Cell Development and Differentiation** **Session Chair: Ellen De Obaldia**
1:30-1:50	S1A. Sheena Baratono "*IFN and TLR9 signaling block lymphocytic differentiation of CLPs in the repeated TLR9 stimulation model of Macrophage Activation Syndrome"*
1:50-2:10	S1B. Qi Yang *"T cell factor 1 is required for group 2 innate lymphoid cell generation"*
2:10-2:30	S1C. Will Bailis *"Notch signaling concurrently promotes multiple inflammatory helper T cell fates while inhibiting regulatory T cell differentiation"*
2:30-2:50	S1D. Ellen De Obaldia *"T cell development requires constraint of C/EBPa by Notch target and transcriptional repressor Hes1"*
2:50-3:10	**Break** 1st and 2nd year students: Easel/poster set-up, Penthouse Ballroom

3:10-4:30	**Session II: Signaling in Immune Cells** **Session Chair: Amanda Schmidt**
3:10-3:30	S2A. Carolyn Gray *"Negative feedback regulation of NF-κB-Inducing kinase (NIK) is proteasome-dependent but does not require cellular inhibitors of apoptosis (cIAPs)"*
3:30-3:50	S2B. Claire O'Leary *"Nedd4 family interacting proteins activate Nedd4 E3 ubiquitin ligases in T cells to limit effector T cell function''*
3:50-4:10	S2C. Kate Weissler *"Variations in TCR signaling by peptides can affect the formation and activity of regulatory T cell in vivo*"
4:10-4:30	S2D. Amanda Schmidt *"Diacylglycerol-mediated signals promote natural regulatory T cell generation"*
4:30-4:40	**Break**
4:40-5:40	**Session III: Professional Development** Gangadhar Sunkara, Ph.D. Executive Director, Department of Drug Metabolism, Pharmacokinetics/Pharmacodynamics Novartis Institutes for Biomedical Research Ronald Herbst, Ph.D. Senior Director Respiratory, Inflammation & Autoimmunity Research MedImmune, Inc.
6:00	**Grand Hotel registration and room check-in, Main Lobby** ***Please set up posters for the remainder of the conference in Penthouse Ballroom.***
6:00-7:30	**Dinner: "Little Italy" Buffet**
7:45-8:45	**Session IV: Faculty Talks**
7:45-8:15	David Feldser, Ph.D. Assistant Professor of Cancer Biology University of Pennsylvania, School of Medicine *"Deconstructing tumor immune surveillance with mouse models of lung cancer"*

8:15-8:45 Henry Daniell, Ph.D.
Professor & Director of Translational Research
University of Pennsylvania, School of Dental Medicine
Departments of Biochemistry and Pathology
"Oral delivery of autoantigens or antigens bioencapsulated in plant cells to investigate mechanisms of conferred tolerance or immunity"

8:50-12:00 AM **Social**

SATURDAY, OCTOBER 19, 2013

8:00-9:00 AM **Breakfast Buffet**

9:00-10:40 **Session V: Regulation of Infection**
Session Chair: Laurel Monticelli

9:00-9:20 S5A. Ramin Herati
"Peripheral CXCR5+PD1+ response predicts influenza vaccine antibody responses in young adults but not older adults"

9:20-9:40 S5B. Michael Askenase
*"*Systemic conditioning of monocyte progenitors imparts regulatory function to monocytes during acute mucosal infection*"*

9:40-10:00 S5C. Naomi Philip
"*Caspase-8, RIPK1 and FADD control cell death and caspase-1 processing during Yersinia infection*"

10:00-10:20 S5D. Erika Crosby
"*Bystander CD8 T cells promote increased disease severity during Leishmania major infection in an NKG2D-dependent manner"*

10:20-10:40 S5E. Laurel Monticelli
"*Innate lymphoid cells orchestrate mucosal tissue repair through an amphiregulin-EGFR pathway"*

10:40-11:00 **Break**

11:00-11:10 **Introduction to Keynote Speaker:** Avinash Bhandoola, M.B., B.S., Ph.D.

11:10-12:10 **Keynote Speaker: Max D. Cooper, M.D.**
Professor of Pathology and Laboratory Medicine
Georgia Research Alliance Eminent Scholar in Developmental Immunology
Emory University School of Medicine
"Evolution of adaptive immunity"

12:30-1:30 PM	**Lunch: Pub Buffet**
1:30-3:30	**Free time to explore Cape May**
3:30-5:30	**Poster Session**
5:30-7:00	**Dinner: "The Jersey Shore"**
7:00-8:30	**Session VII: Faculty Talks**
7:00-7:45	Gregory Beatty, M.D., Ph.D. Assistant Professor, University of Pennsylvania School of Medicine, Department of Medicine, Hematology/Oncology Division *"Harnessing macrophages for cancer therapy"*
7:45-8:30	Laura Su, M.D., Ph.D. Assistant Professor, University of Pennsylvania School of Medicine, Department of Medicine, Rheumatology Division *"Single cell analysis of antigen-specific T cell repertoire in health and disease"*
8:30	**Announcement of Awards for Best Oral Presentation and Best Poster**
REMINDER:	***Please take down your posters tonight.***
9:00-12:00 AM	**Social**

SUNDAY, OCTOBER 20, 2013

8:00-11:00 AM	**Breakfast Buffet**
REMINDER:	***Please check out of your room by 11 AM.***

***** END OF CONFERENCE *****

****

SAVE THE DATE:

27th Annual Immunology Graduate Group Retreat
October 17-19, 2014
The Grand Hotel
Cape May, NJ

****

ABSTRACTS FOR ORAL PRESENTATIONS

S1A. IFN and TLR9 signaling block lymphocytic differentiation of CLPs in the repeated TLR9 stimulation model of Macrophage Activation Syndrome

Sheena Baratono[1], Nianshen Chu[2], Edward Behrens[2]
[1]Immunology Graduate Group of the University of Pennsylvania and the [2]Department of Pediatric Rheumatology, Children's Hospital of Philadelphia, Philadelphia, PA 19104

Severe systemic inflammation resulting from cytokine storm illnesses is seen in a variety of auto-inflammatory and infectious diseases. This inflammation leads to widespread organ pathology including hepatitis, pan-cytopenia, fever, and cytokinemia. Utilizing a novel mouse model developed in the lab, where mice are administered 5 doses of the TLR9 agonist CpG over ten days, we can recapitulate the aforementioned signs of cytokine storm syndromes.

Given the pan-cytopenias, we are interested in exploring the effects of systemic inflammation on hematopoiesis. Data using our mouse model show a marked reduction in B lymphopoiesis, which we found intriguing given the low B cell counts in many patients with cytokine storm related syndromes. While B lymphopoiesis is decreased, myelopoiesis appears unchanged. Our data also show that the defect in B cell development occurs as early as the common lymphoid progenitor (CLP) committed B cell precursor stage. These cells are defined as Ly-6D^{+} CLPs and have the preferential ability to differentiate into B cells. Sorted Ly-6D+ CLPs plated on OP9 stromal cells demonstrate decreased B cell yields upon *in vitro* CpG exposure in a TLR9 intrinsic manner. However, T cell differentiation by these same cells on OP9-dl1 stromal cells is intact. This TLR9 intrinsic effect is masked *in vivo*, likely by other inflammatory signals such as IFNγ. Additionally, Ly-6D+ CLPs sorted from PBS or CpG treated mice show diminished capability to differentiate into B cells *in vitro* on OP9 stromal cells suggesting that CLPs exposed to the *in vivo* inflammatory environment demonstrate diminished capability to differentiate into B cells. Moreover, we have shown that IFNγ is necessary for the reduction of Ly-6D+ CLPs and B cells *in vivo* and that it has potent cell intrinsic anti B cell effects *in vitro*. *We hypothesize that there is a defect in the ability of CLPs to differentiate into B cell progenitors, and that the decreases at all stages of development are dependent on direct TLR9 and IFNγ signaling.*

S1B. T cell factor 1 is required for group 2 innate lymphoid cell generation

Qi Yang[1], Laurel A. Monticelli[2], Steven A. Saenz[2], Anthony Wei-Shine Chi[1], Gregory F. Sonnenberg[2], Jiangbo Tang[3], Maria Elena De Obaldia[1], Will Bailis[1], Jerrod L. Bryson[1], Kristin Toscano[1], Jian Huang[4], Angela Haczku[4], Warren S. Pear[1], David Artis[2], Avinash Bhandoola[1]
[1]Department of Pathology and Laboratory Medicine, [2]Department of Microbiology, [3]Department of Cancer Biology, [4]Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

Group 2 innate lymphoid cells (ILC2) are innate lymphocytes that confer protective type 2 immunity during helminth infection and are also involved in allergic airway inflammation. Here we report that ILC2 development required T cell factor 1 (TCF-1, the product of the Tcf7 gene), a transcription factor also implicated in T cell lineage specification. Tcf7$^{-/-}$ mice lack ILC2, and were unable to mount ILC2-mediated innate type 2 immune responses. Forced expression of TCF-1 in bone marrow progenitors partially bypassed the requirement for Notch signaling in the generation of ILC2 in vivo. TCF-1 acted through both GATA-3-dependent and GATA-3-independent pathways to promote the generation of ILC2. These results are reminiscent of the critical roles of TCF-1 in early T cell development. Hence, transcription factors that underlie early steps of T cell development are also implicated in the development of innate lymphoid cells.

S1C. Notch signaling concurrently promotes multiple inflammatory helper T cell fates while inhibiting regulatory T cell differentiation

Will Bailis, Yumi Yashiro-Ohtani, Terry C. Fang, Maria Elena De Obaldia, Robin D. Hatton, Avinash Bhandoola, Casey T. Weaver, David Artis, Warren S. Pear
Department of Pathology and Laboratory Medicine, Abramson Family Cancer Research Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.

Notch signaling is classically understood to instruct bipotential cell fate decisions. We have recently identified a novel mechanism for the Notch pathway in the regulation of cell specification, in which Notch concurrently regulates the differentiation of multiple cell fates. Rather than preferentially instructing the differentiation of one Th cell lineage, Notch simultaneously binds and regulates the signature genes of the Th1, Th2, and Th17 genetic programs and acts to sensitize activated $CD4^+$ T cells to differentiating cytokines. We now find that, in addition to positively regulating these inflammatory Th cell subsets, Notch signaling negatively regulates the regulatory T cell fate by desensitizing cells to TGFβ-derived signals. Moreover, studies using $Hes1^{-/-}$ chimeras suggest that Notch likely negatively regulates regulatory T cell differentiation indirectly *via* Hes1. Overall, our data provide a new paradigm for Notch in hematopoiesis, with Notch simultaneously orchestrating multiple lineage programs by tuning cellular sensitivity to environmental differentiation cues, rather than selectively driving the development of a single fate.

S1D. T cell development requires constraint of C/EBPa by Notch target and transcriptional repressor Hes1

Ellen De Obaldia, Jeremiah Bell, Yumi Yashiro-Ohtani, Xinxin Wang, Jonathan DeLong, Dan Zlotoff, Dil Afroz Sultana, Avinash Bhandoola
Perelman School of Medicine at the University of Pennsylvania, Department of Pathology and Laboratory Medicine, Philadelphia, PA

Intrathymic Notch signaling induces expression of T-cell genes and discourages progenitors from adopting alternative fates. The importance of actively repressing alternative gene expression programs in T-cell development is unclear. To examine the role of repression in T-cell development, we focused on Hes1, a Notch target and bHLH transcriptional repressor. In addition to the described T lineage developmental defect, we found that Hes1-deficient multipotential fetal liver progenitors co-cultured with Notch ligands generated many myeloid cells; whereas, Notch almost completely inhibited myelopoiesis from wild-type progenitors. Critically, Hes1-deficient progenitors failed to downregulate expression of a myeloid regulator, C/EBPa, in response to Notch signals. Thus, we hypothesized that failure to inhibit a myeloid gene expression program precludes T cell development in the absence of Hes1. This idea predicts that progenitors lacking C/EBPa, and thus devoid of myeloid potential, would not require Hes1 to undergo T cell development. Indeed, genetic deletion of C/EBPa restored *in vivo* T-cell development from Hes1-deficient progenitors to wild type levels. These results definitively establish the significance of constraining myeloid lineage gene expression programs early in T-cell development.

S2A. Negative feedback regulation of NF-κB-Inducing kinase (NIK) is proteasome-dependent but does not require cellular inhibitors of apoptosis (cIAPs)

Carolyn M. Gray, Michael J. May
The Department of Animal Biology and The Mari Lowe Center for Comparative Oncology, The University of Pennsylvania School of Veterinary Medicine

NF-κB activation occurs by two mechanisms, the classical and non-canonical pathways. Classical NF-κB signaling requires NEMO and IKKβ, whereas the non-canonical signaling pathway requires IKKα and upstream kinase NIK. This constitutively active kinase is undetectable in resting cells. Thus the central events that regulate non-canonical NF-κB signaling precisely control the stability of NIK, and defining these mechanisms is crucial for the development of novel therapeutic strategies aimed at selectively blocking this pathway. Basal NIK associates with TRAF3, TRAF2, and cIAP1/2. In this complex the cIAPs ubiquitylate basal NIK, leading to its degradation. Upon receptor ligation the E3 activity of the cIAPs is redirected and NIK levels stabilize, activating non-canonical NF-κB by phosphorylation of IKKα. A negative feedback mechanism has been described to terminate this signal. In this model active IKKα phosphorylates both p100 and NIK. Though the mechanism remains unclear, the phosphorylation of active NIK leads its turnover. In this study we have established a strategy to precisely differentiate between the degradation of newly synthesized endogenous NIK and active NIK in LTβR-stimulated cells. We demonstrate that signal-induced NIK is rapidly degraded through the proteasome. Furthermore, using a novel smac mimetic compound that eliminates cIAP1 and cIAP2, we find that unlike the basal regulatory mechanism, active NIK turnover does not require the cIAPs. Our results definitively establish that the negative feedback control of non-canonical NF-κB signaling by IKKα-mediated NIK turnover occurs through a novel proteasome-dependent and cIAP-independent mechanism.

S2B. Nedd4 family interacting proteins activate Nedd4 E3 ubiquitin ligases in T cells to limit effector T cell function

Claire O'Leary, Chris Riling, Paula Oliver
University of Pennsylvania, Children's Hospital of Philadelphia

E3 ubiquitin ligases, such as the catalytic Nedd4-family ligases, tune signaling pathways in activated T cells to regulate differentiation and cytokine production. These ligases often function with adaptors. We have found that Nedd4-family interacting protein 1 (Ndfip1) and Ndfip2 promote Nedd4-family E3 ligase catalytic activity by relieving autoinhibition. Our in vitro studies indicate that Ndfip1 and Ndfip2 have overlapping function. In vivo, Ndfip1 negatively regulates T cell activation and TH2 cytokine production. We generated Ndfip2-/- mice to examine the in vivo role of Ndfip2. Unlike Ndfip1, Ndfip2 is not a prominent negative regulator of T cell activation or TH2 polarization. However, loss of Ndfip2 exacerbated the inflammatory Ndfip1-/- phenotype. This suggested that, like Ndfip1, Ndfip2 limits inflammation. Supporting this, Rag1-/- mice reconstituted with WT and Ndfip1/Ndfip2 doubly deficient (DKO) fetal liver developed more severe inflammation than mice reconstitued with WT and Ndfip1-/- fetal liver. To probe the mechanism by which Ndfip2 limits T cell-mediated inflammation we compared cocultured DKO and WT CD4+ T cells to cocultured Ndfip1-/- and WT CD4+ T cells. DKO T cells, but not Ndfip1-/- T cells, outcompete cocultured WT cells, implicating a role for Ndfip2 in T cell persistence or proliferation. These studies suggest that Ndfip1 and Ndfip2 are pleiotropic modulators of inflammation, with overlapping and unique roles in T cell function.

S2C. Variations in TCR signaling by peptides can affect the formation and activity of regulatory T cell *in vivo*

Kate Weissler, Felipe Bedoya, Vickey Garcia, Liz Kropf, Andrew J. Caton
The Wistar Institute, Philadelphia, PA

Regulatory T cells (Tregs) are crucial for preserving immune homeostasis and are believed to play an important role during the immune response to a viral infection. There is good evidence that stimulation through the TCR is necessary both for the formation and activation of Tregs, but how variations in TCR signaling in response to self and/or viral peptides can impact these processes is not well understood. We show that both deletion and Treg induction can follow peripheral recognition of a self-antigen by conventional CD4+ T cells. Self-antigen expressed at a low level is optimal for Treg induction, while the same self-antigen expressed at relatively higher levels favors deletion. Interestingly, these effects of varying self-antigen expression on peripheral Treg formation resemble the effects of low versus high self-antigen expression on Treg formation in the thymus. To analyze the role of TCR signaling in Treg activation *in vivo*, we have been using a model of influenza virus infection. We find that a viral peptide induces little or no conversion of conventional CD4+ T cells into Tregs and that Tregs recognizing a low abundance self-peptide fail to accumulate at infection sites or affect the anti-viral response. However, thymically-generated Tregs that are strongly reactive with a viral peptide are activated via the TCR to differentiate and modulate the immune response to the viral infection. Interestingly, when the Tregs can also interact with a self-peptide that is expressed at high levels, their ability to differentiate and modulate the immune response to the virus is impaired. These studies are defining the effects that TCR signaling by viral and self-peptides can exert on the formation and activity of Tregs in different contexts *in vivo*.

S2D. Diacylglycerol-mediated signals promote natural regulatory T cell generation

[1]Amanda Schmidt, [1]Tao Zou, [2]Rohan Joshi, [1]Theresa Leichner, [2]Gary Koretzky, [1]Taku Kambayashi
[1]Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [2]Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA

Natural regulatory T cells (nTregs) are important for maintaining peripheral tolerance. nTregs are thought to develop from thymocytes receiving strong TCR-mediated signals. However, the specific signaling pathways from the TCR leading to nTreg development are not fully understood. TCR engagement leads to activation of phospholipase C-γ1, which generates the lipid second messenger diacylglycerol (DAG) from phosphotidylinositol 4,5-bisphosphate. In this study, we identify DAG as a critical signaling molecule in nTreg development. Using mice that lack DAG kinase-ζ (DGKζ), a negative regulator of DAG, we demonstrate that enhanced DAG-mediated signals result in a significant increase in thymic CD25+Foxp3–CD4+ nTreg precursors and Foxp3+CD4+ nTregs in a cell-autonomous manner. DGKζ-deficient T cells exhibited increased c-Rel nuclear translocation and extracellular signal-regulated kinase (ERK) phosphorylation after TCR stimulation, suggesting that these downstream pathways may contribute to the nTreg enhancement. Indeed, diminution of c-Rel or blockade of ERK phosphorylation abrogated the increased nTreg generation in DGKζ-deficient thymocytes. Additionally, the amount of ERK phosphorylation could be directly correlated with TCR-induced Foxp3 induction in immature thymocytes, and nTreg development was augmented in mice bearing selectively enhanced ERK activation. These data suggest that TCR-driven DAG signals promote nTreg development by activation of the ERK and c-Rel signaling pathways.

S5A. Peripheral CXCR5+PD1+ response predicts influenza vaccine antibody responses in young adults but not older adults

Ramin Sedaghat Herati[1], Morgan A. Reuter[2], Douglas V. Dolfi[2], Kathleen D. Mansfield[2], Raj K. Kurupati[3], Senthil Kannan[3], Hildegunde Ertl[3], Kenneth E. Schmader[4], Michael R. Betts[2], David H. Canaday[5], E. John Wherry[2]

[1]Department of Medicine, Univ of Pennsylvania Perelman School of Medicine, Philadelphia, PA, [2]Institute for Immunology and Department of Microbiology, Univ of Penn, Philadelphia, PA, [3]Wistar Institute, Philadelphia, PA, [4]Division of Geriatrics, Duke University Medical Center, Durham, North Carolina, [5]Division of Infectious Disease, Case Western Reserve Univ, Cleveland, OH

Although influenza vaccination is recommeded for all adults annually, the incidence of vaccine failure, defined as weak or absent increase in neutralizing antibody titers, is increased in the elderly compared to young adults. The T follicular helper subset of CD4 T cells provide B cell help in germinal centers and are necessary for class-switched antibody responses. Previous studies suggested a role for circulating T follicular helper cells (cTfh) following influenza vaccination in adults. Here, we identified a subset expressing CXCR5 and Programmed Death 1 (PD-1) which were highly similar to lymphoid Tfh based on phenotype, transcriptional profile, and functional ability. cTfh from elderly adults were present at reduced frequency, had decreased in vitro B cell help ability, and greater expression of activation marker inducible costimulator (ICOS) compared to young adults. At seven days after inactivated influenza vaccination, cTfh from young adults correlated with influenza vaccine-specific IgM and IgG responses in young adults but not in elderly adults. In sum, we have identified aging-related changes in cTfh which manifested as reduced influenza vaccine responses. Future rational vaccine design efforts should incorporate Tfh measurement as an immune correlate of protection, particularly in the setting of aging.

S5B. Systemic conditioning of monocyte progenitors imparts regulatory function to monocytes during acute mucosal infection

Michael H. Askenase, John R. Grainger, Yasmine Belkaid

Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease

Ly6C^{hi} CX3CR1^{int} monocytes are recruited to sites of inflammation in a wide range of infectious and inflammatory models. The current paradigm suggests that upon acute infection with an intracellular pathogen, Ly6C^{hi} monocytes rapidly enter inflamed tissue, where they secrete inflammatory cytokines and participate in pathogen killing. However, we have recently described a more complex function of these cells during acute *Toxoplasma gondii* infection, including production of anti-inflammatory mediators such as IL-10 and prostaglandin E2, which limit pathology and ensure host survival.

Innate cells in mucosal tissue respond rapidly to an invading pathogen, and provide signals that recruit peripheral inflammatory cells, but whether early innate signals can condition the differentiation of these cells prior to their arrival in the tissue is not well understood. We hypothesized that systemic inflammatory signals condition monocyte precursors early during *T. gondii* infection, altering monocyte differentiation to reprogram the responses of Ly6C^{hi} monocytes to local signals upon recruitment to the inflamed intestine.

To assess this hypothesis, we compared monocyte responses to infection with the intestinal parasite *T. gondii* and to DSS colitis. We found that monocytes recruited to the intestine during *T. gondii* infection demonstrated a unique phenotype characterized by altered chemokine receptor expression and increased anti-inflammatory function. Intriguingly, monocytes and their precursors in the blood and bone marrow also adopted these features, suggesting that these cells were educated before they entered inflamed tissue. IFN-γ is required for this systemic conditioning of monocytes and the acquisition of their unique phenotype correlated closely with serum levels of IFN-γ. These data suggest that during acute Th1 infections, systemic IFN-γ signaling conditions monocyte differentiation to produce cells with balanced inflammatory and anti-inflammatory function upon recruitment to the site of infection.

S5C. Caspase-8, RIPK1 and FADD control cell death and caspase-1 processing during *Yersinia* infection

Naomi H. Philip[1,2], Christopher P. Dillon[4], Annelise Snyder[2], Meghan Wynosky-Dolfi[2], Patrick Fitzgerald[4], Douglas R. Green[4], Igor E. Brodsky[1,2,3]

[1]Immunology Graduate Group, [2]Department of Pathobiology, [3]Institute for Immunology, University of Pennsylvania, Philadelphia, PA, [4]St. Jude Children's Research Hospital, Memphis, TN

Pathogens manipulate various host cell processes and signaling pathways through the activity of specific virulence factors that enter the host cell cytosol. For instance, the Gram-negative bacteria *Yersinia pseudotuberculosis* injects proteins termed Yops that can remodel the actin cytoskeleton and inhibit the NF-κB and MAPK signaling pathways. This raises an important question: how do infected cells activate a productive inflammatory response despite the presence of these microbial virulence factors? Immunogenic forms of programmed cell death can eliminate the pathogen's replicative niche and provide pro-inflammatory signals necessary for effective immunity. However, the precise mechanism by which cell death in the context of infection mediates protective immunity is not well understood. Cell death is a major consequence of infection with *Y. pseudotuberculosis*, which makes *Yersinia* an attractive model to study the immune response to pathogens and how they evade anti-microbial defenses. *Yersinia*-induced cell death requires the effector YopJ, a potent inhibitor of NF-κB and MAPK signaling. Cell death by apoptosis, pyroptosis and necroptosis are regulated by caspases. We find a novel requirement for caspase-8, receptor interacting protein 1 (RIPK1) and Fas-associated protein with death domain (FADD) in *Yersinia*-induced cell death and pro-inflammatory caspase-1 activation. Moreover, mice deficient in caspase-8 are highly susceptible to *Yersinia* infection. We hypothesize that activation of these pathways during *Yersinia* infection may induce specific pro-inflammatory signals that shape innate and adaptive responses and promote microbial clearance.

S5D. Bystander CD8 T cells promote increased disease severity during *Leishmania major* infection in an NKG2D-dependent manner

Erika J. Crosby[1], E. John Wherry[2], Phillip Scott[1]

[1]Department of Pathobiology, University of Pennsylvania; [2]Department of Microbiology, University of Pennsylvania

T cells are recruited to inflamed tissue independent of their antigen specificity, but whether T cells with irrelevant specificities (bystander T cells) contribute to the immune response is unclear. We found that Lymphocytic choriomeningitis virus (LCMV)-specific CD8 T cells from a previous LCMV infection were recruited in large numbers to leishmanial lesions in mice infected with *L. major*. Importantly, LCMV immune mice developed larger leishmanial lesions than mice that had received *L. major* alone. Associated with the increased disease severity was a significant infiltration of CD8 T cells, neutrophils, and monocytes into the lesions with no change in parasite control. CD8 T cells were required for the increased disease pathology, as LCMV immune mice depleted of CD8 T cells prior to infection with *L. major* no longer exhibited the increased pathology. Furthermore, bystander CD8 T cells within the lesions expressed both granzyme B and the NK cell receptor NKG2D. Engagement of the NKG2D receptor on memory CD8 T cells has been shown to be costimulatory, but in some cases it can mediate direct cell cytotoxicity independent of antigen specificity. Several of the Rae-1 family of ligands for NKG2D were upregulated within the lesional skin after infection with *L. major.* Strikingly, blocking NKG2D interactions during *L. major* infection of LCMV immune mice resulted in decreased disease severity. Taken together, this work demonstrates that bystander CD8 T cells expressing NKG2D are recruited into the leishmanial lesion and induce immunopathology in an NKG2D dependent manner with no increase in protection. Ongoing work is focused on understanding how NKG2D promotes increased pathology by CD8 T cells.

S5E. Innate lymphoid cells orchestrate mucosal tissue repair through an amphiregulin-EGFR pathway

Laurel A. Monticelli[1,2], Lisa C. Osborne[1,2], Gregory D. Rak[1,2], Jerome M. Molleston[1], Lance W. Peterson[1,2], Dietmar M.W. Zaiss[3], E. John Wherry[1,2], David Artis[1,2,4]

[1]Department of Microbiology and [2]Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [3]Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, University of Utrecht, 3584 CL Utrecht, The Netherlands; [4]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

While recent studies identified a role for Group 2 innate lymphoid cells (ILC2) in promoting pathologic type 2 inflammation in the skin, lung and intestine, the tissue-protective roles of ILCs at barrier sites remain poorly understood. In a previous study, we demonstrated that ILC2s could promote tissue homeostasis following influenza virus-induced lung injury through expression of an epidermal growth factor receptor (EGFR) ligand Ampiregulin (Areg). However, the upstream signals that regulate ILC2-intrinsic Areg expression and how the Areg-EGFR pathway influences tissue remodeling and host recovery during viral infection remain unknown. In new studies we demonstrate that IL-33 is a primary stimulus for ILC2 Areg expression *in vivo* and that administration of recombinant IL-33 or infection with influenza virus induces EGFR activation specifically in bronchiolar airway epithelial Clara cells in an ILC2-dependent manner. Strikingly, chemical or genetic inhibition of the Areg-EGFR pathway results in severely impaired lung function and a failure to initiate airway epithelial repair leading to increased host mortality, suggesting that this pathway is essential for host recovery from influenza infection. Notably, the tissue-protective functions of ILC2s are not limited to the airways, as modulation of the ILC2-Areg-EGFR pathway could promote tissue reparative responses during DSS-induced intestinal colitis. Taken together, these data indicate a critical role for ILC2s in mediating mucosal tissue homeostasis though an IL-33-Areg-EGFR axis and suggest that ILC2s provide a functional link between the signaling pathways that balance inflammation and tissue repair at barrier sites.

ABSTRACTS FOR POSTER PRESENTATIONS

P1. Regulation of iTH17 development by AKT2

Lauren Banks[1], Martha Jordan[3], Gary Koretzky[4]
[1]*Cell and Molecular Biology Graduate Group,* [3]*Department of Pathology and Laboratory Medicine, and* [4]*Department of Medicine, University of Pennsylvania, Philadelphia, PA*

Understanding the homeostasis of induced TH17 (iTH17) cells is of increasingly appreciated importance due to their implication in both autoimmune diseases and host defense from extracellular pathogens. Recent work by Dang *et al.* (Cell, 2011) and Shi *et al.* (JEM, 2011) demonstrated an important role of the transcription factor Hypoxia Inducible Factor 1α (HIF1α) in the development of iTH17 cells. Deletion of HIF1α resulted in impaired transcription of the canonical TH17 cytokine, IL-17A and functionally impaired RORγt, an essential transcription factor in the developmental program of iTH17 cells. HIF1α is thought to be activated by mTORC1, also demonstrated to be important for iTH17 differentiation and a well-known downstream target of AKT2. AKT has three isoforms and is a serine threonine kinase responsible for a number of cellular processes including growth and metabolism. In our efforts to dissect the signaling pathways important for iTH17 development, our lab recently found an isoform-specific requirement for AKT2 in the *in vitro* induction of iTH17 cells. However, mice deficient in AKT1, the other highly expressed AKT isoform in T cells, only had a moderate impairment of iTh17 cell development. Based on these findings, we hypothesized that *AKT2 is acting through a Hif1α-dependent mechanism to influence iTH17 development.* In support of our hypothesis, we found decreased levels of HIF1α protein in iTH17 cells deficient in AKT2, but not AKT1. Additionally, we found decreased mRNA expression of known transcriptional targets of HIF1α. In order to determine if increasing the level of HIF1α protein in AKT2 deficient T cells could restore their ability to become iTH17 cells, we used both pharmacologic and physiologic methods of stabilizing HIF1α protein. Despite increased levels of HIF1α protein, we did not see a restoration of IL-17A production in AKT2 deficient TH17 cells. Future directions include examining the AKT2- HIF1α pathway in iTh17 differentiation using a double-deficient mouse and gene profiling with metabolic pathway analysis to identify cellular processes commonly and uniquely regulated by AKT1, AKT2, and HIF1α.

P2. IL-33 regulates group 2 innate lymphoid cells and metabolic homeostasis in white adipose tissue

Jonathan R. Brestoff, Laurel A. Monticelli, Steven A. Saenz, Elia Tait Wojno, Brian S. Kim, David Artis
Institute for Immunology and Department of Microbiology, Perelman School of Medicine

Obesity is an increasingly prevalent disease worldwide that is associated with increased triglyceride storage in adipocytes and increased white adipose tissue (WAT) mass. Emerging research indicates that various innate and adaptive immune cells in WAT critically regulate metabolic homeostasis and are dysregulated in the context of obesity. Group 2 innate lymphoid cells (ILC2) were recently identified in murine WAT, however the roles of ILC2s in WAT remain largely unknown. Here we identify both human and murine WAT ILC2s that express GATA-3 and interleukin (IL)-33 receptor (IL-33R). WAT obtained from severely obese human patients had markedly decreased WAT ILC2 frequencies compared to non-obese controls. Further, obese mice fed a high fat diet (HFD) had significantly decreased WAT ILC2 frequencies and numbers compared to non-obese mice fed a control diet. Taken together these findings provoke the hypothesis that loss of ILC2s contributes to the development of obesity. Therefore, to test whether activation of ILC2s regulates metabolic homeostasis, mice were treated with the ILC2 activating cytokine IL-33, and WAT-associated ILC2 responses and WAT mass were examined. Strikingly, IL-33 treatment was associated with increased ILC2s in WAT and decreased WAT mass. Conversely, loss-of-function studies employing IL-33-deficient (*Il33*$^{-/-}$) mice demonstrated increased weight gain, increased triglyceride levels in adipocytes and decreased serum free fatty acid concentrations compared to wild-type controls in the context of a HFD. Collectively, these findings suggest that the IL-33-ILC2 axis may regulate lipid homeostasis in WAT and suggest that targeting this axis might represent a novel anti-obesity therapeutic strategy.

P3. Caspase-11 controls inflammasome activation in response to the specialized secretion systems of bacterial pathogens

Cierra N. Casson[1], Alan M. Copenhaver[1], Erin E. Zwack[2], Hieu T. Nguyen[1], Till Strowig[3], Bahar Javdan[1], William P. Bradley[1], Thomas C. Fung[1], Richard A. Flavell[3], Igor E. Brodsky[2], Sunny Shin[1]
[1] Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2] Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [3] Department of Immunobiology and Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT

Inflammasome activation is important for antimicrobial defense because it induces cell death and regulates the secretion of IL-1 family cytokines, which play a critical role in mediating inflammatory responses. The inflammasome activates caspase-1 to process and secrete IL-1β. However, the mechanisms governing IL-1α release are less clear. Recently, a non-canonical inflammasome was described that activates caspase-11 and mediates cell death and release of IL-1α and IL-1β. Caspase-11 activation in response to a wide variety of Gram-negative bacteria requires priming to upregulate protein expression and TIR-domain-containing adaptor-inducing interferon-β (TRIF)-dependent interferon production. Additionally, intracellular lipopolysaccharide (LPS) is a known activator of the caspase-11-dependent inflammasome. Whether additional bacterial signals trigger caspase-11 activation is unknown. Many bacterial pathogens use specialized secretion systems to translocate effector proteins into the cytosol of host cells. These secretion systems can also deliver flagellin into the cytosol, which triggers caspase-1 activation and pyroptosis. However, even in the absence of flagellin, specialized secretion systems induce inflammasome activation and the release of IL-1α and IL-1β, but the pathways that mediate this response are unclear. We observe rapid IL-1α and IL-1β release and cell death in response to the type IV or type III secretion systems of *Legionella pneumophila and Yersinia pseudotuberculosis*. Whereas IL-1β secretion requires caspase-1, IL-1α secretion and cell death require caspase-11. Interestingly, caspase-11 promotes IL-1β release in response to the type IV secretion system through the NLRP3/ASC inflammasome, yet caspase-11-dependent release of IL-1α is independent of both the NAIP5/NLRC4 and NLRP3/ASC inflammasomes as well as TRIF and type I interferon signaling. Furthermore, we find both overlapping and non-redundant roles for IL-1α and IL-1β in mediating neutrophil recruitment and bacterial clearance in response to pulmonary infection by *L. pneumophila*. Our findings demonstrate that virulent, but not avirulent, bacteria trigger a rapid caspase-11-dependent innate immune response important for host defense.

P4. Germline autoreactivity of VH1-46 antibodies to desmoglein 3 may explain their predominance in pemphigus vulgaris

Michael J. Cho[1], A.S. Lo[3], Eun Jung Choi[1], Arielle R. Nagler[1], Xuming Mao[1], Courtney B. Rubin[1], Marshall R. Posner[2], Lisa A. Cavacini[3], Aimee S. Payne[1]
[1] Univ. of Pennsylvania School of Medicine, Philadelphia, PA; [2] Mt. Sinai School of Medicine, New York, NY; [3] Harvard Medical School, Boston, MA

Pemphigus vulgaris (PV) is a potentially fatal blistering disease caused by autoantibodies (autoAbs) to desmoglein 3 (Dsg3). To better understand how Dsg3 autoreactive Abs develop, we cloned anti-Dsg3 Abs from four PV patients using phage display (n=2) or heterohybridoma (n=2) technologies. We identified VH1-46 anti-Dsg3 Abs from all four patients, comprising five unique heavy chain complementarity determining region (CDR) 3 sequences. All five Abs caused suprabasal blisters in human skin explants. F706 IgG4 and F779 IgG1 bound Dsg3 extracellular (EC) 1 domain, while 3.2, 4.2, and PVE4-8 bound Dsg3 EC1, 3, and 4. Surprisingly, most VH1-46 Abs had relatively few replacement somatic mutations. To determine if somatic mutations are required for Dsg3 binding, we reverted VDJ somatic mutations to their germline (GL) sequences. 3.2GL, 4.2GL and PVE4-8GL still bound Dsg3 by ELISA and/or immunofluorescence, confirmed by surface plasmon resonance. Analysis of mutations in F706GL and F779GL, which lost Dsg3 binding, revealed two identical acidic amino acid CDR mutations. Site-directed mutagenesis indicated that these acidic residues are necessary and sufficient for Dsg3 binding. Mutagenesis of 4.2GL and PVE4-8GL, which retained Dsg3 binding in the absence of somatic mutations, demonstrated that acidic residues in the heavy chain CDR3, presumably introduced during VDJ recombination, were necessary for Dsg3 binding. Our data indicate that VH1-46 autoAb gene usage is common in PV, which may be due to Dsg3 autoreactivity of some naive VH1-46 Abs and/or the requirement for a limited number of acidic amino acid mutations in the CDRs to confer Dsg3 binding.

P5. Regulated cytoskeletal control of ICAM-1 mobility on the dendritic cell plasma membrane enhances T cell activation

William A. Comrie, Sarah Boyle, Shuixing Li, Janis K. Burkhardt
Children's Hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania

T cell activation involves actin-dependent assembly and centripetal movement of signaling microclusters at the Immune Synapse (IS). Model stimulatory surfaces that limit microcluster dynamics enhance TCR signaling, but the possibility that antigen presenting cells can modulate T cell signaling by regulating ligand distribution is largely unexplored. Dendritic cells (DCs) form a multi-focal IS rather than the concentric pattern seen with freely mobile ligands, suggesting that DCs can control receptor-ligand localization. We have measured the lateral mobility of T cell ligands on the DC membrane, and find that ICAM-1, the key ligand for the integrin LFA-1, is selectively constrained via interactions with the actin cytoskeleton. ICAM-1 mobility decreases further upon LPS-induced DC maturation, concomitant with upregulation moesin and α-actinin 1, two actin-binding proteins that bind the cytoplasmic tail of ICAM-1. ICAM-1 co-localizes with moesin at punctate sites on the DC surface. Mutation of the ICAM-1 cytoplasmic tail or suppression of moesin and/or α-actinin liberates ICAM-1 lateral mobility. Importantly, liberating ICAM-1 from cytoskeletal constraints decreases the ability of DCs to induce the proliferation of naïve CD4 T cells. Taken together, our data show that the DC actin cytoskeleton actively controls ligand mobility and thus enhances T cell activation, and raise the possibility that DCs modulate T cell signaling by actively regulating molecular dynamics at the IS.

P6. Bystander cells aid immunity to *L. pneumophila*

Alan Copenhaver, Cierra Casson, Hieu Nguyen, Matthew Duda, Sunny Shin
Perelman School of Medicine, University of Pennsylvania

Many intracellular pathogens use secretion systems to inject virulent factors that interfere with host cells processes, including vesicle trafficking and host protein synthesis, which would be expected to limit cytokine and chemokine production. It is important then to understand how the innate immune system can overcome virulence activities and mount a successful immune response. *Legionella pneumophila*, the etiological agent of Legionnaire's disease, is an excellent model intracellular bacterium, as it has not evolved to evade the mammalian immune response. Legionella uses a type IV secretion system (T4SS) to inject effector proteins into host cells that allows it to modulate host cell processes and create for itself an intracellular, replicative niche. Previous work performed by our lab has found that the innate immune system can distinguish between virulent and avirulent Legionella, as within 24 hours post infection, the immune system mounts an increased pro-inflammatory cytokine response to wild type (WT) Legionella and not to mutant bacteria lacking a T4SS during *in vitro* and *in vivo* infection. These cytokines include IL-6, TNF, and IL-12, which are critical for immune control of bacterial infection. However, it is not known whether cells injected by the T4SS or un-injected cells contribute to cytokine production. We have developed a fluorescence-based assay that allows us to detect injection by the T4SS of Legionella into host cells and the single cell level. Interestingly, our data indicate that un-injected, bystander cells produce IL-6, TNF, and IL-12 in response to virulent Legionella, but not injected cells during *in vitro* and *in vivo* infection. Our results suggest that bystander cells contribute to innate immunity against Legionella. Current research is focused on how injected and un-injected cells communicate with each other to coordinate anti-Legionella immunity.

P7. Pre-B cells suppress Rag expression in response to DNA damage

Megan Fisher, Craig Bassing
Immunology Graduate Group, University of Pennsylvania

B and T cells must create functional antigen receptor genes by V(D)J recombination, a process in which RAG proteins make DNA double-strand breaks (DSBs) at specific gene segments. Regulation of this process is necessary to prevent translocations and other aberrant events. Data from our lab showed that pre-B cells induced *in vitro* to undergo Igκ recombination only induce RAG DSBs at half of the Igκ alleles in the population, corresponding to one broken allele per cell. Inhibition of the DNA-damage response protein ATM leads to RAG cutting of the second Igκ allele in most cells. This response corresponds with an increase in RAG mRNA and protein levels. These data suggest that RAG DSBs induced in pre-B cells signal through ATM to suppress RAG transcription and prevent further V(D)J recombination. This response could be specific to RAG DSBs at the Igκ locus, or it could be triggered by DSBs anywhere caused by other types of DNA stress. To distinguish between these possibilities, we induced primary pre-B cells to express RAG and initiate V(D)J recombination in the presence or absence of an ATM inhibitor. We also exposed these cells to ionizing radiation (IR) to induce many DSBs throughout the genome. We found that IR leads to decreased levels *Rag1* and *Rag2* mRNA, as well as decreased mRNA expression of Gadd45, a DNA damage sensor that drives Rag1 and Rag2 transcription. This response was dependent on ATM kinase activity. In lymphoid cells, IR DSBs signal through ATM to induce Gadd45a expression. My data indicate that pre-B cells have evolved tissue-specific mechanisms that co-opt the conserved DNA damage response to inhibit RAG function in response to DSBs. My ongoing research involves elucidating these mechanisms and determining their contribution to suppressing Ig translocations.

P8. Lymphoid tissue-resident commensal bacteria elicit distinct mucosal and systemic immune cell responses

Thomas C. Fung, Matthew R. Hepworth, David Artis, Gregory F. Sonnenberg
Immunology Graduate Group, Institute for Immunology, University of Pennsylvania

The mammalian intestine is colonized by trillions of commensal bacteria that contribute to essential host functions such as metabolism, resistance to pathogens and development of the immune system. In healthy mammals, most commensal bacteria are restricted to the intestinal lumen and physically separated from the epithelial surface and underlying immune cells. However, some commensal bacteria, such as segmented filamentous bacteria, are found in close contact with the epithelium of the small intestine. Recently, a population of commensal bacteria belonging to the genus *Achromobacter* has been shown to constitutively reside on the interior of intestinal lymphoid tissues of healthy mice and humans, identifying a previously unappreciated anatomic location for commensal bacteria colonization. However, how lymphoid tissue-resident commensal bacteria access and colonize lymphoid tissues without triggering pathological inflammation has not been studied. Using an *in vitro* co-culture method, we demonstrate that *Achromobacter xylosoxidans* can colonize murine dendritic cells (DCs) and promote the production of cytokines that are associated with host-protective immune cell responses. Based on these data, we hypothesize that *A. xylosoxidans* can elicit robust host-protective immune cell responses in intestinal lymphoid tissues *in vivo*. Indeed, mice mono-associated with *A. xylosoxidans* display increased frequencies of IL-22-producing innate lymphoid cells (ILCs), $CD4^+$ T helper 17 (Th17) cells and regulatory T cells (Tregs) present in the colon lamina propria, gut-associated lymphoid tissues, and extraintestinal lymphoid organs such as the spleen. Collectively, these data suggest that *A. xylosoxidans* represents a previously unappreciated group of lymphoid tissue-resident commensal bacteria that can prime mucosal and systemic immune cell responses, and may play a critical role in immunity, inflammation or tissue homeostasis.

P9. Interleukin-22 mediates protection during a murine model of cutaneous leishmaniasis

Ciara Gimblet[1], David Artis[2], Phillip Scott[1]
[1]*Department of Pathobiology, University of Pennsylvania, Philadelphia, PA, USA.*
[2]*Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA*

Cutaneous leishmaniasis is a disease characterized by ulcerating skin lesions that are normally self-healing. However some patients develop more severe disease that fails to resolve. While parasite control by the immune response has been well studied, the mechanisms of how lesions resolve, or in some cases fail to resolve, is not well understood. IL-22, a member in the IL-10 family of cytokines, has been shown to contribute to the wound healing process through maintenance of the epithelial barrier. In this study, we investigated whether IL-22 contributed to lesion resolution during *Leishmania* infection. We infected IL-22 deficient mice in the ear with *L. major* and found that these mice developed more severe disease compared with C57BL/6 control mice. The lesions of the IL-22 deficient mice had significantly more inflammation, which resulted in tissue loss at the site of infection. However, we found that parasite burdens were similar in the wild-type and IL-22 deficient mice, suggesting that the increased inflammation was not due to an increased parasite burden. Instead, the lesions from the IL-22 deficient mice expressed significantly higher levels of IL-1α, IL-1β, and S100A8/S100A9, molecules associated with tissue damage, increased epithelial cell death, and delayed wound healing. These studies suggest that during *L. major* infection, IL-22 plays a previously unappreciated role in controlling Leishmania-induced inflammation, presumably by protecting epithelial cells from cell death and maintaining the epithelial barrier for an efficient wound healing response.

P10. The role of skin memory CD4 T cells in protection against *Leishmania major*

Nelson Glennie, Venkat Yeramilli, Phillip Scott
University of Pennsylvania

Cutaneous infection with *Leishmania major* results in the generation of a memory response that is highly protective against subsequent disease. While central and effector memory T cells within lymphoid compartments are known to play a crucial role in this response, the importance of memory T cells present in peripheral tissues remains poorly defined. We have identified *L. major*-responsive CD4 T cells present in uninflamed skin sites after the resolution of cutaneous disease. This population has enriched expression of canonical skin-residency markers CD69 and CD103, can be detected as early as 2 weeks after *L. major.* infection, and persists in infected mice long-term. Using an adoptive transfer model we have shown enhanced recruitment of immune cells to challenge sites containing skin-localized memory T cells over their naive counterparts. Taken together, these data suggest that memory CD4 T cells in the skin may play a critical role in protection against cutaneous leishmaniasis and therefore have important implications for leishmania vaccine design.

P11. Role of non-core Rag1 in B cell development

Julie E. Horowitz, Craig H. Bassing
Immunology Graduate Group, University of Pennsylvania; Department of Pathology and Laboratory Medicine, Center for Childhood Cancer Research, Children's Hospital of Philadelphia

The RAG1/RAG2 (RAG) endonuclease initiates V(D)J recombination at antigen receptor (AgR) loci by binding and cleaving between germline AgR gene segments and flanking recombination signal sequences. RAG proteins are comprised of indispensable "core" endonuclease domains and dispensable "non-core" regions. Humans with mutation or deletion of RAG1 non-core residues exhibit primary immunodeficiencies associated with autoimmunity, atopy, and limited/altered AgR repertoire. Mice expressing core Rag1 protein (*Rag1*$^{C/C}$ mice) display impaired early T and B cell development associated with reduced levels of IgH and TCRb rearrangements. These phenotypes may arise from impaired RAG endonuclease activity, reduced RAG accessibility to gene segments, and/or impaired cellular proliferation, survival, or differentiation following RAG cleavage. To begin to distinguish among these possibilities, we have analyzed late B cell development in Rag1$^{C/C}$ mice. We observe that Rag1$^{C/C}$ mice exhibit impaired late B cell development with pronounced loss of Igl^{+} B cells. We observe no defect in pre-BCR mediated proliferation of developing B cells in Rag1$^{C/C}$ mice. We show that expression of the pro-survival BCL2 protein but not of a pre-assembled IgH transgene rescues Igl^{+} B cell development in Rag1$^{C/C}$ mice. In mechanistic studies, we find that Rag1$^{C/C}$ pre B cells have decreased levels of germline Igl transcripts and that BCL2 expression in pre B cells fails to rescue Igl transcripts, yet uncovers decreased germline Igk transcripts. Further, we find increased levels of germline Igl transcripts and enhanced nuclease sensitivity of Igk and Igl loci in pre B cells expressing full-length catalytically inactive Rag1 relative to Rag1-deficient pre B cells. Collectively, these data demonstrate a role for non-core Rag1 regions in promoting AgR locus accessibility prior to recombination to establish a diverse primary AgR repertoire.

P12. Genome integrity and the maintenance of antigen-specific CD8 T cell populations

Jonathan Johnnidis, E. John Wherry
Departments of Biology and Microbiology, and Institute for Immunology, University of Pennsylvania

To effect defense against exogenous and endogenous perturbagens, the adaptive immune system leverages the tremendous proliferative capacity with which it is endowed to generate terminally differentiated cells that potently eliminate or suppress threats to organismal health. While considerable, this proliferative capacity is not infinite but can be depleted with age and following repeated rounds of Ag-driven proliferation -- for example in the cases of recurring acute infections or chronic infections.

Underlying the capacity to generate terminally differentiated progeny is the integrity of genomically encoded information. Herein we probe genomic integrity homeostasis during the immune response to viral infection. We find that $CD8^+$ T cells experience significant DNA damage in the course of their attempts to control viral replication, and that relative to downstream progeny cells, precursor cells are endowed with superior DNA damage responsiveness. We suggest that this characteristic of amplified genome integrity surveillance in precursor cells may be a fundamental feature of the long-term maintenance of Ag-experienced T cell populations.

P13. A cell-intrinsic role for Ndfip1 in limiting TH17 differentiation and proinflammatory cytokine production

Awo AK Layman[1], Paula Oliver[2]
[1,2] *University of Pennsylvania School of Medicine, Immunology Graduate Group.* [2] *Children's Hospital of Philadelphia*

Ubiquitination and subsequent degradation of effector pathway proteins is an important way of terminating an effector cell response. Three classes of ubiquitinating 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 an adaptor protein that activates the catalytic function of several related E3 ubiquitin ligases. Previous work from our lab showed that mice lacking Ndfip1 have increased numbers of T_H17 cells and that Ndfip1 is expressed in T cells. Thus, we hypothesized that Ndfip1 has a T cell-intrinsic role in limiting T_H17 cell differentiation and proinflammatory cytokine production. Using a mixed chimera model, we show that T cells that lack Ndfip1 are much more likely to produce IL-17A and GM-CSF, two proinflammatory cytokines produced by T_H17 cells. Additionally, Ndfip1-deficient T cells produce more IL-17A and GM-CSF when cultured under T_H17 polarizing conditions. These data may help to explain why mice that lack Ndfip1 are more susceptible to IL-17-mediated pathology in a DSS colitis model, compared to their Ndfip1-sufficient counterparts. This work reveals a biologically relevant role for Ndfip1 in limiting T_H17 differentiation and GM-CSF production, as well as associated inflammatory pathology. Future work will identify components of Ndfip1-mediated ubiquitin complexes and determine the underlying mechanism by which Ndfip1 limits proinflammatory cytokine production by T_H17 cells.

P14. TCR signaling by CD4+ T cells is required for maintenance of Foxp3+ regulatory T cells

Theresa Leichner, Atsushi Satake, Taku Kambayashi
Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine

Regulatory T cells (Tregs) control conventional T cell (Tconv) activation and Treg numbers need to be kept in an appropriate proportion to Tconvs for maintenance of peripheral tolerance. Treg homeostasis is highly dependent on their IL-2-mediated proliferation in the periphery. Since Tconvs but not Tregs produce IL-2, IL-2 could serve as a gauge for Tregs to adjust their numbers accordingly to the size of the Tconv pool in the periphery. As the production of IL-2 by Tconvs is dependent on TCR signaling, we hypothesized that TCR signaling by Tconvs in response to self MHC class II (MHC-II) molecules is required for Treg maintenance.

Using MHC-II-deficient bone marrow chimeric mice, we found that Tregs cannot be maintained in the absence of MHC-II. The drop in Treg numbers was associated with Tconv activation and the development of severe dermatitis, gut pathology, and weight loss. The decrease in Treg numbers and the onset of this disease was delayed by the injection of IL-2 or by adoptive transfer of more Tregs. Furthermore, in a cell transfer model in which TCR signals were diminished in Tconvs alone, Tregs were unable to be maintained, with a related IL-2 mRNA decrease in the Tconv signaling mutants. These results suggest that TCR signaling by Tconvs in response to self MHC-II molecules is critical for the maintenance of Tregs and that the production of IL-2 is a potential sensor for the indexing of Treg numbers to Tconv. This TCR signaling requirement points to a potential role of positive selection in producing Tconvs bearing TCRs with adequate affinity to self MHC-II complexes in Treg maintenance. Indeed, T cells from MHC-II mismatched chimeras only produced IL-2 against DCs of the MHC haplotype they were positively selected on. However, the strength of TCR signal received during thymic development by Tconvs did not correlate with their ability to maintain Tregs, as $CD5^{hi}$ and $CD5^{lo}$ Tconvs maintained Tregs at a similar ratio in an adoptive transfer setting. Thus, although TCR signaling by Tconvs is required for Treg maintenance, all positively selected Tconvs possess sufficient ability to signal through the TCR for the maintenance of Tregs.

P15. Prime-boost vaccination with heterologous influenza strains focuses antibody responses to conserved epitopes

Susanne L. Linderman[1,2], Colleen B. Sullivan[1], Scott E. Hensley[1,2]
[1]*The Wistar Institute and* [2]*Insitute for Immunology, University of Pennsylvania, Philadelphia, PA 19104*

Primary influenza antibodies are typically directed against the variable globular head of the hemagglutinin (HA) protein. However, since HA rapidly acquires mutations, humans are repeatedly exposed to antigenically drifted strains over time. Our previous studies suggest that influenza antibody specificities in humans are influenced by prior influenza exposures. Here, we tested the hypothesis that influenza antibody repertoires can be shifted to more conserved areas of HA by sequential vaccination with H1N1 strains that have dramatic antigenic differences. To address this, we vaccinated mice with the PR8 strain and then revaccinated with either the PR8 strain or an antigenically distinct PR8 mutant, termed S12a. We found that PR8/S12a heterologous vaccination elicited more cross-reactive HA antibodies compared to PR8/PR8 homologous vaccination. S12a-specific antibody secreting cells were only elicited by PR8/S12a vaccination. Surprisingly, PR8/S12a vaccination also elicited more PR8-specific antibody secreting cells compared to PR8/PR8 vaccination. Ongoing studies are evaluating the fine specificities and the neutralization efficacy of antibodies elicited by these vaccination regimens.

P16. IL-21 blocks B cell death from BCR-delivered TLR9 agonists and upregulates both AID and T-bet

Martin S. Naradikian, Michael A. Oropallo, Michael P. Cancro
University of Pennsylvania

A growing literature implicates the B cell receptor (BCR), Toll-like receptors (TLR), and survival cytokines or costimulation in the etiology of SLE and other humoral autoimmune diseases. Our lab has found that BCR-delivered TLR9 ligands yield a unique post-proliferative cell death that limits responses to DNA-linked antigens. Furthermore, death via this mechanism is rescued by the survival cytokine BLyS. However, here we show that BLyS is not unique in its capacity to rescue this cell death. Indeed, the T cell-derived survival signals including CD40L, IL-4, and IL-21 similarly rescue B cells from BCR delivered TLR9 agonists. All of these also induce AID, which is required for class switch recombination and somatic hypermutation. However, IL-21 uniquely upregulates T-bet. Since T-bet has previously been described as necessary for switching to the pathogenic isotype, IgG_{2a}, these findings suggest that IL-21 may be involved in the production of DNA autoantibodies.

P17. Sensitivity of mitogen-activated protein kinase responses in macrophage Toll-like receptor signaling

Caleb E. Ng, Rachel A. Gottschalk, Ronald N. Germain
Immunology Graduate Group, University of Pennsylvania; Lymphocyte Biology Section, Laboratory of Systems Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health

Toll-like receptors (TLRs) mediate sensing of common pathogen associated molecular patterns by the innate immune system. Signal transduction by TLRs results in changes in gene expression and the production of cytokines. Using single-cell techniques, we observe temporal heterogeneity in the production of the cytokines TNF-alpha, IL-6, and IL-12 upon ligation of TLR4 in bone marrow-derived macrophages. TLR signaling proceeds through multiple pathways, including the highly evolutionarily conserved mitogen-activated protein kinase (MAPK) phosphorelay cascades. MAPK signaling has been a topic of intense study in numerous systems. However, a quantitatively detailed description of MAPKs in innate immune signaling has yet to be reported. We report single-cell analyses of the kinetics and sensitivity of two MAPKs, extracellular response kinase (ERK) and p38. These data will provide the foundation for a more detailed model of signal transduction by TLRs.

P18. Cytotoxic T cells mediate pathology and metastasis in cutaneous leishmaniasis

Fernanda O. Novais[1], Lucas P. Carvalho[2], Joel W. Graff[3], Daniel P. Beiting[4], Gordon Ruthel[1], David S. Roos[4], Michael R. Betts[5], Michael H. Goldschmidt[1], Mary E. Wilson[3], Camila I. de Oliveira[6], Phillip Scott[1]

[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 [2]Instituto Nacional de Ciência e Tecnologia de Doenças Tropicais-INCT-DT(CNPq/MCT), Serviço de Imunologia, Hospital Universitario Prof. Edgard Santos, Universidade Federal da Bahia Salvador,Bahia,Brazil.[3]Iowa City VA Medical Center, Iowa City, Iowa 52246 [4]Department of Biology, University of Pennsylvania, Philadelphia, PA 19104 [5]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 [6]Centro de Pesquisas Gonçalo Moniz, Fundaçao Oswaldo Cruz, Salvador, Brazil

Disease progression in response to infection can be strongly influenced by both pathogen burden and infection-induced immunopathology. While current therapeutics focus on augmenting protective immune responses, identifying therapeutics that reduce infection-induced immunopathology are clearly warranted. Despite the apparent protective role for murine CD8+ T cells following infection with the intracellular parasite *Leishmania,* CD8+ T cells have been paradoxically linked to immunopathological responses in human cutaneous leishmaniasis. Transcriptome analysis of lesions from *Leishmania braziliensis* patients revealed that genes associated with the cytolytic pathway are highly expressed and CD8+ T cells from lesions exhibited a cytolytic phenotype. To determine if CD8+ T cells play a causal role in disease, we turned to a murine model. These studies revealed that disease progression and metastasis in *L. braziliensis* infected mice was independent of parasite burden and was instead directly associated with the presence of CD8+ T cells. In mice with severe pathology, we visualized CD8+ T cell degranulation and lysis of *L. braziliensis* infected cells. Finally, in contrast to wild-type CD8+ T cells, perforin-deficient cells failed to induce disease. Thus, we show for the first time that cytolytic CD8+ T cells mediate immunopathology and drive the development of metastatic lesions in cutaneous leishmaniasis.

P19. PD-1 antagonizes CD8+ T cell exhaustion by preventing excessive proliferation and loss of Tbet^{hi} progenitors during chronic infection

Pamela Odorizzi, Michael Paley, Burton Barnett, Makoto Kurachi, E. John Wherry

Department of Microbiology and Institute for Immunology, The University of Pennsylvania Perelman School of Medicine, USA

Chronic viral infections, such as HIV, HBV and HCV, are a major public health threat and cause significant morbidity and mortality worldwide. Lack of immune control during these infections is associated with CD8 T cell exhaustion. A major feature of exhaustion is the expression of multiple inhibitory receptors, notably PD-1, on virus-specific CD8 T cells. Importantly, blocking inhibitory receptor pathways during the chronic phase of infection improves CD8 T cell responses and reduces viral burden. These findings emphasize the importance of inhibitory receptors in anti-viral immune responses and suggest the possibility of targeting these pathways in vaccinations and therapeutics. However, advancements in this area have been hindered by a lack of basic mechanistic insight into inhibitory receptor pathways and how these receptors shape the CD8 T cell response to chronic infection. To address this question, we generated LCMV-specific CD8 T cells (P14 cells) deficient in PD-1. Upon co-transfer with WT P14 cells, PD-1 KO P14 cells expanded to a greater degree than WT P14 cells during the early stages of chronic LCMV infection but contracted dramatically 14 days post-infection. PD-1 KO P14 cells were functionally distinct from WT P14 cells in the chronic phase of infection, displaying elevated inhibitory receptor expression, reduced proliferation and impaired cytokine production, but maintaining enhanced cytotoxic ability. Furthermore, PD-1 deficiency resulted in dramatic loss of Tbet^{hi} progenitor cells during the chronic phase of infection, leading to an ultimate failure in long-term CD8+ T cell maintenance. Similar changes were observed following therapeutic PD-1 blockade. These studies suggest a critical role for PD-1 in tempering and sustaining CD8 T cell responses during chronic infection and have important implications in the design of vaccines and therapeutics targeting inhibitory receptor pathways.

P20. Development of a canine model of anti-tumor CAR therapy

M. Kazim Panjwani[1], Nicola Mason[2]
Immunology Graduate Group[1] and Department of Pathobiology, School of Veterinary Medicine[2], 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. 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 human CD19-specific single chain variable fragment (scFv). Our initial studies assessed canine CAR function in primary human T cells, given the high construct homology and ease with which human T cells can be transduced with lentiviruses. Primary human T cells were transduced with lentiviruses containing each of the 3 cCAR constructs or GFP, and co-cultured with K562s bearing human CD19 (hKt19s) or control K562 cells. T cells modified with each cCAR construct 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 each cCAR. To effectively re-direct canine T cells, it has been necessary to develop and optimize a lentiviral transduction protocol. We have now been able to achieve stable transduction of 15-20% canine T cells using lentiviral GFP and are in the process of transducing canine T cells with cCARs. Developing the spontaneous canine model for re-directed T cell therapy in solid tumors will help elucidate CAR-tumor interactions, allow us to investigate ways to circumvent tumor-mediated immunosuppression and improving efficacy of genetically re-directed T cells.

P21. Innate immune regulation of intestinal epithelial stem cells

Lance Peterson[1,2], Laurel Monticelli[1,2], David Artis[1,2]
[1]Department of Microbiology and Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. [2]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA 19104, USA.

A constant dialogue between the luminal microbiota, immune cells, and epithelial cells contributes to protection against microbial pathogens and the maintenance of intestinal immune homeostasis. Mucosal immune cells play an important role in mediating barrier immunity through their regulation of epithelial cell function and homeostasis. Multipotent intestinal epithelial stem cells (IESCs), which renew the entire epithelial surface, represent a potential target of immune-mediated regulation of epithelial cell function. Using *in vivo* and *in vitro* models to assess IESC proliferation, differentiation and self-renewal, we are investigating the contribution of specific innate immune cell and cytokine signaling pathways to the regulation of the IESC niche in the context of health and disease.

Employing IESC reporter mice we observed that in response to DSS-induced intestinal damage and inflammation, IESC number and proliferation were significantly reduced. We observed clusters of innate lymphoid cells (ILCs) in the intestinal lamina propria, and treatment with exogenous recombinant IL-33 promoted ILC2 responses and amphiregulin-dependent protection of mice from DSS-induced intestinal damage. Through the genetically targeted disruption of IESC-specific signaling, we are investigating the *in vivo* role of ILC2-derived cytokines and growth factors in regulating IESCs in the context of intestinal damage, inflammation and tissue repair. In ongoing studies employing primary IESC cultures we are testing the direct influence of ILC2s on IESCs. Using these *in vitro* and *in vivo* approaches, we anticipate that we will make new insights into the influence of immune cell-IESC interactions on the maintenance of intestinal barrier function and tissue homeostasis.

P22. Ndfip1 and its peptide mimics are de-repressors of Itch and related E3 ubiquitin ligases

Chris Riling[1], Hari Kamadurai[2,7], Suresh Kumar[3,7], Claire O'Leary[1,7], Erika E. Manion[3], Mingjie Ying[4], Brenda Schulman[5], Paula Oliver[6]

[1]University of Pennsylvania, School of Medicine; [2]St. Jude Children's Research Hospital; [3]Progenra Inc, Malvern PA; [4]The Children's Hospital of Philadelphia, Cell Pathology Division; [5]Howard Hughes Medical Institute; [6]University of Pennsylvania, School of Medicine, Department of Pathology and Laboratory Medicine; [7]these authors contributed equally

Nedd4 family E3 ubiquitin ligases regulate a diverse array of biologic processes including sodium and iron homeostasis, tumor suppression, and immune function. These ligases can be regulated by autoinhibition and through their association with a small family of interacting proteins, Ndfip1 and Ndfip2. Here we show that these two regulatory processes are functionally linked. We demonstrate that in the absence of Ndfip1, Itch can bind an E2 but the E2 is unable to transfer ubiquitin onto the catalytic cysteine of Itch, thus defining the limiting step of autoinhibition. This is because Itch is autoinhibited by an intramolecular interaction between its HECT and WW domains. Ndfip1, via its PY motifs, binds multiple WW domains of Itch, releasing the HECT, and thus allowing ubiquitin charging by the E2. This mode of autoinhibition may be unique to a subset of Nedd4-family members as Ndfip1 and its peptide mimics are required for the activation of some but not all Nedd4-family E3 ubiquitin ligases *in vitro*. This formally redefines Ndfip1 as a derepressor of autoinhibition for Nedd4-family members. These data reveal strategies for therapeutically activating Nedd4-family E3 ligases. Based on the biologic function of Nedd-4 family members that use this mechanism, such therapeutics may be particularly useful for the treatment of inflammatory diseases.

P23. Evaluation of T-bet and perforin dysregulation in HIV/SIV-specific responsive CD8+ T cells in rhesus macaque model of HIV/SIV infection

Emily R. Roberts, Michael R. Betts

Perelman School of Medicine and School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

T-bet and perforin have been described to be involved in CD8+ T cell effector functions, which include clearance of target cells such as HIV-infected CD4+ T cells. Chronic HIV progressors display dysregulation of both T-bet and perforin expression in HIV-specific responsive CD8+ T cells as compared to elite controllers. The frequency of perforin expressing HIV-specific responsive CD8+ T cells in HIV+ patients inversely correlates with viral load. Thus, the decreased ability to upregulate perforin in HIV-specific responding CD8+ T cells described in chronic progressors consequently may contribute to the inferior control of viral load as compared with elite controllers. The expression profiles of T-bet and perforin in HIV-specific responsive CD8+ T cells have yet to be described during the acute phase of infection.

As an animal model, Rhesus macaques (RM) demonstrate some characteristics of chronic immune activation during SIV infection that have been described during HIV infection. However, the possibility of dysregulation of T-bet and perforin in SIV specific CD8+ T cells has not been characterized in SIV infected RM. In this study, we will evaluate PBMCs from three to four RM pre-SIV infection, and at four time points during the acute phase of infection. The expression profile of T-bet and perforin in the CD8+ T cell compartment will be evaluated in these animals using fluorescence cytometry. Data generated in RM will be compared to data generated from a cohort of acutely infected HIV+ patients. Validation of similar dysregulation of T-bet and perforin in the HIV/SIV-specific responsive CD8+ T cell compartment during acute infection will support the use of RM as an animal model to study CD8+ T cell dysfunction in the context of HIV/SIV infection.

P24. Dicer regulates CD4 and CD8 silencing and lineage commitment during T cell development

Levi J. Rupp[1], Brenna L. Brady[1], Andrea C. Carpenter[2], Remy Bosselut[2], Craig H. Bassing[1]
[1]Perelman School of Medicine at the University of Pennsylvania, [2]Laboratory of Immune Cell Biology, National Institutes of Health

T cell development involves a highly regulated process of lineage specification and commitment. T-cell specific deficiency of the RNA processing enzyme Dicer results in significant apoptosis of developing thymocytes but has not been reported to affect CD4/CD8 lineage commitment. By rescuing cell death in Dicer deficient thymocytes (either by transgenic BCL-2 expression or p53 deficiency) we have shown that Dicer is required for appropriate CD4 and CD8 co-receptor silencing and lineage commitment. Specifically, Dicer deficient BCL-2 transgenic mice (LckCre;EμBCL-2;Dicer$^{flox/flox}$) contain an aberrant population of mature peripheral CD4+CD8+ T cells. Bone marrow chimeras and TCR transgenic studies demonstrated that the CD4+CD8+ population contains both MHCI and MHCII-restricted cells. Runx3 is the "master regulator" of the CD8 lineage and is required for appropriate CD4 silencing. Sorted CD4+CD8+ cells from Dicer$^{-/-}$ OT-I mice exhibited reduced levels of Runx3 protein, suggesting that an inability to initiate Runx3 expression causes impaired CD4 silencing and lineage commitment in Dicer deficient MHCI-restricted cells. Furthermore, *in vitro* TCR stimulation of Dicer$^{-/-}$ OT-I CD4+CD8+ cells led to generation of CD4+CD8- T cells. Thus, Dicer deficiency allows re-direction of MHCI-restricted cells to the CD4 lineage and may permit un-coupling of lineage commitment and thymic egress. Parallel studies in MHCII-restricted TCR transgenic mice aim to test this hypothesis.

P25. microRNAs as regulators of anti-viral CD8 T cells responses

Erietta Stelekati[1], Donald T. Gracias[2], Jennifer L. Hope[2], Mohammed-Alkhatim Ali[1], Peter D. Katsikis[2], E. John Wherry[1]
[1]Department of Microbiology and Institute for Immunology, University of Pennsylvania Perelman School Medicine, Philadelphia, PA 19104, U.S.A.
[2]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19129, U.S.A.

CD8 T cells are crucial mediators of antiviral immunity and memory CD8 T cells provide protection upon secondary challenge. During chronic infections dysfunctional "exhausted" T cells replace the functional memory cells observed in acute infections. 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 role of microRNAs in regulating anti-viral CD8 T cell responses remains largely unknown. Here, we asked how microRNAs regulate anti-viral CD8 T cell responses. We performed microarray analysis of LCMV-specific CD8 T cells at different stages of acute or chronic infection and identified clusters of microRNAs differentially expressed at different stages of an anti-viral response as well as clusters of microRNAs differentially expressed in CD8 T cells responding to acute versus chronic infection. Further investigation of the first is important to understand and optimize anti-viral CD8 T cell responses, while examination of the latter will provide insight into CD8 T cell dysfunction during chronic infections.

P26. Aryl hydrocarbon receptor activity promotes the control of parasite burdens during chronic toxoplasmosis

Sagie Wagage, Gretchen Harms Pritchard, Arielle Glatman Zaretsky, Christopher A. Hunter
Department of Pathobiology, University of Pennsylvania

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that has been shown to affect multiple aspects of the immune response. A number of putatitve AHR ligands are generated during infection with *Toxoplasma gondii*, raising the possibility that sensing through this transcription factor could affect the immune response to the parasite. When challenged with *T. gondii*, *Ahr*$^{-/-}$ mice developed higher parasite burdens during chronic infection. However these mice had no defects in multiple aspects of the effector immune response, including antibody production and T cell activation. These findings led to the question of whether *Ahr*$^{-/-}$ mice had a cell intrinsic defect in controlling parasite growth. Taken together, these results indicate that sensing through the AHR contributes to the control of parasite replication following *T. gondii* infection.

P27. M2 macrophages are associated with *Leishmania major* infection

Tiffany Weinkopff, Phillip Scott
Department of Pathobiology, University of Pennsylvania, Philadelphia, PA, USA

Leishmania major is an intracellular protozoan parasite that induces cutaneous lesions in the skin. Control of the parasite is dependent upon the activation of macrophages, leading to production of nitric oxide and reactive oxygen species. Thus, in a healing infection one finds macrophages that are polarized towards an M1 phenotype characterized by the expression of iNOS. However, we found that lesions from C57BL/6 mice infected with *L. major* not only expressed genes associated with M1 macrophages, but we also detected elevated expression of M2 genes, including Arg1, Ym1, mannose receptor, dectin-1 and transglutaminase 2. Correlating with the expression of M2 genes, we also saw expression of cytokine genes, such as IL-4, IL-13, IL-10 and TGFβ, that are known to skew macrophages towards an M2 phenotype. While M2 macrophages may provide a safe haven for parasites since they do not produce nitric oxide, they also might promote resolution because of their wound healing capacity. To understand their role in leishmaniasis, we examined how M2 macrophages interact with the parasite. We generated M2 macrophages by intraperitoneal injection of an IL-4 complex, isolated cells and evaluated their ability to control parasites. We also assessed whether these cells can be activated for parasite killing. In addition, M2 macrophages were elicited at the site of infection with IL-4 complex to determine how these cells function *in vivo* during *L. major* infection. Preliminary results indicate that parasite growth is not enhanced in M2 macrophages. Moreover, we found that M2 macrophages could be activated by IFN-g to restrict parasite growth. Unexpectedly, our results suggest both M1 and M2 macrophages may be involved in parasite control during leishmaniasis.

P28. Diacylglycerol kinase deficiency enhances NK cell function without affecting NK cell tuning

EnJun Yang[1], Amanda Schmidt[1], Taku Kambayashi[1]
Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Signaling through immunotyrosine-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 both NK cell function and Ly49 receptor acquisition during NK cell development, the 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. We focused our study on two different DGK isoforms (a and ζ) that are most abundantly expressed in lymphocytes.

Compared to WT NK cells, immunoreceptor-activated DGKζ-deficient NK cells displayed an ~2-fold increase in IFNg production and degranulation. Only a small increase (~20%) in NK cell function was observed in DGKa-deficient NK cells, suggesting that DGKζ plays a more prominent role than DGKa in NK cell function. 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 that had an inducible deletion of DGKζ. Immunoreceptor-activated DGKζ-deficient NK cells displayed increased ERK phosphorylation, a key signaling molecule activated downstream of DAG.

Our data presented here are in contrast to NK cells from mice lacking other negative regulators of signaling such as SHP-1, which are hyporesponsive and display increased Ly49 receptor expression. Moreover, DGKζ-deficient NK cells that are transferred into MHC Class I deficient hosts downregulate their responsiveness to a similar extent as WT cells, 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 the self tolerance of NK cells.

P29. Irradiation reduces homing of bone marrow progenitors LMPP and CLP to the thymus

Shirley L. Zhang, Sugata Manna, Daniel Aaron Zlotoff, Jerrod L. Bryson, Avinash Bhandoola
Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Development of T cells in the thymus requires continuous importation of T lineage progenitors from the bone marrow via circulation. Following bone marrow transplant (BMT), recovery of a normal peripheral T cell pool depends on production of naïve T cells in the thymus; however, delivery of progenitors to the thymus limits T lineage reconstitution. Here, we examine homing of progenitors to the thymus using a short-term homing assay (STHA) in which we analyze the thymus for donor derived thymocytes at very early time points. In unirradiated mice, we use STHA to verify that LMPP and CLP are the only progenitors that home to the thymus after 24 hours. Approximately 4 or 5 cells out of 10,000 donor lymphoid progenitors are able to settle the thymus as we determine using limit dilution analysis of STHA. Surprisingly, following irradiation conditioning, we find homing to the thymus decreases more than 10-fold. Although thymic epithelial cells (TECs) continue to produce chemokine mRNA after irradiation, CCL25 and CCL21 are absent from luminal surface of the endothelium. Finally, we show that pretreatment of bone marrow progenitors with CCL25 and CCL21 partially restores homing to the thymus after irradiation conditioning.