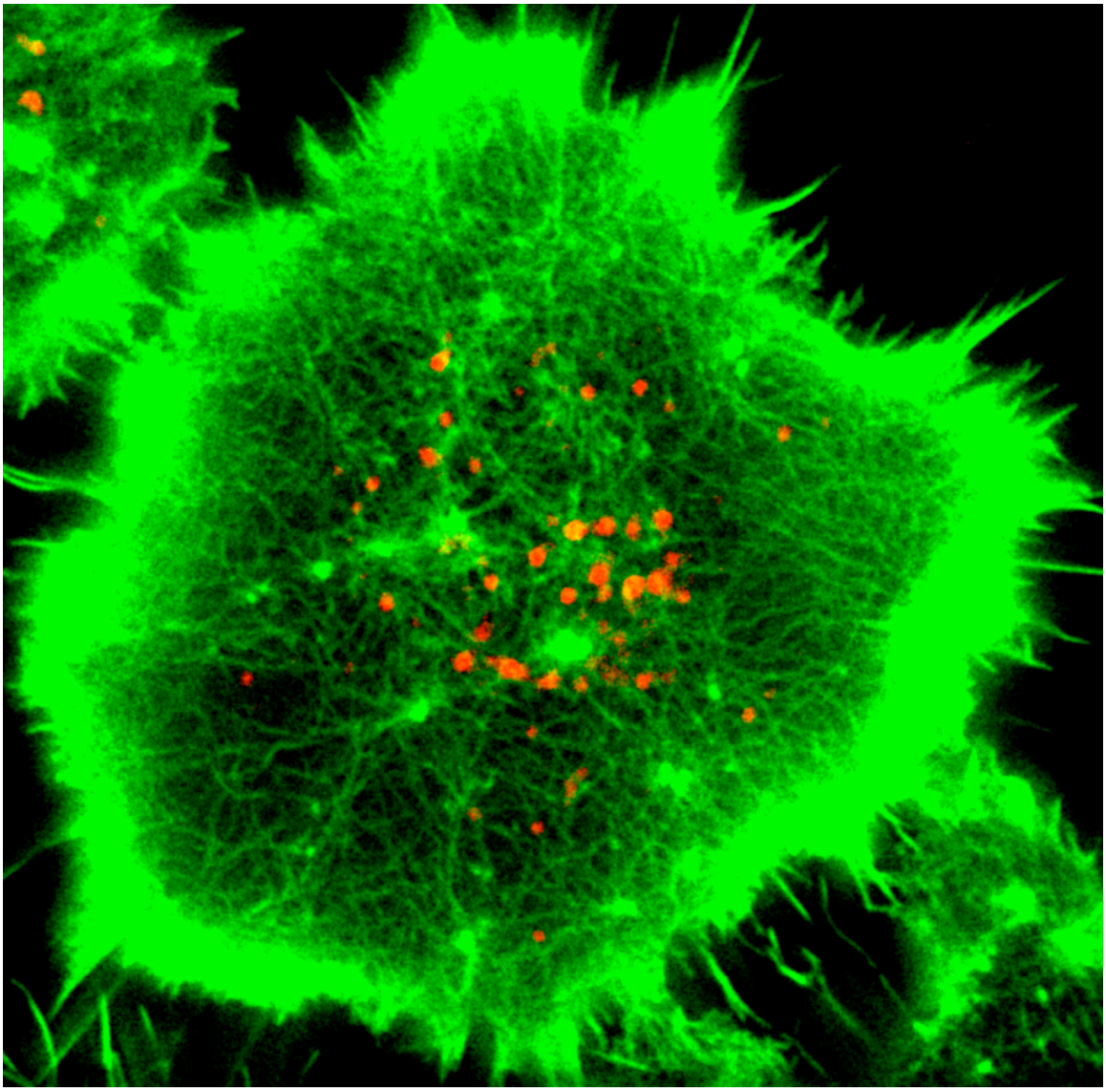
24th Annual Immunology Graduate Group Retreat



October 28-30, 2011
Willow Valley Resort and Conference Center
Lancaster, PA

The Immunology Graduate Group gratefully acknowledges the financial support of all our contributors for the 24th Annual Retreat:

Grant Support

- P01 CA 93615-01 "Temporal and Spatial Organization of Signaling Complexes in T and B cells" project grant
- T32 AI 055428 "Immune System Development and Regulation" training grant
- T32 AI 070077 "VMD-PhD training in infectious disease-related research"
- T32 CA 009140 "Training Program in Immunobiology of Normal and Neoplastic Lymphocytes"

Institutes, Centers, Departments, and Divisions

- Abramson Cancer Center Immunobiology Program
- Combined Degree and Physicians Scholar Program
- Department of Pathobiology at the School of Veterinary Medicine
- Division of Gastroenterology and The Center for Molecular Studies in Digestive and Liver Diseases, Children's Hospital of Philadelphia
- Division of Rheumatology, Children's Hospital of Philadelphia
- Institute for Immunology
- Institute for Regenerative Medicine
- Joseph Stokes, Jr. Research Institute
- The Department of Pathology at Penn Dental School
- The Penn Center for AIDS Research
- The Wistar Institute

Corporate Sponsorship

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Cover Photo

Super-resolution stimulated emission depletion (STED) fluorescence micrograph of an activated human natural killer cell demonstrating actin filaments (green) and perforin-containing lytic granules (red). Image taken by Dr. Emily Mace in the laboratory of Jordan Orange.

24th Annual Immunology Retreat
Friday to Sunday, October 28-30, 2011
Willow Valley Resort and Conference Center
2416 Willow Street Pike, Lancaster, PA 17602-4898

Friday, October 28, 2011

Please 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. All sessions to be held in Statesman Hall A/B/C/D.

11:00-12:00 PM	**Retreat registration and program pick-up, Main Lobby**
12:00-1:20	**Lunch, Smorgasbord, The Willow Tree**
1:20-1:30	**Welcome, John Wherry, IGG Chair**
1:30-2:50	**Session I: Regulation of T-cell responses** **Session Chair: Lisa Korn**
1:30-1:50	Lisa Korn "*A role for MHC II-independent gut flora-derived signals in the homeostasis of gut Treg"*
1:50-2:10	Natalia Ramos-Hernandez *"Ndfip1 is required for the peripheral tolerance of CD4+ T cells"*
2:10-2:30	Olivia Perng *"CD4+ T cell affinity can affect gender bias, disease severity and B cell requirement in inflammatory arthritis"*
2:30-2:50	Alison Beal "*TGF-β silences IL-4 production via an Ndfip1-dependent mechanism to provide a window of opportunity for iTreg differentiation"*
2:50-3:10	**Break** 1st and 2nd year students: Easel and posterboard set-up
3:10-4:30	**Session II: Immune responses during infection** **Session Chair: Karla Wiehagen**
3:10-3:30	Sean Spencer "*Gastrointestinal eosinophils regulate mucosal CD4+ T cell responses and are controlled by the dietary metabolite retinoic acid"*
3:30-3:50	Sagie Wagage "*Regulation of natural killer cell IL-10 expression"*

3:50-4:10	Jill Angelosanto "*Progressive loss of CD8 T cell function during chronic infection is irreversible and non-preventable"*
4:10-4:30	Karla Wiehagen "*A role for Foxp4 in T cell cytokine responses, but not development"*
4:30-4:40	**Break**
4:40-6:00	**Session III: Signaling in immune cells** **Session Chair: Mike Oropallo**
4:40-5:00	Rebecca May "*Dual signaling pathways dependent upon the adaptor protein SLP-76 lead to distinct NK cell effector functions"*
5:00-5:20	Alexander Babich "*F-actin retrograde flow at the immunological synapse is essential for T cell activation"*
5:20-5:40	Mike Oropallo "*Complexed BCR-TLR9 agonists induce a unique proliferation associated cell death in B cells which is rescued by BLyS"*
5:40-6:00	Kaycie Hopkins "*Genome-wide screening in Drosophila reveals the mRNA decapping enzyme DCP2 is anti-viral against Rift Valley Fever Virus"*
6:00	**Willow Valley registration and room check-in, Main Lobby** ***Please set up posters for the remainder of the conference in Statesman C/D***
6:00-7:30	**Dinner, Smorgasbord, The Willow Tree**
7:45-8:50	**Session IV: Keynote**
7:45 – 7:50	Introduction to Keynote Speaker: John Wherry. Ph.D.
7:50 – 8:50	Keynote Speaker, Philippa Marrack, Ph.D. Professor of Immunology, Biochemistry and Molecular Biology, and Medicine; HHMI Investigator National Jewish Health University of Colorado *"How T cell receptors do their job"*
8:50-12:00	**Social, Statesman Hall A/B/C/D**

Saturday, October 29, 2011

8:00-9:00 AM Breakfast, Smorgasbord, The Willow Tree

9:00-10:00	**Session V: T-cell development and migration** **Session Chair: Brittany Weber**
9:00-9:20	Jiyeon Kim "*A T cell receptor signaling mutant reveals two distinct populations of Th17 cells"*
9:20-9:40	Emily Chen *"ERM proteins play a role in T cell migration and adhesion"*
9:40-10:00	Brittany Weber "*A critical role for TCF-1 in T-lineage specification and differentiation"*
10:00-10:30	**Break**
10:30-12:00	**Session VI: NIH Talks**
10:30-10:45	Remy Bosselut, M.D., Ph.D. Senior investigator, Laboratory of Immune Cell Biology Introduction
10:45-11:15	Mike Lenardo, M.D. Chief, Molecular Development of the Immune System Section *"New Discoveries Immune Function through Human Genetics"*
11:15-11:35	Hyun Park, Ph.D. Investigator, Experimental Immunology Branch *"Post-transcriptional regulation of common γ-chain (γc) expression and signaling"*
11:35-11:55	Helen Su, M.D., Ph.D. Chief, Human Immunological Diseases Unit *"DOCK8 functions in human disease"*
11:55-12:15	Kristin Tarbell, Ph.D. Chief, Immune Tolerance Section *"Using DCs to induce T cell tolerance in the context of autoimmune diabetes"*
12:15-1:30pm	**Lunch, Smorgasbord, The Willow Tree**
12:00-3:30	**Free time to explore Lancaster area**

3:30-5:30	**Poster Session, Statesman Room C/D**
5:30-7:00	**Dinner, Smorgasbord, The Willow Tree**
7:00-9:00	**Session VII: Faculty Talks**
7:00-7:30	Jean-Baptiste Telliez, Ph.D. Associate Research Fellow Pfizer Pharmaceuticals *"JAK signaling pathways in inflammatory & autoimmune diseases"*
7:30-8:00	Igor Brodsky, Ph.D. Assistant Professor of Microbiology *"Cell survival and death in immunity to bacterial infection"*
8:00-8:30	Sunny Shin, Ph.D. Assistant Professor of Microbiology *"Innate immune sensing of bacterial virulence"*
8:30-9:00	Carolina Lopez, Ph.D. Assistant Professor of Pathobiology *"Virus v/s host; is there any loser?"*
9:00	**Announcement of Awards for Best Oral Presentation and Best Poster**
9:05-12:00 AM	**Social, Statesman Hall A/B/C/D**

Sunday, October 30, 2011

8:00-11:00 AM	**Breakfast, Smorgasbord, The Willow Tree**
REMINDER:	**Please check out of your room by 12 PM. Please take down your posters on Saturday night.**

END OF CONFERENCE

SAVE THE DATE
25th Annual Immunology Graduate Group Retreat
November 2-4, 2012
Willow Valley Resort and Conference Center

Abstracts for Oral Presentations:

1. Lisa Korn, Harper Hubbeling, Josh Steinberg, and Terri M. Laufer
   "*A role for MHC II-independent gut flora-derived signals in the homeostasis of gut Treg"*

2. Natalia Ramos-Hernandez, Hilda Ramón and Paula M. Oliver
   "Ndfip1 is required for the peripheral tolerance of CD4+ T cells"

3. Olivia Perng, Donald Simons, Malinda Aitken, Lori Mroz, Victoria Garcia, Liz Kropf, and Andrew J Caton
   "CD4+ T cell affinity can affect gender bias, disease severity and B cell requirement in inflammatory arthritis"

4. Alison Beal, Natalia Ramos-Hernández, Chris R. Riling, Erin A. Nowelsky, and Paula M. Oliver
   "*TGF-β silences IL-4 production via an Ndfip1-dependent mechanism to provide a window of opportunity for iTreg differentiation"*

5. Sean Spencer, Jason Hall, John Grainger, Shruti Naik, and Yasmine Belkaid
   "*Gastrointestinal eosinophils regulate mucosal CD4+ T cell responses and are controlled by the dietary metabolite retinoic acid"*

6. Sagie Wagage, Jonathon S. Silver, Christopher A. Hunter
   "*Regulation of natural killer cell IL-10 expression"*

7. Jill Angelosanto, Shawn Blackburn, Alison Crawford, and E. John Wherry
   "*Progressive loss of CD8 T cell function during chronic infection is irreversible and non-preventable"*

8. Karla Wiehagen, Evann Corbo, Shanru Li, Elizabeth Staub, Christopher A. Hunter, Ed Morrissey, and Jonathan S. Maltzman
   "*A role for Foxp4 in T cell cytokine responses, but not development"*

9. Rebecca May, CJ Hsu, Mariko Okumura, Gary Koretzky and Taku Kambayashi
   "*Dual signaling pathways dependent upon the adaptor protein SLP-76 lead to distinct NK cell effector functions"*

10. Alexander Babich, Shuxing Li, Bruce D. Freedman, and Janis K. Burkhardt
    "*F-actin retrograde flow at the immunological synapse is essential for T cell activation"*

11. Mike Oropallo, Krishna Moody, Kerstin Kiefer, Ann Marshak-Rothstein, and Michael P. Cancro
    "*Complexed BCR-TLR9 agonists induce a unique proliferation associated cell death in B cells which is rescued by BLyS"*

12. Kaycie Hopkins, Laura M McLane, Tariq Maqbool, Beth Gordesky-Gold, and Sara Cherry
    "*Genome-wide screening in Drosophila reveals the mRNA decapping enzyme DCP2 is anti-viral against Rift Valley Fever Virus"*

13. Jiyeon Kim, Jennifer E Smith-Garvin, Martha S Jordan, and Gary A Koretzky
"*A T cell receptor signaling mutant reveals two distinct populations of Th17 cells"*

14. Emily Chen, Meredith H. Shaffer, and Janis K. Burkhardt
"ERM proteins play a role in T cell migration and adhesion"

15. Brittany Weber, Anthony Wei-Shine Chi, Alejandro Chavez, Yumi Yashiro-Ohtani, Qi Yang, Olga Shestova, and Avinash Bhandoola
"A critical role for TCF-1 in T-lineage specification and differentiation"

Abstracts for Posters:

P1. Michael Askenase, John Grainger, Nicolas Bouladoux, and Yasmine Belkaid
"Recruited monocytes balance inflammatory and regulatory responses to commensal microbiota during oral T. gondii infection"

P2. Will Bailis, Steven Saenz, Chris Siebel, David Artis, and Warren Pear
"Notch signaling is required to suppress the T cell interferon gamma response during type 2 inflammation"

P3. Anupam Banerjee and David Michael Allman
"EBF-1 restricts T-lineage potential by directly repressing GATA3 in Ly6D$^+$ common lymphoid progenitors"

P4. Burton E. Barnett, Maria L. Ciocca, Radhika Goenka, Lisa G. Barnett, Junmin Wu, Janis K. Burkardt, Terri M. Laufer, Michael P. Cancro, and Steven L. Reiner
"Unequal inheritance of fate determinants by dividing germinal center B cells"

P5. Lisa G. Barnett, Radhika Goenka, Jonathan S. Silver, Christopher A. Hunter, Michael P. Cancro, and Terri M. Laufer
"MHC class II antigen presentation by multiple, distinct antigen presenting cells is required for follicular helper T cell differentiation"

P6. Scott W. Canna, Michele Paessler, Portia Kreiger, Katharine Slade, Sheila Rao, and Edward M Behrens
"IL-10 mainly derived from activated hepatic T-cells suppresses Toll-like Receptor 9-mediated Macrophage Activation Syndrome"

P7. Shannon A. Carty, Scott M. Gordon, Jiyeon S. Kim, Tao Zou, Jennifer Smith-Garvin, Eric S. Alonzo, Ethan Haimm, Derek B. Sant'Angelo, Gary A. Koretzky, Steven L. Reiner, and Martha S. Jordan
"Requirements for PLZF and IL-4 in the development of innate-like CD8+ T cells"

P8. Irene Chernova, Alexandra Bortnick, and David Allman
"Heterogeneity of the Bone Marrow Plasma Cell Pool"

P9. William A Comrie and Janis K. Burkhardt
"The dendritic cell F-actin network and ERM family member moesin function to constrain the lateral mobility of ICAM-1 and enhance T cell activation"

P10. Evann Corbo-Rodgers, Karla R. Wiehagen, Elizabeth S. Staub, Jonathan S. Maltzman
"The method of CD4 memory T cell generation determines signaling requirements for persistence"

P11. Erika Crosby, E. John Wherry, and Phillip Scott
"Influence of Viral Specific Memory Cells on Leishmania major Infection"

P12. Maria Elena De Obaldia, J. Jeremiah Bell, Daniel A. Zlotoff, Dil Afroz Sultana, and Avinash Bhandoola
"Hes1-mediated constraint of C/EBPα is essential for T cell development"

P13. Dawson M. Gerhardt, Kosta Pajcini, Stacey Rentschler, Rajan Jain, Olga Shestova, Michael J. Chen, Jon A. Epstein, Nancy A. Speck, and Warren S. Pear
"Important functions for the Notch1 transcriptional activation domain (TAD) in hematopoietic development"

P14. Radhika Goenka, Andrew H. Matthews, Patrick J. O'Neill, Jean L. Scholz, Warren J. Leonard, William Stohl, and Michael P. Cancro
"Positive selection during affinity Maturation relies on T cell derived BLyS"

P15. Claudia Gonzalez-Lombana and Philip Scott
"IL-10 limits IL-17 induced immunopathology following L. major infection"

P16. Carolyn M. Gray and Michael J. May
"A role for NEMO in non-canonical NF-κB signaling"

P17. Julie E. Horowitz and Craig H. Bassing
"A role for the N-terminal domain of Rag1 in immature B cell development"

P18. Jonathan B. Johnnidis and E. John Wherry
"Exploring the Role of Aging Pathways in Antiviral CD8 T cell Differentiation"

P19. Andy L. Johnson, Alison Crawford, Jill Angelosanto, Michael Paley, Christopher Hergott, and E. John Wherry
"Metabolic Regulation of CD8 T Cell Exhaustion"

P20. Rohan P Joshi, Tao Zou, Taku Kambayashi, Matthew Riese, and Gary A Koretzky
"Diacylglycerol kinase zeta but not diacylglycerol kinase alpha suppresses nTreg development"

P21. Laurel A. Monticelli, Gregory F. Sonnenberg, Michael C. Abt, Theresa Alenghat, Carly G.K. Ziegler, Travis A. Doering, Jill M. Angelosanto, Brian J. Laidlaw, Cliff Y. Yang[3], Taheri Sathaliyawala, Masaru Kubota, Damian Turner, Joshua M. Diamond, Ananda W. Goldrath, Donna L. Farber, Ronald G. Collman, E. John Wherry, and David Artis
"Innate lymphoid cells promote lung tissue homeostasis following acute influenza virus infection"

P22. Ryan H. Moy, Margaret Nakamoto, Jie Xu, Shelly Bambina, Ari Yasunaga, Spencer S. Shelly, Beth Gold, and Sara Cherry
"Virus recognition by Toll-7 activates antiviral autophagy in Drosophila"

P23. Shruti Naik, Nicolas Bouladoux, Mariam Quinones, Wolfgang Kastenmuller, Rosalba Salcedo, Giorgio Trinchieri, Julie A. Segre , and Yasmine Belkaid
"Cutaneous commensals control skin immunity via a distinct IL-1/CD40 dependent mechanism"

P24. Martin S Naradikian, William J Quinn III, and Michael P Cancro
"Osteoclastogenesis and APRIL production requirements for plasma cell survival"

P25. Fernanda Novais, Camila Indiani de Oliveira, and Phillip Scott
"The role of CD8 T cells in Leishmania braziliensis infection"

P26. Shaun O'Brien, Rajan Thomas, Katie Williams, and Andrew Wells
"Ikaros as regulator of naïve CD8+ T cell activation and differentiation"

P27. Aisling O'Hara Hall, Cristina Tato, Elia D. Tait Wojno, Beena John, Guillaume Oldenhove, Claudia Gonzalez Lombana, Nicolas Bouladoux, Laurence A. Turka, Steven L. Reiner, Daniel Cua, Yasmine Belkaid, M. Merle Elloso, and Christopher A. Hunter
"A role for IL-27 in the development of T-bet+ Treg required to limit infection-induced pathology"

P28. Claire O'Leary, Ami LaRoche, Chris Riling, and Paula Oliver
"Adaptor interactions of Ndfip2 with Nedd4-family E3 ligases in T cells"

P29. Pamela Odorizzi, Shawn Blackburn, and E. John Wherry
"Investigating the Mechanisms of Inhibitory Receptor Blockades in Chronic Viral Infection"

P30. Michael A. Paley, Scott M. Gordon, Steve L. Reiner, and E. John Wherry
"T-box transcription factors coordinate terminal differentiation and self-renewal in CD8 T cells during chronic viral infection"

P31. Sheila Rao, Katharine Slade, Gary A. Koretzky, and Edward M. Behrens
"A role for the protein tyrosine kinase Syk during TNFα secretion"

P32. Amanda Schmidt, Tao Zou, Martha Jordan, Jonathan Maltzman, and Taku Kambayashi
"Differential requirement of SLP-76 signaling in regulatory T cell development and function"

P33. Erietta Stelekati, Haina Shin, Travis Doering, Dan Beiting, Doug Dolfi, Jennifer Liboon, Hao Shen, David Roos and E. John Wherry
"Bystander chronic infection impairs the development of optimal CD8+ T cell memory"

P34. Natalie Steinel and Craig Bassing
"A Role for ATM in Antigen Receptor Allelic Exclusion"

P35. Kate Weissler, Felipe Bedoya, and Andrew Caton
"The regulatory T cell response to influenza infection is driven by recognition of cognate antigen"

P36. Katie L. Williams, Amaya I. Wolf, Krystyna Mozdzanowska, and Jan Erikson
"Localization of Antibody Secreting Cells Following Influenza Infection"

P37. Shirley L. Zhang, Daniel A. Zlotoff, Sugata Manna, and Avinash Bhandoola
"Thymic Settling after Bone Marrow Transplantation"

P38. Rena Zheng, Annarita Miccio, and Gerd Blobel
"The non-conventional cyclin G2 as target of the GATA1/FOG1/NURD transcription factor complex"

Abstracts for Oral Presentations

1.
"A role for MHC II-independent gut flora-derived signals in the homeostasis of gut Treg"
Lisa L. Korn, Harper Hubbeling, Josh Steinberg, and Terri M. Laufer

Regulatory T cells (Tregs) prevent autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis through a variety of mechanisms. Like all CD4 T cells, CD4 Tregs expressing the transcription factor Foxp3 develop in the thymus, though Tregs can also be generated in the periphery from naïve CD4 T cells. T cell receptor (TCR)-major histocompatibility complex class II (MHC II) signals are necessary for thymic Treg generation and provide antigen-specific activation and homeostatic signaling the periphery. Yet the homeostasis of Tregs in the periphery is not well understood, and the role of MHC II in their maintenance is unclear.

The gut is an important site of Treg induction and function. MHC II is expressed constitutively on the small intestine epithelium, while dendritic cells in the lamina propria, Peyer's patches, and associated mesenteric lymph nodes are important in generating Tregs. To study the role of MHC II in the homeostasis of gut Tregs, we examined the Tregs in mice with absent or limited MHC II expression. K14/A_b^b (K14) mice have MHC II restricted to cortical thymic epithelium; CD4+ T cells, including Tregs, develop in the thymus but are not exposed to peripheral MHC II.

Interestingly, the percentage of CD4+ T cells that are Tregs increases in the guts of mice that lack peripheral TCR-MHC II interactions. This accumulation begins after weaning, when the mucosal immune system is exposed to new commensal bacteria. Additionally, depletion of gut flora by antibiotics in adult mice restores the Treg frequency to those in WT mice. Preliminary analyses suggest that increased Treg division in the gut does not occur in the absence of MHC II. However, the Treg population may turn over at an increased rate. These data suggest that gut flora-derived signals may play a previously unappreciated role in Treg homeostasis that is independent of MHC II-TCR signaling.

2.
"Ndfip1 is required for the peripheral tolerance of CD4+ T cells"
Natalia Ramos-Hernández, Hilda Ramón, and Paula M. Oliver

Ndfip1 is an adaptor protein for the Nedd4-family of E3 ligases. Our lab has shown that in CD4+ T cells, Ndfip1 is required for Itch-mediated degradation of JunB. Thus, activated CD4+ T cells lacking Ndfip1 have increased levels of JunB and are biased towards Th2-cytokine production. Elevated IL4 production by Ndfip1-/- T cells also prevents inducible regulatory T cell (iTreg) differentiation. Consequently, Ndfip1-/- mice develop a Th2-mediated inflammatory disease at sites of environmental antigen exposure and die by 14 weeks of age. Here we present data suggesting that the functions of Ndfip1 in CD4 T cells go beyond promoting Itch-mediated degradation of JunB.

Ndfip1-deficient CD4+ T cells are hyperresponsive to T cell receptor signaling. Upon stimulation through their T-cell receptors (TCR) in vitro, in the absence of CD28-costimulatory signals, Ndfip1-/- CD4+ T cells produce abundant amounts of IL-2, express

high levels of the IL-2Ra, and proliferate. Interestingly, this defect is not dependent on the E3 ubiquitin ligase Itch.
Supporting that this may also occur in vivo, T cells in Ndfip1-/-CD28-/- mice have an activated phenotype, produce IL-4 and induce Th2-mediated disease. While IL-4 production can exacerbate the hyperresponsive of these cells, IL-2 production and excessive proliferation also occur in cells lacking both Ndfip1 and IL-4. Thus, IL-4 production is not causing this defect.
Furthermore, in an antigen-specific model of oral tolerance we have found that while Ndfip1$^{+/+}$ CD4 T cells become tolerant to antigen, Ndfip1$^{-/-}$ T cells become activated, expand and cause GI inflammation. We hypothesize that Ndfip1 negatively regulates CD4 T cell activation and tolerance to non-pathogenic 'environmental' antigens.

3.
"CD4+ T cell affinity can affect gender bias, disease severity and B cell requirement in inflammatory arthritis"
Olivia A Perng, Donald Simons, Malinda Aitken, Lori Mroz, Victoria Garcia, Liz Kropf, and Andrew J Caton
The University of Pennsylvania and The Wistar Institute

CD4+ T cells make a crucial contribution to the development of inflammatory arthritis in both in humans and in mouse models. However, how variations in the affinity with which T cells recognize target antigens might shape disease development and influence treatment modalities is poorly understood. We have examined these phenomena in mouse models of autoimmune arthritis: TS1xHACII and TS1(SW)xHACII mice express influenza hemagglutinin (HA) as a neo-self peptide and co-express transgenic TCRs that have either high affinity (TS1xHACII mice) or low affinity (TS1(SW)xHACII mice) for the HA-derived MHC class II determinant S1. Despite extensive deletion of the autoreactive HA-specific TCRs, autoimmune arthritis spontaneously develops in both strains, and in each case arthritis can be prevented by IL-17 blockade. Notably, mice expressing the lower affinity TCR display less severe extra-articular disease manifestations, and a prominent female sex bias emerges among arthritic individuals. In addition, B cells are required for arthritis development in the low affinity setting; by contrast, there is no such B cell requirement in the high affinity setting, and in this case the disease is accompanied by higher levels of systemic pro-inflammatory cytokines. These studies demonstrate that the overall affinity of the CD4+ T cell response to an autoantigen can play a prominent role in guiding the pathways that can lead to inflammatory arthritis development. They also provide a basis for the gender basis and/or extra-articular manifestations that can accompany inflammatory arthritis, and may explain why treatment modalities targeting particular pathways (e.g. cytokines vs. B cells) can exhibit different efficacies in arthritis patients.

4.
"TGF-β silences IL-4 production via an Ndfip1-dependent mechanism to provide a window of opportunity for iTreg differentiation"
Allison M. Beal (1,3), Natalia Ramos-Hernández (2), Chris R. Riling (2), Erin A. Nowelsky (1) and Paula M. Oliver (1,2,3)
The Children's Hospital of Philadelphia, Cell Pathology Division; (2) University of Pennsylvania, School of Medicine; (3) Department of Pathology and Laboratory Medicine

Pathways that regulate T cell tolerance to environmental antigens are not well understood. Ndfip1 is an adaptor that promotes polyubiquitylation by the E3 ubiquitin ligase Itch. Mice that lack either Ndfip1 or Itch develop a severe Th2-mediated inflammatory disease at sites

of environmental antigen exposure. De novo differentiation of naïve T cells into regulatory T cells (iTregs) that express the transcription factor Foxp3 helps to maintain tolerance to environmental antigens. We show that mice lacking Ndfip1 contain fewer iTregs. In vitro, Ndfip1-deficient T cells undergoing iTreg differentiation express normal levels of Foxp3 during the first 48 hours of iTreg differentiation; however, these levels fail to be sustained. Abortive iTreg induction is caused by IL-4 production by Ndfip1-/- T cells. Supporting this, when Ndfip1-deficient T cells lack IL-4, iTreg differentiation is restored. Thus, Ndfip1 is required to dampen IL-4 production during iTreg differentiation. We demonstrate that Ndfip1 is transiently upregulated during iTreg differentiation in a TGF-β-dependent manner. Once expressed, Ndfip1 promotes Itch-mediated degradation of JunB, thus preventing IL-4 production. Based on these data, we propose that TGF-β signaling induces Ndfip1 expression to dampen IL-4 production and thus permits iTreg differentiation.

5.

"Gastrointestinal eosinophils regulate mucosal CD4+ T cell responses and are controlled by the dietary metabolite retinoic acid"

Sean Spencer[12], Jason Hall[2], John Grainger[2], Shruti Naik[12], and Yasmine Belkaid[2]
1: Immunology Graduate Group, University of Pennsylvania 2: Mucosal Immunology Section, Laboratory of Parasitic Diseases, NIH

Eosinophils comprise a sizeable portion of resident immune cells within the healthy GI tract of both mice (>15%) and humans (10-20eos/hpf). Despite their prominence, the role of eosinophils in the regulation of GI immune responses remains unclear. Thus we investigated the role of eosinophils in promoting mucosal immune responses. Administration of vaccine to mice selectively lacking eosinophils (dblGATA1), resulted in a decreased accumulation of antigen specific CD4+ T cells in the small intestine as compared to cohoused controls while responses in the draining lymph nodes were largely unaffected. This suggests that eosinophils are important for the orchestration of tissue immune responses after vaccination. Microarray gene analysis of tissue resident gastrointestinal eosinophils revealed a signature of activation with increased levels of chemokines (MIP1-α, MIP1-β) and cytokines (IL-1α, IL-1β, and IL-6) that have been shown to be important for mounting effective immune responses in the gut.
Although eosinophils are recruited to the small intestine, it is unclear what factors are regulating this process. We found that eosinophil frequencies and IL-1β production were independent of commensal organisms. On the other hand, we find a 3-fold reduction in eosinophils and absent IL-1β production in Vitamin A deficiency. A short treatment (4 days) with the Vitamin A metabolite retinoic acid partially restores both eosinophil numbers and IL-1β production. All together, our data suggest that tissue resident eosinophils have an unexpected role as central regulators of vaccine induced mucosal immunity.

6.

"Regulation of natural killer cell IL-10 expression"

Sagie Wagage, Jonathon S. Silver, and Christopher A. Hunter
Department of Pathobiology, University of Pennsylvania

Proper regulation of inflammatory immune responses is critical for surviving infection. This is exemplified by studies in IL-10KO mice, which succumb to immune-mediated pathology during infection with *T. gondii* in spite of their ability to control parasite burdens. Previous work has shown that IL-10 derived from $CD4^+$ $FoxP3^-$ T cells is sufficient to rescue infected IL-10 KO mice. However numerous cell types are capable of producing IL-10, and dendritic

cells, T_{FH} cells, and Tregs express IL-10 during toxoplasmosis. Interestingly, natural killer cells make up a substantial proportion of IL-10 producing cells during early infection. IL-12 has previously been shown to promote IL-10 production by NK cells, but other factors influencing NK cell IL-10 production are not well understood. Lymphokine activated killer cells (LAKs) produce IL-10 when stimulated with IL-2 and IL-12 or IL-21 and can be used to study NK cell IL-10 production. The aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, promotes NK cell IL-10 expression. Treatment with an AHR antagonist inhibited IL-10 production by LAKs, and AHR agonists enhanced IL-10 production. NK cells from infected mice restimulated with an AHR agonist expressed increased levels of IL-10. IL-27, an IL-12 family member that promotes T cell IL-10 production, may also contribute to NK cell IL-10 expression during infection. Along with previously published work, these results suggest that the AHR is an evolutionarily conserved regulator of IL-10 expression by innate and adaptive immune cells.

7.

"Progressive loss of CD8 T cell function during chronic infection is irreversible and non-preventable"

Jill Angelosanto, Shawn Blackburn, Alison Crawford, and E. John Wherry

During an acute infection, activated CD8 T cells begin a complicated process of differentiation from naïve to cytotoxic effector cells and finally to antigen experienced memory cells. During a chronic infection, however, this process is disrupted and CD8 T cells become dysfunctional or exhausted. Exhausted cells are unable to efficiently produce cytokines or perform effector functions, but it is unknown whether the defects of exhausted cells develop progressively during chronic antigen exposure and/or if exhausted CD8 T cells undergo an irreversible fate decision early in infection. We addressed these questions by examining the memory development potential of antigen specific CD8 T cells at various stages of chronic infection. We found that virus specific CD8 T cells removed from infection at day 30 were lost after transferred to antigen free mice. At 15 days post infection (pi), antigen specific T cells isolated from a chronic infection develop dysfunctional memory while day 8 effectors retain the ability to become functional memory cells if removed from infection. This progressive loss of memory potential cannot be prevented through altered priming conditions, as optimal priming was found insufficient to prevent exhaustion when effector CD8 T cells transferred to a chronic host became exhausted. Finally, to examine the lineage origin of exhausted CD8 T cells, we sought to determine if exhausted cells arose from terminal effectors or memory precursors. CD8 T cell effector subsets were sorted based on KLRG1 expression and transferred into chronic hosts. Analysis at 30 days post-transfer found not only that the $KLRG1^{lo}$ population alone survived, but also a subset of these cells upregulated $KLRG1^{hi}$ in the chronic host, giving rise to short term effector cells. These studies demonstrate that memory and exhaustion are cell fates developed progressively throughout infection with plasticity of antigen specific CD8 T cells early in infection, but a loss of this ability to change after the effector phase.

8.

"A role for Foxp4 in T cell cytokine responses, but not development"

Karla R. Wiehagen*, Evann Corbo*, Shanru Li*, Elizabeth Staub*, Christopher A. Hunter†, Ed Morrissey*, and Jonathan S. Maltzman*
Department of Medicine

Transcription factors regulate T cell fates at every stage of development and differentiation. Foxp4, a member of the Foxp family of forkhead transcription factors, is expressed in

lymphocytes, but its function is unknown. We used a Cre-loxp mediated approach to evaluate the cell autonomous role for Foxp4 in T lymphocytes. T cell development, peripheral cellularity and cell surface phenotype are normal in the absence of Foxp4. Foxp3+ T regulatory cells develop normally without Foxp4 expression, and naïve Foxp4 deficient CD4 T cells can differentiate to inducible T regulatory cells in vitro. Despite a three-fold increase in Foxp4 mRNA levels following in vitro activation, deletion of Foxp4 does not affect proliferative responses. When challenged with a chronic pathogen, Toxoplasma gondii. Foxp4 deficient T cells exhibited decreased production of IFNγ in an antigen specific recall response, but retain the ability to control the infection. We conclude that Foxp4 is dispensable for T cell development, but necessary for T cell cytokine recall responses to antigen in chronically infected mice.

9.

"Dual signaling pathways dependent upon the adaptor protein SLP-76 lead to distinct NK cell effector functions"

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University of Pennsylvania

Proximal signaling pathways that lead to NK effector function are incompletely understood. Thus, we aimed to dissect the proximal signaling pathways downstream of the Ly49D activating receptor and focused our studies on SLP-76, an adaptor molecule which is important in signaling downstream of ITAM-containing receptors. When NK cells were activated through Ly49D, SLP-76 was phosphorylated and recruited to the plasma membrane. Furthermore, SLP-76 knockout (KO) NK cells exhibited diminished ERK and Akt phosphorylation compared to wildtype (WT) NK cells after Ly49D stimulation. These biochemical defects correlated with decreased IFNg production and granule exocytosis by SLP-76 KO NK cells. To rule out developmental defects, NK cells were inducibly deleted of SLP-76. These NK cells displayed defective IFNg production and granule exocytosis, suggesting that SLP-76 plays an important role in Ly49D-mediated NK cell function. We next explored the mechanisms by which SLP-76 relocalizes from the cytosol to the plasma membrane. As this process depends on adaptor molecules LAT and NTAL in T cells, we tested whether LAT and NTAL were similarly crucial for SLP-76 function in NK cells. Like SLP-76 KO NK cells, LAT/NTAL double KO (DKO) NK cells displayed significant functional defects, suggesting that LAT/NTAL might be required for SLP-76 activation. Surprisingly, however, membrane recruitment and phosphorylation of SLP-76 were intact in LAT/NTAL DKO NK cells following Ly49D stimulation, suggesting that NK cells use a novel LAT/NTAL-independent pathway leading to SLP-76 phosphorylation and membrane recruitment. This novel LAT/NTAL independent pathway leads to distinct NK cell effector functions including proliferation. Together, these results demonstrate a critical role of SLP-76 in NK cell activation downstream of multiple signaling pathways emanating from the Ly49D activating receptor.

10.

"F-actin retrograde flow at the immunological synapse is essential for T cell activation"

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T cell activation relies on extensive cytoskeletal remodeling at the immunological synapse (IS), the contact zone between a T cell and an antigen presenting cell. T cell receptor

engagement triggers the assembly of signaling complexes called microclusters (MCs) on the T cell side of the IS. Signaling in MCs leads to activation of PLCγ1, which signals release of calcium from ER stores and subsequent calcium influx through CRAC channels. Actin and myosin IIA localize to the IS and regulate MC dynamics and sustained calcium signaling; however the link between actin dynamics and calcium signaling is poorly understood. Using live cell microscopy and pharmacological inhibitors, we demonstrate that actin polymerization is the primary driver of retrograde flow of the actomyosin network; myosin IIA activity promotes long-term maintenance of the IS but is not required for F-actin retrograde movement and turnover. Moreover, we found that calcium signaling requires ongoing F-actin retrograde flow, but is not dependent on myosin IIA contractility. Further investigation showed that perturbation of actin retrograde flow inhibits calcium signaling at a point distal to PLCγ1 activation, most likely at the level of CRAC channel regulation. Localization of mitochondria at the IS is altered in response to F-actin inhibition, correlating with the loss of sustained calcium entry. Since IS-proximal mitochondria are needed to maintain CRAC channel activity, our results support a model in which ongoing F-actin flow brings mitochondria into proximity to CRAC channels at sites of TCR engagement, thereby promoting sustained calcium signaling.

11.

"Complexed BCR-TLR9 agonists induce a unique proliferation associated cell death in B cells which is rescued by BLyS"

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Combined B cell receptor (BCR) and Toll-like receptor 9 (TLR9) signaling has been implicated in the T cell independent activation of B cells in the presence of both foreign and self DNA. Previous research using AM14 mice, which have a transgene encoding a BCR typical of pathogenic Rheumatoid factor producing B cells, has demonstrated that these cells are induced to proliferate by chromatin containing immune complexes (chromatin-ICs). This work revealed a requisite for combined BCR and TLR signaling in the onset of RF production. We have recently found that although AM14 RF B cells stimulated with chromatin-ICs indeed divide, they die rapidly within 48-72 hrs of the initial stimulus. This cell death is unique to complexed BCR and TLR stimulation, as BCR agonist (anti-IgM) alone, TLR9 agonist (CpG ODN) alone, or both BCR + TLR9 agonists uncomplexed induces cells to continue to survive and proliferate robustly within this time frame. Furthermore, WT cells also undergo a proliferation associated cell death following complexed BCR:TLR9 stimulation, implying that combined BCR:TLR9 signaling is not sufficient to allow for T cell independent activation of B cells. Importantly, we have found that addition of the survival cytokine BLyS rescues survival of B cells proliferating in response to chromatin-ICs likely through BLyS binding to BR3. Finally, we have begun to examine the apoptotic pathway downstream of chromatin-IC stimulation, and have found that it is cell intrinsic and involves caspases 9, 3, and PARP.

12.

"Genome-wide screening in Drosophila reveals the mRNA decapping enzyme DCP2 is anti-viral against Rift Valley Fever Virus"

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All medically relevant arthropod-borne viruses (arboviruses) that are currently known have been shown to encode an RNA genome; these viruses are a poorly understood global threat to human health. This is due in part to our lack of understanding about the interplay between these viruses and cellular host factors. The emerging bunyavirus RVFV can cause fatal hemorrhagic disease in infected humans and has high mortality rates among livestock. In the hope of illuminating potential therapeutic targets or novel immune pathways, we have recently completed a genome-wide RNAi screen in *Drosophila* to identify host factors affecting RVFV replication. We have identified the mRNA decapping enzyme DCP2 as a conserved host factor that restricts the replication of RVFV and another bunyavirus, LaCrosse virus, in both *Drosophila* and *Aedes aegypti* mosquito cells. We hypothesize that DCP2 restricts viral replication through its normal metabolic turnover of cellular mRNAs, which RVFV requires as a substrate for viral RNA transcription via cap snatching. Interestingly, sequencing reveals that RVFV primarily snatches cellular mRNAs related to cell cycling, and further RNAi experiments show that cell cycle arrest following S phase increases RVFV replication efficiency. We hypothesize that RVFV takes advantage of the clearance of DNA synthesis mRNAs in P bodies following S phase as an optimal pool of cap snatching substrates.

13.

"A T cell receptor signaling mutant reveals two distinct populations of Th17 cells"

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Interleukin-17 (IL-17) producing CD4+ T cells (Th17 cells) are essential for immune responses in mucosal and epithelial sites which are the first line of host defense. Th17 cells also play a critical role in the pathogenesis of many autoimmune diseases, and recent clinical trials modulating Th17 cell function and/or differentiation in psoriasis and Crohn's disease have been successful. Most studies to date have focused on elucidating the cell extrinsic requirements for differentiation of Th17 cells from naïve CD4+ T cells in peripheral effector sites. Here we report a population of Th17 cells, "natural Th17 cells" (nTh17), that acquire effector function during development in the thymus, thereby distinguishing them from conventional Th17 cells which require antigen encounter and differentiation in the periphery. We demonstrate that these nTh17 cells are present and indeed develop in the thymus using fetal thymic organ culture. nTh17 cells express surface markers consistent with an innate and/or activated phenotype, and their development is dependent on selection by MHC class II in the thymus. TCR repertoire analysis of nTh17 cells revealed unique characteristics in TCR gene usage compared to conventional Th17 cells. A mouse model with a mutation in the TCR signaling protein SLP-76 (Y145F mice) further highlights the difference between the two Th17 populations. Y145F mice show enrichment of nTh17 cells in the thymus compared to wild-type mice. However, in the periphery of these mice, CD4+ T cells in the small intestinal lamina propria fail to produce IL-17, correlating with the defective Th17 differentiation of Y145F naïve CD4+ T cells cultured *in vitro* under conditions promoting conventional Th17 cell differentiation. Using mixed radiation bone marrow chimeras, we found that the aberrant Th17 phenotype in the thymus and periphery of Y145F mice is cell-intrinsic. Taken together, our findings define a Th17 population that is developmentally distinct from conventional Th17 cells and potentially functions at the interface of innate and adaptive immunity. Current studies focus on determining the developmental requirements and function of nTh17 cells.

14.

"ERM proteins play a role in T cell migration and adhesion"

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T cells circulate between the blood and lymph nodes in search of cognate antigen. This process is dependent on their ability to form a protruding leading edge and a constricted tail-like structure termed the uropod. Cell polarity is a crucial characteristic for T cells to migrate through blood vessels, adhere to endothelium, extravasate into lymph nodes and migrate within lymph nodes. Members of the ezrin, radixin and moesin (ERM) family of actin-binding proteins are important regulators of polarity in many cell types. We therefore analyzed the role of these proteins in regulating T cell shape and migration.

T cells express two ERM family members, ezrin and moesin. Both ezrin and moesin localize to the uropod of migrating T cells. To test the function of these proteins in migrating T cells, we purified CD4+ T cells from conditional ezrin knockout mice and suppressed moesin in these cells using siRNA. Ezrin and moesin-deficient T cells exhibited defective binding to fibronectin, and formed a uropod half as efficiently as wild type control cells in response to fibronectin-coated surfaces. Double-deficient cells were also impaired in their ability to migrate toward a chemokine gradient in vitro and to home to lymph nodes in vivo. Taken together, these results show that ezrin and moesin are important to generate polarized T cells capable of normal integrin-dependent adhesion and efficient movement in vitro and in vivo.

15.

"A critical role for TCF-1 in T-lineage specification and differentiation"

Brittany Nicole Weber, Anthony Wei-Shine Chi, Alejandro Chavez, Yumi Yashiro-Ohtani, Qi Yang, Olga Shestova and Avinash Bhandoola
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Within the thymus, Notch signals impose the T cell fate on incoming multipotent progenitors, but the downstream effectors and mechanisms that impart T lineage specification and lineage commitment are not well understood. We have found that transcription factor T cell factor- 1 (TCF-1) is a critical regulator in T lineage specification. TCF-1 is highly expressed in the earliest thymic progenitors, and its expression is upregulated by Notch signals. TCF-1 deficient bone marrow progenitors failed to give rise to T cells when cultured with Notch ligands in vitro, whereas inhibition of B and myeloid development by Notch signaling still occurred. Hence TCF-1 is critical for T lineage progression but dispensable for suppression of alternative fates mediated by Notch signals. Using competitive BM chimera studies in vivo, we found that TCF-1-/- progenitors are blocked at the early thymic progenitor (ETP) stage, suggesting that TCF-1 regulates critical targets essential for early stages of T cell development. To identify the gene targets of TCF-1 we ectopically expressed TCF-1 in progenitors, and cultured them in the absence of T-inductive Notch signals. Characterization of these TCF-1-transduced cells revealed expression of many T-lineage genes, including T-cell-specific transcription factors *Gata3* and *Bcl11b*, and components of the T-cell receptor. These data reveal TCF-1 as a critical regulator that is not only essential for normal T cell development but is sufficient to establish many components of T cell identity.

Abstracts for Posters:

P1.
"Recruited monocytes balance inflammatory and regulatory responses to commensal microbiota during oral T. gondii infection"
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The mammalian intestine is home to immense colonies of commensal microflora. These commensals are essential for host nutrient absorption and normal immune function, but as potent stimulators of inflammation, they pose a threat if exposed to the immune system in an uncontrolled manner. The intestinal epithelial barrier prevents microbial translocation and enables the immune system to carefully control the context in which the luminal content of the intestine is exposed to the immune system. As the intestinal mucosa is a point of entry for many viral, microbial, and parasitic pathogens, this control is necessary for the intestinal immune system to balance tolerance of food antigens and commensal microflora with the ability to respond swiftly to infection without compromising the ability of the gut to perform functions essential for life: nutrient absorption and water absorption.

Acute *T. gondii* infection causes destruction of the epithelial barrier and translocation of gut resident microbes into the lamina propria of the small intestine and draining lymphoid tissues. This results in severe immunopathology and appears to permanently alter the immune homeostasis of the small intestine. In order to better understand what drives pathogenesis during acute intestinal infection, we stimulated small intestinal macrophages and dendritic cells from healthy and *T. gondii* infected mice with microbial and parasitic products and examined their ability to respond to these signals by secreting pro-inflammatory and regulatory cytokines. Our data reveal dramatic alterations in the ability of the innate compartment to respond to microbial stimuli during infection. Furthermore, our results support the idea of a partitioning of cells by their ability to respond parasite or commensal microbiota.

P2.
"Notch signaling is required to suppress the T cell interferon gamma response during type 2 inflammation"
Will Bailis, Steven Saenz, Chris Siebel, David Artis, and Warren Pear
University of Pennsylvania

$CD4^+$ T helper 2 (Th2) cells and the associated type 2 inflammatory response are required for anti-helminth immunity and promoting allergic inflammation and airway hyperresponsiveness. T cell specific pan-Notch inhibition impairs Th2 differentiation and the generation of a productive inflammatory response against gastrointestinal helminth infection. However, the influence of individual Notch receptors on T cells and the importance of individual Notch ligands during type 2 inflammation remains unclear. To clarify the role that Notch signaling plays during type 2 inflammatory responses, we took advantage of a series of inhibitory monoclonal antibodies to systemically inhibit Notch signaling mediated by individual Notch receptors or initiated by individual ligands in a model of helminth infection. Inhibiting Notch1 signaling, and to a lesser extent Notch2, prevented helminth clearance, resulted in an elevated T cell interferon gamma (IFNγ) response, and dysregulated the type 2 inflammatory response against *Trichurus muris* infection. Preliminary *in vitro* data suggests that the elevated T cell IFNγ response observed *in vivo* is likely a direct effect of

anti-Notch receptor antibodies on $CD4^+$ T cells. Furthermore, our anti-Notch ligand studies have evinced a role for Delta-like1 (DLL1) and Delta-like4 (DLL4) in promoting helminth clearance and suppressing the T cell IFNγ response during *T. muris* infection, identifying a novel role for DLL1 and DLL4 in regulating Th2 immunity. Collectively, our data support a model in which signaling through both Notch1 and Notch2 on $CD4^+$ T cells, potentially mediated by DLL1 and/or DLL4, is required to suppress the aberrant production of IFNγ during a Th2 response, and in turn, for the development of normal type 2 inflammation.

P3.
"EBF-1 restricts T-lineage potential by directly repressing GATA3 in Ly6D$^+$ common lymphoid progenitors"
Anupam Banerjee and David Michael Allman
Department of Pathology and Laboratory Medicine

The molecular mechanisms governing progressive restriction of alternate lineage options during B-cell development in the bone marrow are unclear. We have recently utilized Pax5 knockout fetal liver chimeras to show that Early B cell Factor-1 (EBF) promotes early steps of B-lineage commitment and represses T-lineage development independently of Pax5. Quantitative in vitro assays on the OP9-DL4 stromal system confirmed that ectopic expression of EBF-1 in Pax5-knockout ($Pax5^{-/-}$) and $EBF\text{-}1^{-/-}$ primary fetal liver LSKs inhibits their ability to generate $CD44^+CD25^+$ early T-lineage cells. Gene expression analyses on clonal $EBF\text{-}1^{-/-}$ and $E2a^{-/-}$ $flt3^+$ $IL7R\alpha^+$ fetal liver progenitors transduced with EBF-1 retrovirus revealed a significant decline in the transcript levels of GATA3, an obligate transcription factor for T-lymphoid fate, whereas Notch-1 levels remained relatively unchanged. ChIP analyses revealed that EBF-1 bound the GATA3 locus in vivo. Moreover, utilizing an inducible EBF-1-ER fusion construct in the presence of cycloheximide as well as an EBF-engrailed fusion protein, we uncovered a direct role for EBF-1 in GATA3 repression. The biological significance of such an inhibitory effect of EBF-1 was highlighted in fetal liver chimera experiments wherein $Ly6D^+$ common lymphoid progenitors (CLPs) regained T-lineage potential in the absence of EBF-1, consistent with having similar levels of GATA3 to their uncommitted $Ly6D^-$ counterparts. These experiments suggest that EBF-1 initiates T-lineage restriction in B-lineage committed precursors by downregulating the critical T-cell transcription factor GATA3. Experiments to directly implicate a role for EBF-1 in B-fate maintenance are currently undergoing in the lab.

P4.
"Unequal inheritance of fate determinants by dividing germinal center B cells"
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B cells that encode high-affinity, protective antibodies are generated in the germinal center (GC) reaction, a microanatomical structure that includes GC B cells and follicular helper T cells (T_{FH}). GCs are seeded by a small number of antigen specific B cells that compete for selective signals. The selection of GC B cells to proliferate and differentiate into plasma cells and memory B cells relies on prolonged, motile contacts with T_{FH}. In other instances where cells undergo prolonged external contacts, polarity cues are imparted that lead to asymmetric division. We hypothesized T_{FH} may provide polarity cues during these interactions, in addition to mitogenic and differentiative signals, so that GC B cells may

divide asymmetrically to generate diversity. Using confocal microscopy we observe that GC B cells asymmetrically segregate and unequally inherit the ancestral polarity regulator PKCz, and the receptor for interleukin 21 (IL-21R) and Bcl6, which are responsible for initiating and maintaining the GC B cell fate, respectively. We observe that neither B cells undergoing homeostatic proliferation nor GC B cells from mice deficient in leukocyte adhesion, ICAM-1$^{-/-}$ mice, divide asymmetrically, suggesting that cell-to-cell adhesions are responsible for initiating polarity. These adhesions may be occurring between a B cell and a T cell, as ligation of CD40 *in vitro* results in a high frequency of asymmetric divisions. Further examination of ICAM-1$^{-/-}$ mice revealed that while there is only a moderate reduction in T_{FH} and GC B cells, the number of antibody secreting cells is severely diminished compared to wild-type mice. Together, these data support a model where, in addition to canonical signals, GC B cells receive polarity cues from T_{FH} that result in the unequal inheritance of fate determinants by daughter B cells, leading to divergent differentiation.

P5.

"MHC class II antigen presentation by multiple, distinct antigen presenting cells is required for follicular helper T cell differentiation"

Lisa G. Barnett[1], Radhika Goenka[1], Jonathan S. Silver[2], Christopher A. Hunter[2], Michael P. Cancro[1], and Terri M. Laufer[1,3]
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Follicular helper T cells (T_{FH}) are a subset of $CD4^+$ helper T cells that are critical participants in the germinal center reaction and without them, germinal centers and long-lived plasma cells fail to form. In this study we aim to delineate the antigen presenting cells that mediate T_{FH} differentiation. The contributions of antigen presentation by dendritic cells (DCs) and B cells in T_{FH} development and function were determined by by ablating or restricting antigen presentation to $CD11c^+$ DCs or $CD19^+$ B cells. We find that DCs are required for T cell priming as well as T_{FH} differentiation, as both T cell expansion and T_{FH} development are impaired upon DC depletion. By restricting MHCII antigen presentation solely to $CD11c^+$ DCs, we find that DCs are sufficient for the generation of a novel T_{FH} intermediate (termed pre-T_{FH}) which expresses some characteristic makers of T_{FH}, such as the transcription factor Bcl6 and the chemokine receptor CXCR5. However, DC restricted priming is not sufficient for imparting full T_{FH} effector function, as these pre-T_{FH} cells fail to produce IL-21 and IL-4. MHCII antigen presentation restricted to B cells induces minimal $CD4^+$ T cell priming and cannot initiate T_{FH} differentiation. These data support a multi-step model for T_{FH} differentiation, in which DCs must prime and differentiate naïve $CD4^+$ T cells towards a T_{FH} intermediate which then must receive additional signals from distinct APCs in order to complete full effector differentiation.

P6.

"IL-10 mainly derived from activated hepatic T-cells suppresses Toll-like Receptor 9-mediated Macrophage Activation Syndrome"

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Background: Macrophage Activation Syndrome (MAS) is a potentially fatal cytokine storm syndrome that complicates multiple rheumatic illnesses. We have recently shown the critical role of IL-10 as a regulator of MAS in an infection-free murine model. The current study

dissects the mechanisms by which IL-10 exerts this profound regulatory effect. Methods: As previously described, the Toll-like Receptor 9 (TLR9) agonist CpG1826 was injected repeatedly to induce MAS. Cellular IL-10 production was assessed by means of both intracellular flow cytometry and a YFP reporter system. Transgenic mice and dendritic cell (DC)-depleted mice were utilized as detailed. Results: CpG given during IL-10R blockade resulted in fulminant MAS, complete with excess hemophagocytosis. Additionally, serum IFNγ, IL-10, IL-6 and IL-12 levels in IL-10R blocked mice were dramatically elevated compared to IL-10 sufficient mice. Investigating the source of this protective IL-10, we found that TLR9-stimulation resulted in induction of IL-10 by T-cells, DCs, and NK cells. Activated ($CD44^{hi}CD69^{hi}CD62L^{lo}$) hepatic T-cells, particularly CD8+ T-cells, showed the most impressive expansion. Both $Rag1^{-/-}$ and DC-deficient mice developed enhanced MAS in response to CpG, correlating with reduced IL-10. These data are consistent with DC/T-cell interactions as a possible mechanism for IL-10 induction. Additionally, numbers of hepatic CD4+FoxP3+ T-cells (Tregs) increase dramatically with CpG treatment, and decrease with IL-10R blockade, suggesting their possible role as IL-10 responsive effector cells. Conclusions: IL-10 is a critical regulator of TLR9-induced MAS. Activated hepatic CD8 T-cells are an important source of IL-10 in this model, and DCs may be critical for their differentiation. Tregs may be important IL-10 dependent regulators of disease. These studies support the critical role of IL-10 in prevention of fulminant MAS, explicate an important regulatory pathway in this disease, and continue to identify targetable cell populations and pathways for treating MAS patients.

P7.

"Requirements for PLZF and IL-4 in the development of innate-like CD8+ T cells"

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Conventional T cells exit the thymus as naïve cells with low expression of surface markers typically associated with activation and a requirement for antigen stimulation in the periphery in order to acquire effector function. Non-conventional thymocytes that possess characteristics typically associated with innate immune cells are termed innate-like lymphocytes (ILLs) and, in contrast to conventional T cells, acquire effector function during thymic development prior to peripheral activation and express surface markers typically expressed on activated or memory T cells, such as CD44, CD122 and NK1.1. SH2 domain-containing leukocyte phosphoprotein of 76 kDa (SLP-76) nucleates a signaling complex critical for T-cell receptor (TCR) signaling and is necessary for T cell development. Mice with a tyrosine to phenylalanine mutation at a crucial phosphorylation site at position 145 in SLP-76 (Y145 mice) develop an expanded population of $CD8^+CD44^+CD122^+$ ILLs, characterized by the expression of the T-box transcription factor Eomesodermin (Eomes) and rapid production of IFN-γ after *ex vivo* stimulation. Using mixed bone marrow chimeras, we demonstrate that the development of $CD8^+$ ILLs is directed by cell-extrinsic factors. Y145F mice also develop an expanded population of CD4+ thymocytes that produce IL-4 via a mechanism dependent on promyelocytic leukemia zing finger (PLZF), a known transcriptional regulator of innate cell function. We find that blockade of IL-4 signaling through by administration of IL-4 neutralizing antibodies or deletion of PLZF in Y145F mice results in loss of $CD8^+$ ILL formation. Furthermore, we demonstrate that CD8+ ILLs are

present in low numbers in wild-type mice, as demonstrated in fetal thymocyte organ culture. We are currently developing an in vitro culture system to investigate the sufficiency of IL-4 in driving CD8+ ILL development. Together, these data shed light on the cell-intrinsic and cell-extrinsic factors that drive $CD8^+$ ILL development.

P8.
"Heterogeneity of the Bone Marrow Plasma Cell Pool"
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Long-lived plasma cells (PCs) are key mediators of humoral immunity that are responsible for maintaining antibody titers and are believed to populate unique survival niches in the bone marrow (BM). Current models predict that BM PCs consist exclusively of long-lived, slowly renewing cells. However, we find that the majority of BM PCs experience rapid rates of turnover. Furthermore, though plasma cell differentiation is thought to require silencing of the B-cell gene expression program, the rapidly renewing PC population retains expression of the canonical B-cell markers B220 and CD19. Surprisingly, many antigen-specific cells enter and persist in this pool for months post-immunization. Together these data suggest that BM niches are continuously repopulated by newly generated plasma cells, and offer new insights into the cellular basis of antibody titer maintenance.

P9.
"The dendritic cell F-actin network and ERM family member moesin function to constrain the lateral mobility of ICAM-1 and enhance T cell activation"
William A Comrie and Janis K. Burkhardt
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Immunology Graduate Group, University of Pennsylvania

During an immune response, T cells must come together and form synapses with cognate antigen presenting cells (APCs) in order to become activated. Different synaptic patterns are formed depending on the nature of the APC. B cells form an ordered synapse characterized by mobile microclusters of signaling molecules that move centripetally to build an ordered array with concentric rings of actin, LFA-1, and CD28/CD3. On the other hand, dendritic cells (DCs) form a stable multifocal synapse that lacks an ordered concentric pattern. It is unknown how DCs maintain the multifocal pattern, or how it affects T cell activation. Here, we test the hypothesis that the DC actin cytoskeleton modulates the mobility of key signaling molecules in order to enhance T cell activation. We show that the DC actin cytoskeleton differentially modulates the lateral mobility of MHCII, ICAM-1, and CD80 within the plasma membrane. ICAM-1 is maintained in a highly immobile state and MHCII remains untethered to the DC cytoskeleton, while CD80 adopts an intermediate phenotype. Studies to identify the proteins that constrain the mobility of these molecules are underway. One candidate is moesin, a protein known for crosslinking transmembrane proteins to the cortical actin network. We find that moesin is highly up-regulated upon DC maturation, and co-localizes with ICAM-1 at actin-rich sites on the DC membrane. Perturbation of moesin function using either siRNA or a dominant negative mutant alters DC ligand mobility and diminishes T-cell activation.

P10.

"The method of CD4 memory T cell generation determines signaling requirements for persistence"

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CD4+ memory T cells are formed in response to infection or vaccination, provide protection to the host against re-infection, and persist through a combination of enhanced survival and slow homeostatic turnover. We developed a temporally controlled system to study the requirements for tonic T cell receptor (TCR) signals in memory populations by timed deletion of the adaptor SLP-76. In previously published studies, where memory cells were generated *in vitro*, SLP-76 conditional knockout (cKO) CD44hi memory cells have a substantial defect in homeostatic turnover, and reduced persistence. To extended these studies and directly assess antigen-specific (AgSp) memory cells generated by *in vivo* infection, conditional mice were infected with lymphocytic choriomeningitis virus (LCMV) and CD4+ memory T cells were identified based on staining with I-A^b:GP61 peptide tetramer reagents. In marked contrast to *in vitro* generated memory T cells, GP61+AgSp memory cells persist greater than 100 days in the absence of SLP-76. Therefore, AgSp memory CD4+ T cells generated by viral infection have distinctly different requirements for SLP-76 and tonic TCR signals for their persistence. Experiments are ongoing, including other infectious models, to better define what allows SLP-76 deficient CD4 memory T cells to exhibit prolonged survival. It is important for the field to recognize that the factors governing memory cell persistence are dependent upon either the method of generation or TCR specificity, and that these findings may influence the way we induce memory T cells through vaccination.

P11.

"Influence of Viral Specific Memory Cells on Leishmania major Infection"

Erika Crosby, E. John Wherry, and Phillip Scott
University of Pennsylvania

One of the hallmarks of adaptive immunity is the development of a highly specialized, pathogen specific immune response that is maintained long term in the form of immunological memory. Despite this specificity, an important question is whether these persistent memory cells can influence the immune response at an unrelated inflammatory site. In this work, we utilized lymphocytic choriomeningitis virus (LCMV) Armstrong and *Leishmania major* (*L. major*) as our model infections to investigate the influence of viral specific memory cells on an unrelated infection. By utilizing a transgenic P14 CD8+ T cell transfer system, we first assessed the ability of LCMV Armstrong activated cells to migrate to an *L. major* infected ear lesion. While naïve P14 CD8+ T cells were unable to migrate to an *L. major* lesion, P14 CD8+ T cells from a mouse that had been infected with LCMV Armstrong 30 days prior migrated readily to the lesion. In addition to migrating to the lesion, these LCMV memory cells also had significantly increased granzyme B protein levels. Having seen that LCMV memory cells were able to enter an *L. major* lesion, we asked whether following an acute viral infection, viral CD8+ memory T cells could be found in the lesion and/or influence parasite control. Mice were infected with LCMV Armstrong and 30 days later infected with *L. major*. After 2 weeks, there was a significant increase in both the number and percentage of CD8+ T cells and a 4-log decrease in parasite numbers in the lesion of coinfected mice compared to mice infected with *L. major* alone. These data indicate that viral memory cells are able to influence the course of an unrelated infection.

Further studies are needed to determine the exact mechanism by which these memory cells are conferring protection.

P12.

"Hes1-mediated constraint of C/EBPα is essential for T cell development"

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Notch signaling in the thymus induces T lineage-specific gene expression and discourages progenitors from adopting alternative gene expression programs. We found that the Notch target and transcriptional repressor Hairy and Enhancer of Split homolog-1 (Hes1) constrains myeloid potential in T cell progenitors via repression of the myeloid lineage transcription factor CCAAT/enhancer binding protein α (C/EBPα). Hes1-deficient progenitors failed to make normal numbers of T cells upon culture with Notch ligands, but instead gave rise to an abundant population of $Mac1^{+}Gr1^{+}$ granulocytes. Furthermore, T cell development in Hes1-deficient progenitors was restored following genetic deletion of the myeloid lineage transcription factor C/EBPα. These results indicate that an essential function of Hes1 is to constrain progenitors from activating myeloid lineage programs early in T cell development.

P13.

"Important functions for the Notch1 transcriptional activation domain (TAD) in hematopoietic development"

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Notch1 is required to generate the earliest embryonic HSC at embryonic day 9.5 (E9.5) in the aorta-gonad-mesonephros (AGM). The role of Notch1 at later stages of fetal hematopoiesis is less well characterized, owing to the lethal phenotype of Notch1 knockout mice between E9.5-E10.5. The potential role of Notch1 in expansion in fetal liver HSC at E14.5 remains an open and important question. We are addressing this question using a knockin mouse that exhibits hypomorphic Notch1 signaling, yet survives past mid-gestation. Notch1 contains an evolutionarily conserved transcriptional activation domain (TAD) that is required for optimal Notch1 signaling in vitro and for Notch1 induced T-ALL. To determine whether the Notch1 TAD functions in mammalian development, we created knockin mice lacking the TAD (Notch1ΔTAD). Although Notch1ΔTAD embryos were found at the expected frequencies at early embryonic time points, no mice survived beyond birth due to multiple cardiac abnormalities. Because the mice survived beyond E14.5, we investigated HSC emergence in the AGM and development in the fetal liver. Preliminary characterization of Notch1ΔTAD E14.5 fetal liver cells suggests that HSCs, as defined by the LSK and SLAM phenotypes, are markedly decreased. This may be due to defects in HSC production, homing, proliferation or survival. HSCs purified from the fetal liver of Notch1ΔTAD embryos are also impaired in their ability to reconstitute the hematopoietic compartment in competitive bone marrow transplants, suggesting a cell intrinsic defect. In contrast, the emergence of HSCs earlier in embryonic development is relatively intact. Together, these studies suggest important roles for the Notch TAD in mammalian development and suggest that Notch signaling has an important function in fetal liver hematopoiesis.

P14.

"Positive selection during affinity Maturation relies on T cell derived BLyS"

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Protective humoral immunity relies on high affinity B cell clones that are generated and selected during the germinal center (GC) reaction. To determine whether BLyS family member(s) play a role in this process, we assessed their distribution within the GC. We show that majority of the GC is devoid of BLyS despite the presence of ample BLyS in the adjacent follicular regions. The paucity of BLyS correlates with down-regulation of TACI receptor on GC B cells and likewise follicular B cells in TACI-deficient mice bind less BLyS. TACI down-regulation on follicular B cells is mediated by IL-21 in a surrogate thymus-dependent stimulation. Furthermore, some BLyS staining is detectable in the light zone associated by T Follicular Helper cells (T_{FH}). The expression of BLyS by T_{FH} is crucial for appropriate GC evolution, since efficient affinity maturation fails in mixed bone marrow chimeras where T cells are deficient in BLyS. We conclude that positive selection during affinity maturation relies on restricted sources of BLyS on GC T_{FH} that provide survival signals to preserve high affinity clones.

P15.

"IL-10 limits IL-17 induced immunopathology following L. major infection"

Claudia Gonzalez-Lombana and Philip

University of Pennsylvania

Leishmaniasis is a protozoal infection where the disease is a function of both the replication of the parasites, as well as the inflammatory response that the parasites elicit. Indeed some of the most severe forms of leishmaniasis are primarily caused by an uncontrolled inflammatory response. For example, in patients with mucocutaneous leishmaniasis there is an exaggerated Th1 immune response, and very few parasites associated with the lesions. In these patients the severity of the disease has been correlated with low IL-10 cytokine production, less responsiveness to IL-10 and decreased in situ IL-10 receptor expression. We developed a murine model in order to understand how IL-10 regulates *Leishmania*-induced pathology. C57BL/6 mice were infected with *L. major* and IL-10 signaling was blocked by treatment with anti-IL-10 receptor monoclonal antibodies. Blocking IL-10 led to the development of severe disease, in spite of increased parasite control. Similar results were obtained in IL-10 deficient mice. We found that the lesions in anti-IL-10 receptor antibody treated mice contained an increased number of activated monocytes (Gr1-Ly6C+) and neutrophils compared with control mice. In addition, there was an increase in both IFN-g and IL-17 producing CD4 T cells in lesions from mice treated with anti-IL-10 receptor antibody. In order to determine if the increased IL-17 production was critical for the development of pathology, mice were treated simultaneously with anti-IL-10 receptor and anti-IL-17 antibody. These mice developed less severe lesions than mice only treated with anti-IL-10 receptor antibody .Our results suggest that in *L.major* infections IL-10plays a role in suppressing immunopathology by controlling IL-17 production.

P16.

"A role for NEMO in non-canonical NF-κB signaling"

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The NFκB family of transcription factors plays a major role in pro-inflammatory gene activation. Gene deletion studies have revealed two distinct mechanisms to NFκB activation: the classical and the non-canonical pathways. Classical NFκB signaling has been extensively characterized in models of acute inflammation, and aberrant classical NF-κB activity has been described in numerous immune and inflammatory diseases. The classical NFκB pathway depends on both NEMO (NFκB Essential Modulator) and IKKβ (Inhibitor of κB Kinase β), whereas non-canonical NFκB signaling requires IKKα and an upstream protein NIK (NFκB Inducing Kinase), but *not* IKKβ or NEMO. In seeking to understand the mechanisms that control this alternative pathway, we identified a novel role for NEMO in the negative regulation of non-canonical NFκB signaling. Murine Embryonic Fibroblasts (MEFs) and Jurkat T cells lacking intact NEMO exhibit increased p100 processing and stable NIK levels prior to stimulation. Loss of NEMO by siRNA-mediated knockdown recapitulates this dysregulated non-canonical NFκB phenotype in Hela cells. Furthermore, NEMO-deficient MEFs have high basal levels of the non-canonical NFκB transcription factor RelB in the nucleus and elevated transcript levels of the non-canonical NFκB-regulated chemokine *CXCL12.* Unexpectedly, we found that NEMO associates with NIK and we are currently investigating the molecular mechanism of this novel NEMO:NIK interaction. Together our data reveal a novel unanticipated role for NEMO in non-canonical NFκB signaling prompting reassessment of the upstream events that positively and negatively regulate non-canonical NFκB pathway activation. Our initial findings lead us to hypothesize that *NEMO associates with NIK and suppresses the non-canonical NFκB pathway*.

P17.

"A role for the N-terminal domain of Rag1 in immature B cell development"

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Adaptive immunity requires expression of a diverse repertoire of lymphocyte antigen receptors (AgR), which are encoded by germline variable (V), diversity (D), and joining (J) gene segments. In developing lymphocytes, the RAG1/RAG2 endonuclease catalyzes V(D)J recombination through DNA double strand break (DSB) intermediates. RAG-mediated DSBs alter chromatin structure along AgR loci and signal changes in the expression of proteins involved in cellular survival and AgR selection, each of which may be required to prevent immunodeficiency and suppress autoimmunity. To date, RAG1/RAG2 functions have been studied using truncated "core" enzymes—the minimal forms capable of cleaving DNA *in vitro.* However, core RAG proteins do not support normal V(D)J recombination or lymphocyte development in vivo. The RAG1 "non-core" region contains a RING domain with E3 ligase activity that ubiquitylates histones *in vivo*. Naturally occurring *RAG1* mutations that disrupt this E3 ligase activity cause Omenn syndrome, a human immunodeficiency associated with altered AgR repertoire. To elucidate mechanisms by which the RAG1 E3 ligase regulates AgR repertoire, we have begun to analyze $Rag1^{core}$ mice. We find that $Rag1^{core}$ mice contain decreased numbers of pre B cells, immature transitional (T)1, and T2 B cells, normal numbers of more mature marginal zone B cells, and fewer numbers of Igl^{+} B cells. We demonstrate that development of Igl^{+} B cells is not reconstituted with age, enforced cellular survival signals, or expression of an auto-reactive AgR transgene that

forces Igk gene editing and increased development of Igl^+ B cells in wild-type mice. These data are consistent with requirement of RAG1 E3 ligase activity for control of Igl recombination, normal AgR selection, and possibly for the prevention of auto-immunity and immunodeficiency. Ongoing studies will determine the specific role of RAG1 E3 ligase activity in signaling changes in chromatin structure and gene expression in response to RAG DSBs.

P18.
"Exploring the Role of Aging Pathways in Antiviral CD8 T cell Differentiation"
Jonathan B. Johnnidis and E. John Wherry
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Antigen-experienced T cells can be extraordinarily long-lived following acute infections and during chronic infections, yet the mechanisms underpinning this longevity are unknown. SIRT1 is an NAD-dependent deacetylase that integrates metabolic inputs to modulate cellular stress responses, survival, and organismal longevity across species. SIRT1 is upregulated in CD8 T cells following viral infection, and mice with a conditional disruption of the Sirt1 locus in T cells exhibit impaired control of chronic LCMV infection. Sirt1-deficient CD8 T cells differentiate aberrantly in response to both acute and chronic viral infection, and are marked by both quantitative and qualitative defects. Quantitative defects are more pronounced in Ag-specific populations that incur greater degrees of stress, consistent with a role for SIRT1 in potentiating stress responses. The putative SIRT1 downstream target Superoxide Dismutase (SOD) is critical for countering oxidative stress in mitochondria, and is also upregulated in CD8 T cells following viral infection. SOD-deficient mice are known to sustain accelerated loss of genomic integrity with age, and here are shown to exhibit deficiencies in CD8 T cell differentiation in response to chronic infection. The interrelationships of SIRT1 and SOD and their roles in sustaining T cell differentiation and longevity are being further explored.

P19.
"Metabolic Regulation of CD8 T Cell Exhaustion"
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Chronic viral infections such as HIV, HCV and HBV are global epidemics affecting >500 million people. A hallmark of immune responses to chronic infections is the formation of exhausted CD8 T cells. These exhausted T cells have impaired effector functions and upregulate "inhibitory" receptors, leaving them ineffective at controlling infections. Exhausted T cells also display an altered metabolic transcriptional signature. Metabolic processes can influence T cell differentiation, but the importance of altered metabolism and its connection to the other changes in exhausted CD8 T cells has not been investigated. To address these questions, we have begun to examine how metabolic alterations may be linked to inhibitory receptors such as PD-1, CTLA-4, and CD160. These receptors can modulate the PI3K/AKT pathway, which is upstream of the major metabolic regulator mTOR. Compared to other CD8 T cell subsets, LCMV-specific exhausted CD8 T cells displayed delayed mTOR-dependent phosphorylation of ribosomal protein S6 and altered levels of multiple metabolic components including transferrin receptor, neutral amino acid receptor, and mitochondrial mass. Delayed S6 phosphorylation was associated with intermediate, but not high, expression of PD-1 and CD160. This is surprising given that PD-1 may inhibit PI3K and PD-1 blockade improves CD8 T cell function. We are currently examining other

components of AKT/mTOR signaling and the functional consequences of altered mTOR activation in exhausted CD8 T cells with the ultimate goal of identifying potential novel targets for therapeutic treatment of chronic infection.

P20.

"Diacylglycerol kinase zeta but not diacylglycerol kinase alpha suppresses nTreg development"

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Diacylglycerol (DAG), a critical second messenger of T cell receptor (TCR) signaling, is negatively regulated through phosphorylation by diacylglycerol kinases (DGKs). Lack of DGKα and DGKζ, isoforms of DGK expressed in T cells, results in enhanced activation and proliferation of T cells to TCR stimuli. In addition, DGKζ knockout (DGKζKO) mice have more robust responses to viral infection and tumors. Presumably due to excessively strong TCR signaling, deletion of both DGKα and DGKζ results in a severe block in thymocyte development that is not seen with deletion of either isoform alone. Strong TCR signals may also direct developing thymocytes to the natural T regulatory cell (nTreg) fate. Consistent with this, we have found that DGKζ deficiency significantly increases percentages of nTreg cells. The key structural features of DGKζ that are important for nTreg development are unknown. In cell lines, the kinase domain and phosphorylation of the myristoylated alanine-rich C-kinase substrate (MARCKS) domain control the enzymatic activity and localization of DGKζ. To probe the function of these domains, we used retroviruses to transduce DGKζKO bone marrow and re-express mutant DGKζ proteins using bone marrow chimeras. Validating our system, expression of wild-type DGKζ restored suppression of nTreg development compared to empty vector. Expression of kinase dead DGKζ did not suppress development of nTregs, suggesting that kinase activity is important for this function. To our surprise, expression of a non-phosphorylatable MARCKS domain DGKζ did not restore suppression of nTreg development, suggesting that localization of DGKζ may play a critical role in its function. Because DGKα and DGKζ have been shown to localize differently in cell lines, we investigated if DGKα and DGKζ have different roles in nTreg development. Surprisingly, we found that mice lacking DGKα do not have higher percentages of nTregs, even in the presence of DGKζ heterozygosity. Suppression of nTreg development may therefore be a function specific to DGKζ and not DGKα. Current investigations are probing the mechanism behind the observed differences.

P21.

"Innate lymphoid cells promote lung tissue homeostasis following acute influenza virus infection"

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Innate lymphoid cells (ILCs), a recently identified heterogeneous cell population, are critical in orchestrating immunity and inflammation in the intestine but whether ILCs can influence immune responses or tissue homeostasis at other mucosal sites remains poorly characterized. Here we identify a population of lung-resident ILCs in mice and humans that expressed CD90, IL-2Ra, IL-7Ra and IL-33R and produced IL-5 and IL-13 in response to IL-33. Strikingly, murine ILCs accumulated in the lung following influenza virus infection and depletion of ILCs resulted in loss of airway epithelial barrier integrity, decreased lung function and impaired airway remodeling. Global gene expression profiling of lung ILCs revealed strong enrichment for genes regulating wound healing pathways and administration of the lung ILC product amphiregulin, an epidermal growth factor family member, effectively restored airway tissue remodeling in ILC-depleted mice. Collectively, these results demonstrate a critical role for lung ILCs in mediating airway epithelial integrity and respiratory tissue homeostasis following influenza virus infection.

P22.

"Virus recognition by Toll-7 activates antiviral autophagy in Drosophila"

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Detection of pathogens by the innate immune system relies on germline-encoded pattern recognition receptors (PRRs) that recognize conserved molecular signatures known as pathogen-associated molecular patterns (PAMPs). The canonical PRRs are the mammalian Toll-like receptors (TLRs), which were discovered through their homology to *Drosophila* Toll. While immune functions have been assigned to all mammalian TLRs, of the nine *Drosophila* Toll receptors, only Toll has been definitively implicated in antimicrobial defense. Most mammalian TLRs directly bind theirs PAMPs whereas Toll recognizes a host cytokine that is activated upon infection. The mammalian TLRs are also phylogenetically distinct and not involved in development like *Drosophila* Tolls, raising the question of whether they were co-opted for immunity independently. We have identified a role for an additional *Drosophila* Toll receptor, Toll-7, in conferring antiviral immunity to Vesicular Stomatitis virus (VSV). Multiple Toll receptors including Toll-7 are transcriptionally induced by VSV infection. Toll-7 depletion by RNA interference leads to increased viral replication in both flies and cells, as well as decreased survival in VSV-infected flies. More similar to the mode of recognition for mammalian TLRs, Toll-7 binds VSV. This interaction is required to activate autophagy, an ancient cytoplasmic degradative pathway that has previously been shown to restrict VSV. Moreover, Toll-7 mutant flies exhibit diminished induction of autophagy and enhanced susceptibility to VSV infection. Collectively, these data suggest that viral recognition by Toll-7 elicits antiviral autophagy and support a more direct evolutionary relationship between the *Drosophila* Tolls and the mammalian TLRs.

P23.

"Cutaneous commensals control skin immunity via a distinct IL-1/CD40 dependent mechanism"

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Mammals have co-evolved with bacterial communities that inhabit their mucosal and cutaneous barriers. Several studies have identified a central role for the intestinal flora in orchestrating local and systemic immunity, and unveiled the mechanisms underlying host commensal interactions at the gastrointestinal interface. In contrast, the role of resident flora at other barrier sites and the dialogue between microbes and defined tissue microenvironments is poorly understood. Using skin as a model organ we evaluated the impact of commensal microbiota in regulating immune cells in this distinct tissue site. We find that the murine skin and gut house unique communities of bacteria and that resident skin commensals control the balance of effector and regulatory T lymphocytes in the dermis. Terminal differentiation of T cells in the skin is critically dependent upon modulation of resident dendritic cell activation and the innate cytokine milieu by commensals. More specifically, the cutaneous inflammatory set point relies on the MyD88/IL-1 and CD40/CD40L axis. Additionally, our data show that commensal immune control in the skin depends on pathways that are distinct from the intestinal host commensal cross talk. Further, we find that local skin commensals are necessary for triggering pathology and mounting protective responses to dermal pathogen *Leishmania major*. These findings highlight the importance of the flora as a distinctive feature of tissue compartmentalization and reveal unique mechanisms of immune regulation by resident commensal niches. Our data underscore the importance of developing targeted tissue specific therapies that may rely on modulation of local commensal clues to ameliorate disease pathology.

P24.
"Osteoclastogenesis and APRIL production requirements for plasma cell survival"
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Thymus dependent (TD) humoral responses establish the major pool of long-lived plasma cells (PC). These cells are maintained for the lifetime of the host and impart sterilizing immunity via antibody secretion. While the mechanism underlying their maintenance remains elusive, it has recently been shown that Receptor Activator for Nuclear Factor κ B Ligand (RANKL) and the B Lymphocyte Stimulator (BLyS) family of ligands and receptors play key roles in PC longevity. Cancro and colleagues have shown that RANKL expression on long-lived PC induces A Proliferation Inducing Ligand (APRIL) production by myeloid cells. RANK signaling in myeloid cells results in large, multinucleated osteoclasts, but it remains unclear if osteoclastogenesis per se is necessary for APRIL production. Recently, Yu *et al* have shown that IL-4 via STAT6 represses key downstream transcription factors necessary for osteoclastogenesis even in the presence of RANKL. Thus, by adopting an *in vitro* approach, we plan to dissect whether only RANK signals or subsequent downstream factors are required for APRIL production.

P25.

"The role of CD8 T cells in Leishmania braziliensis infection"

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Leishmania braziliensis causes cutaneous lesions, and a subset of infected individuals develop mucosal disease when the parasites metastasize to the nasal and oral mucosa. The factors responsible for the disease in *L. braziliensis* patients remain poorly understood. However, with all forms of leishmaniasis control of the parasites is dependent upon generating a strong CD4 Th1 response; CD8 T cells are also thought to contribute to protection due to their production of IFN-g. Since IFN-g is required to resolve experimental *L. braziliensis* infections in mice, we investigated whether CD8 T cells might be required for control of *L. braziliensis*. BALB/c mice were infected with metacyclic *L. braziliensis* (MHOM/BR/01/BA788) intradermally in the ear. We found that early after infection there was substantial recruitment of CD8 T cells to the site of infection, suggesting that they may be contributing to the immune response. We next tested whether CD8 depletion would alter the course of infection. *L. braziliensis* infected BALB/c mice were treated with anti-CD8 monoclonal antibody or an isotype control antibody, and the course of infection monitored. Surprisingly, we found that BALB/c mice depleted of CD8 T cells exhibited enhanced resistance. Importantly, the loss of CD8 T cells was associated with lower parasite numbers, indicating that CD8 T cells were inhibiting optimal immunity to this parasite. A histological and flow cytometric analysis of the lesions indicated that depletion of CD8 T cells led to an increase in the percent of neutrophils. This finding is consistent with previous results demonstrating a protective role for neutrophils with this infection. Taken together, these data highlight a new role for CD8 T cells in modulating leishmanial infections, and current studies are underway to determine how CD8 T cells regulate neutrophil responses following *L. braziliensis* infection. This work was supported by CNPq, Fiocruz, and the National Institute of Allergy and Infectious Diseases, National Institutes of Health

P26.

"Ikaros as regulator of naïve CD8+ T cell activation and differentiation"

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Naïve CD8+ T cell activation and differentiation requires the integration of TCR, co-stimulatory and "Signal 3" cytokine cues. Upon appropriate stimulation, activated CD8+ T cells undergo initial rounds of cell division and an initial burst of autocrine IL-2. Activated CD8+ T cells terminate their autocrine IL-2 and become dependent on exogenous IL-2 from CD4+ T cells, termed CD4+ help. Our lab is focused on the molecular integration of these 3 signals and has previously identified Ikaros, a chromatin-remodeling factor, in the integration of TCR and CD28 signals in CD4+ T cells. Ikaros has not been described in mature CD8+ T cells and has been mainly characterized as a hematopoietic cell fate determinant, as Ikaros null mice lack T, B, and NK cells. *As CD4 and CD8+ T cells share similar differentiation pathways, we hypothesize that Ikaros regulates CD8+ T cell differentiation via influencing autocrine IL-2 and IFN-γ production.* In CD3/CD28 *in vitro* activated naïve CD8+ T cells with only one copy of Ikaros (Ikaros Het), we observe that they produce robust IFN-γ in comparison to their wild-type counterparts. Interestingly, these Ikaros Het CD8+ T cells do not require the addition of exogenous Signal 3 cytokines as they produce more autocrine IL-2 than activated wild-type CD8+ T cells. This IL-2 acts in a cell-extrinsic fashion, as IL-2

blockade results in IFN-γ ablation by these activated CD8+ T cells. Our data indicates a novel role for Ikaros in mature CD8+ T cells, in that it acts intrinsically via repression of IL-2 and this IL-2 regulation influences the production of IFN-γ. This augmented IL-2 by Ikaros Het CD8+ T cells may obviate the requirement for CD4+ T cell help and may have implications for CD8+ T cell tolerance and autoimmunity.

P27.

"A role for IL-27 in the development of T-bet+ Treg required to limit infection-induced pathology"

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IL-27 is a potent antagonist of T H1 mediated inflammatory responses, but the basis for this effect is not fully understood. Recent studies have identified a population of Foxp3+ T-bet+ CXCR3+ CD4+ T cells that limit TH1 mediated immune pathology. The studies presented here demonstrate that the ability of IL-27 to activate STAT1 promotes Treg expression of T-bet and CXCR3, and that the presence of this specialized population during infection with Toxoplasma gondii was dependent on IL-27. Furthermore, transfer of Treg ameliorates the infection induced CD4+ T cell-mediated pathology observed in IL-27-/- mice. Together, these findings define IL-27 as a key cytokine that promotes the development of Tregs specialized to control TH1 immunity.

P28.

"Adaptor interactions of Ndfip2 with Nedd4-family E3 ligases in T cells"

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Members of the Nedd4-family of HECT-type E3 ubiquitin ligases degrade key transcription factors and regulatory proteins following T-cell activation. Regulation of these ligases is therefore critical to appropriate immune responses. Regulation may be mediated through interaction with adaptor proteins, as shown for Nedd4-family interacting protein 1 (Ndfip1) which regulates the E3 ligase Itch in T cells. Another putative adaptor for Nedd4-family members, Ndfip2, has been shown *in vitro* to bind and activate the catalytic activity of mammalian Nedd4-family E3 ligases. Our initial findings utilizing GST pulldown indicate that Ndfip2 binds to a subset of Nedd4-family E3 ligases in activated T cells, supporting a role for Ndfip2 in regulating E3 ligase function in T cells. In order to interrogate the *in vivo* function of Ndfip2, we generated an Ndfip2 knockout mouse with GFP expressed under the endogenous Ndfip2 promoter. We show that Ndfip2 is upregulated in T cells upon stimulation. While Ndfip2 deficient mice have grossly normal lymphoid compartments, with age Ndfip2 deficient mice show an increased percentage of activated phenotype T cells relative to wild-type controls. Additionally, these cells have increased expression of the transcription factor Eomesodermin *ex vivo.* Further studies will investigate whether Ndfip2-/-

T cells have a primary defect in memory or effector cell differentiation following activation and whether this impacts immune function.

P29.
"Investigating the Mechanisms of Inhibitory Receptor Blockades in Chronic Viral Infection"
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Chronic viral infections, such as HIV, HBV, and HCV, are a major public health problem and cause significant morbidity and mortality worldwide. Lack of immune control during these infections is associated with T cell exhaustion. One of the features of T cell exhaustion is the co-expression of multiple inhibitory receptors, including PD-1, Lag-3, Tim-3, CD160 and 2B4, on antigen-specific CD8+ T cells. Partial reversal of T cell dysfunction can be achieved by blocking one or more inhibitory receptor pathways. For example, blockade of PD-1 restores function of exhausted CD8+ T cells during chronic LCMV infection; furthermore, blockade of both PD-1 and Lag-3 provides synergistic recovery of effector T cell function, proliferation and viral control. The inability to fully reverse exhaustion, however, highlights the need for a more mechanistic understanding of inhibitory receptor blockades. To address this, we are investigating two major questions in the field. First, how do inhibitory receptor blockades influence the cellular and transcriptional pathways downstream of different inhibitory receptors? We expect that inhibitory receptor blockades will initiate distinct transcriptional changes by altering key transcription factors (such as Tbet, Eomes, and BATF) and modulate metabolic pathways that are involved in CD8+ T cell differentiation and activation. Second, what are the CD8+ T cell intrinsic and extrinsic effects of inhibitory receptor blockade? Inhibitory receptors are also expressed on other immune cells, such as regulatory T cells. We predict that inhibitory receptor blockade may alter the function of these cells, which could contribute to the reversal of CD8+ T cell exhaustion. Ultimately, a better understanding of inhibitory receptor blockades will provide insight into the development of therapies for human chronic infections.

P30.
"T-box transcription factors coordinate terminal differentiation and self-renewal in CD8 T cells during chronic viral infection"
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In many biological settings, a cell population must balance terminal differentiation with longevity and self-renewal. CD8 T cells often encounter these competing demands. Unlike acutely resolved infections which allow the CD8 T cell population to return to a state of quiescence, chronic viral infections - where the pathogen persists - place an intense strain on the CD8 T cell population by continuously inciting the generation of terminally-differentiated, antiviral effector cells.

How CD8 T cells maintain a large antiviral population during chronic infection is not well understood. Furthermore, the transcription factors that allow CD8 T cells to balance self-renewal and terminal differentiation in this setting are not known.

Here, we report that two T-box transcription factors, Eomesodermin (Eomes) and T-bet, act to maintain the maximal CD8 T cell response by regulating the persistence of two

complementary subsets. Eomes^{lo} T-bet^{hi} CD8 T cells act as progenitor cells exhibiting limited proliferation, yet give rise to highly proliferative Eomes^{hi} T-bet^{lo} CD8 T cells.

Thus, during chronic infection Eomes and T-bet coordinate the balance between self-renewal and terminal differentiation in order to maintain the CD8 T cell response.

P31.
"A role for the protein tyrosine kinase Syk during TNFα secretion"
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Cells of the innate immune system, such as macrophages and dendritic cells (DCs) secrete a variety of proinflammatory cytokines, including TNFα and IL-6 in response to various stimuli. While they are essential for protective immunity against infection, inappropriate cytokine responses can contribute to acute and chronic inflammation. Therefore, understanding the signaling events involved in this pathway is crucial for treating disease. Membrane-bound TNFα is packaged in the Golgi complex from where it is transported to the recycling endosome. From there, it is delivered to the cell surface at the site of phagocytic cups for its cleavage by TNFα-converting enzyme prior to its release into the intercellular space. The protein tyrosine kinase Syk has been reported to be involved in regulation of cytokine release; however, its exact role is unclear. Using cell lines in which we knocked down Syk and mice harboring CD11c+ DCs genetically engineered to delete Syk, we have found that Syk-specific deletion results in decreased secretion of TNFα following stimulation with the TLR9 agonist CpG DNA, while IL-6 secretion occurs normally. However, CpG-induced TNFα mRNA and intracellular protein levels are normal in the absence of Syk. Interestingly, Syk-deficient cells show an increased level of surface TNFα, suggesting that Syk is important for the cleavage of TNFα from the plasma membrane prior to release into the intercellular space. It has been reported that TNFα is phosphorylated prior to cleavage by casein kinase 1, a kinase activated by calcium signaling. We find that stimulation of Syk-deficient cells with ionomycin in addition to CpG causes a boost in TNFα secretion, similar to that in Syk-sufficient cells. Additionally, mice containing Syk-deficient DCs show decreased secretion of TNFα into the serum following injection with CpG, indicating an in vivo function for Syk in the recognition of viral DNA. These data suggest that Syk has a role upstream of TNFα cleavage and that calcium signaling is important for TNFα secretion. Elucidating the exact function of this kinase in the cytokine secretion pathway may be helpful for designing therapies aimed at inhibiting its activity during inflammatory disease.

P32.
"Differential requirement of SLP-76 signaling in regulatory T cell development and function"
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Regulatory T cells (Treg)s are a subset of T cells that are required for suppression of inappropriately activated T cells. The signal transduction requirements in Treg function have not been fully elucidated. In particular, the requirement for Treg TCR signaling in suppression of conventional T cell activation has recently become controversial. Src homology 2 (SH2) domain-containing leukocyte protein of 76 kDa (SLP-76) is a key adaptor protein required for transduction downstream of the TCR. Through use of mice in which SLP-76 can be conditionally deleted in mature Tregs, we tested the importance of TCR

signaling in Treg function. Compared to SLP-76-sufficient Tregs, SLP-76-deficient Tregs displayed no suppressive ability in *in vitro* suppression assays, suggesting that TCR signaling by Tregs is required for their inhibitory function. Furthermore, using SLP-76 tyrosine mutant mice, we found that tyrosine 145 (Y145) but not Y112/128 of SLP-76 was required for their suppressive function. Surprisingly, however, Tregs developed normally in SLP-76 Y145F mutant mice, suggesting the differential requirement of this tyrosine in Treg development vs. function. These data suggest that while both Treg development and function require SLP-76, the essential downstream pathways that emanate from SLP-76 might be distinct in Treg development versus function.

P33.
"Bystander chronic infection impairs the development of optimal CD8+ T cell memory"
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CD8+ T cells are the hallmarks of protective immune responses against intracellular pathogens and memory CD8+ T cells provide protection upon secondary infections. The formation of optimal memory CD8+ T cells is a complex and dynamic process not thoroughly understood. Epidemiological evidence suggests that chronic infections can compromise immunity against antigenically unrelated infections, however there is no mechanistic understanding of how memory CD8+ T cell development is altered in the presence of co-infection. Here, we tested whether an unrelated "bystander" chronic infection impacted the development of CD8+ T cell memory. We demonstrate that differentiation and function of memory CD8+ T cells against OVA were substantially impaired in mice chronically infected with an antigenically irrelevant pathogen (LCMV or *T. gondii*). Furthermore, adoptive transfer of OVA-specific effector CD8+ T cells into a chronic inflammatory environment strikingly affected the differentiation of those cells into optimally functional memory CD8+ T cells. Gene expression profiling studies suggested that in the context of bystander chronic infection, pathways induced by IFN-I and/or IL-12 were specifically dysregulated in memory CD8+ T cells. Importantly, the molecular signature of those cells was significantly similar to the molecular signature of human naïve CD8+ T cells isolated from HIV-infected individuals. Further, the defective bystander memory CD8+ T cell development in an inflammatory environment was shown to be dependent on the transcription factor T-bet and partially dependent on the transcription factor Blimp1. Finally, chronic inflammation increased the expression of the proapoptotic protein Bim in bystander CD8+ T cells, while inhibiting their IL15-induced rescue from apoptosis *ex vivo*. It is therefore suggested that an established chronic infection can negatively affect the development of bystander memory CD8+ T cell responses. These results pose critical questions for consideration regarding the vaccination of patients with chronic inflammatory conditions.

P34.
"A Role for ATM in Antigen Receptor Allelic Exclusion"
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The majority of lymphocytes rearranges and expresses antigen receptors originating from a single allele, a phenomenon known as allelic exclusion. Feedback inhibition, a major mechanism contributing to allelic exclusion, involves the assembly and expression of an antigen receptor from one allele which suppresses further V-to-(D)J rearrangements on the

homologous allele. For feedback inhibition to successfully mediate allelic exclusion asynchronous rearrangement of homologous alleles is required. Recently we showed data suggesting that ATM may initiate signals which repress additional V(D)J recombination events and enforce asynchronous rearrangement of homologous antigen receptor alleles. To investigate whether ATM enforces allelic exclusion *in vivo*, we examined $ATM^{-/-}$ mouse lymphocytes by FACS for allelic inclusion. Analysis of mice either heterozygous for an engineered allotypic difference at the Igκ locus or expressing allotypically marked IgH alleles showed increased Igκ or IgH allelic inclusion, respectively, in the absence of ATM. Analysis of αβ T cells revealed a slight increase in TCRβ allelic inclusion by FACS, increased bi-allelic Vβ-to-DβJβ rearrangements, as well as increased usage of the 3' Dβ2Jβ2 cluster, suggesting ATM may inhibit secondary Vβ-to-DβJβ rearrangements both *in cis* and *in trans*. Deletion of cyclin D3, which drives G0 phase lymphocytes into the cell cycle, further increased TCRβ allelic inclusion in $ATM^{-/-}$ mice, suggesting that the ability of cyclin D3 to drive cells through G1 phase may cooperate with ATM signals to inhibit bi-allelic Vβ rearrangements. Additionally, $ATM^{-/-}$ mice containing a pre-assembled DJβ complex that reduces TCRβ recombination events and increases Vβ rearrangement frequencies also showed further increased TCRβ allelic inclusion in $ATM^{-/-}$ mice. We hypothesize that ATM promotes antigen receptor allelic exclusion in lymphocytes by enforcing asynchronous V-to-(D)J recombination of homologous antigen receptor alleles to ensure efficient feedback inhibition. In this context, allelic exclusion may have arisen from pressure to maintain genomic stability and suppress transforming lesions during V(D)J recombination.

P35.

"The regulatory T cell response to influenza infection is driven by recognition of cognate antigen"

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Regulatory T cells (Tregs) play a crucial role in maintaining immune homeostasis and in the prevention of autoimmunity. In addition to these well-established functions, thymically-generated Tregs have also been implicated in the control of the immune response to various pathogens. However it remains largely unclear whether Tregs are becoming activated in response to antigen, either from the pathogen or from damaged cells, or as a result of entering an inflammatory environment. Under homeostatic conditions, Tregs require cognate antigen in order to undergo proliferation, and accordingly transferred Tregs specific for the site 1 (S1) epitope of PR/8 influenza virus expanded more in a transgenic mouse that expresses S1 as a self-antigen than in a control mouse. Subsequent infection with an influenza virus that lacks the S1 epitope led to a slight increase in proliferation of transferred Tregs but only in the transgenic mouse. During infection with an influenza virus expressing the S1 epitope, transferred S1-specific Tregs entered the lungs and limited the accumulation of CD8+ T cells, while in contrast Tregs in S1-transgenic recipients were unable to enter the lungs during infection with a virus lacking the S1 epitope unless cognate antigen was present there. This suggests that Treg recruitment and activity during a viral infection is predominantly antigen-driven, rather than a response to inflammation or TLR signals.

P36.

"Localization of Antibody Secreting Cells Following Influenza Infection"

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Anti-viral antibodies induced following influenza infection are important for providing protection against reinfection. During the first few weeks post-infection, anti-viral antibody secreting cells (ASCs) increase in numbers within the lung draining mediastinal lymph node (medLN) and the lung. We have observed that this increase in local anti-viral ASCs is accompanied by an increase in class-switched ASCs not specific for influenza. While anti-viral ASCs within the medLN and lung are thought to be protective, the role of these nonvirus-specific, class-switched ASCs during influenza infection is currently unknown. We have focused on determining if these ASCs are induced locally or are recruited to sites of inflammation during infection. Previous studies have demonstrated that bystander activation of B cells can occur during chronic viral infections, however, whether this also occurs during an acute respiratory viral infection is unclear. We have made use of the well-characterized MD4 mouse strain, containing B cells specific for hen egg lysozyme (HEL), to provide a source of true nonvirus-specific B cells that we can trace. We have generated chimeric mice to determine if a population of nonvirus-specific B cells can be induced to differentiate into ASCs following influenza infection. Our results suggest that although HEL-specific B cells are present in both the medLN and lung after infection, they do not differentiate into ASCs. To determine if nonvirus-specific ASCs are being recruited to inflamed tissue during influenza infection, we have utilized a subcutaneous immunization model to track ASCs generated at distal sites. The results of these studies suggest that subcutaneously generated ASCs are not recruited to either the medLN or lung during influenza infection. While the mechanism responsible for generating nonvirus-specific ASCs within the medLN and lung is still not clear, our data currently suggest that it is not due to bystander activation or recruitment from a distal site.

P37.

"Thymic Settling after Bone Marrow Transplantation"

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The *de novo* generation of naïve T-cells requires continual importation of circulating bone marrow derived progenitors into the thymus. After bone marrow transplantation (BMT), T cells are among the last of the hematopoietic lineages to recover, but the reasons for this delay are not well understood. Thymic settling, under normal physiologic conditions is a selective process that requires either chemokine receptor CCR7 or CCR9 and selectin ligand PSGL-1. The mechanisms of thymic settling shortly after BMT are unknown. We show that thymic settling is briefly CCR9/CCR7-independent after BMT, but is still dependent on PSGL-1. Despite the relaxed requirement for CCR9 and CCR7, we find that thymic settling after BMT is surprisingly inefficient when compared to physiologic conditions. This inefficiency is likely due to the direct damage of the thymic stroma by irradiation. These results together indicate that irradiation alters both the mechanism and efficiency of thymic settling.

P38.

"The non-conventional cyclin G2 as target of the GATA1/FOG1/NURD transcription factor complex"

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Transcription factor GATA1 and its cofactor FOG1 interact to regulate erythropoiesis and megakaryopoiesis. FOG1 binds to nucleosome remodeling and deacetylase complex (NuRD). We previously created FOG1 knock-in mice with engineered point mutations that disrupt the interaction between FOG and NuRD. Homozygous FOG1 knock-in (ki/ki) mice exhibit defective erythropoiesis and megakaryopoiesis. Erythroid progenitors and mature erythrocytes are reduced in number with an increased proportion of mutant erythroid progenitors residing in the G1/G0 phase of the cell cycle. In an effort to understand the mechanisms by which cellular maturation and cell cycle arrest are perturbed in mutant animals, we identified cyclin G2 (CCNG2), a nonconventional cyclin, as a potential GATA1/FOG1/NuRD target gene. CCNG2 mRNA levels are elevated in FOG1 ki/ki bone marrow and fetal liver erythroid precursors. In a conditional GATA1 inducible erythroid cell line CCNG2 expression increases with induction of wild type GATA1 but not with GATA1 containing a point mutation disrupting its interaction with FOG1. Thus, both GATA1 and FOG1 control the regulation of CCNG2. Chromatin immunoprecipitation (ChIP) showed that GATA1 binds to the promoter and an upstream region of the CCNG2 locus. Taken together, these findings establish CCNG2 as novel GATA/FOG1/NuRD target that is likely involved in the cell cycle control of erythroid progenitors. Studies are underway examining the in vivo function of CCNG2 in progenitor cells.