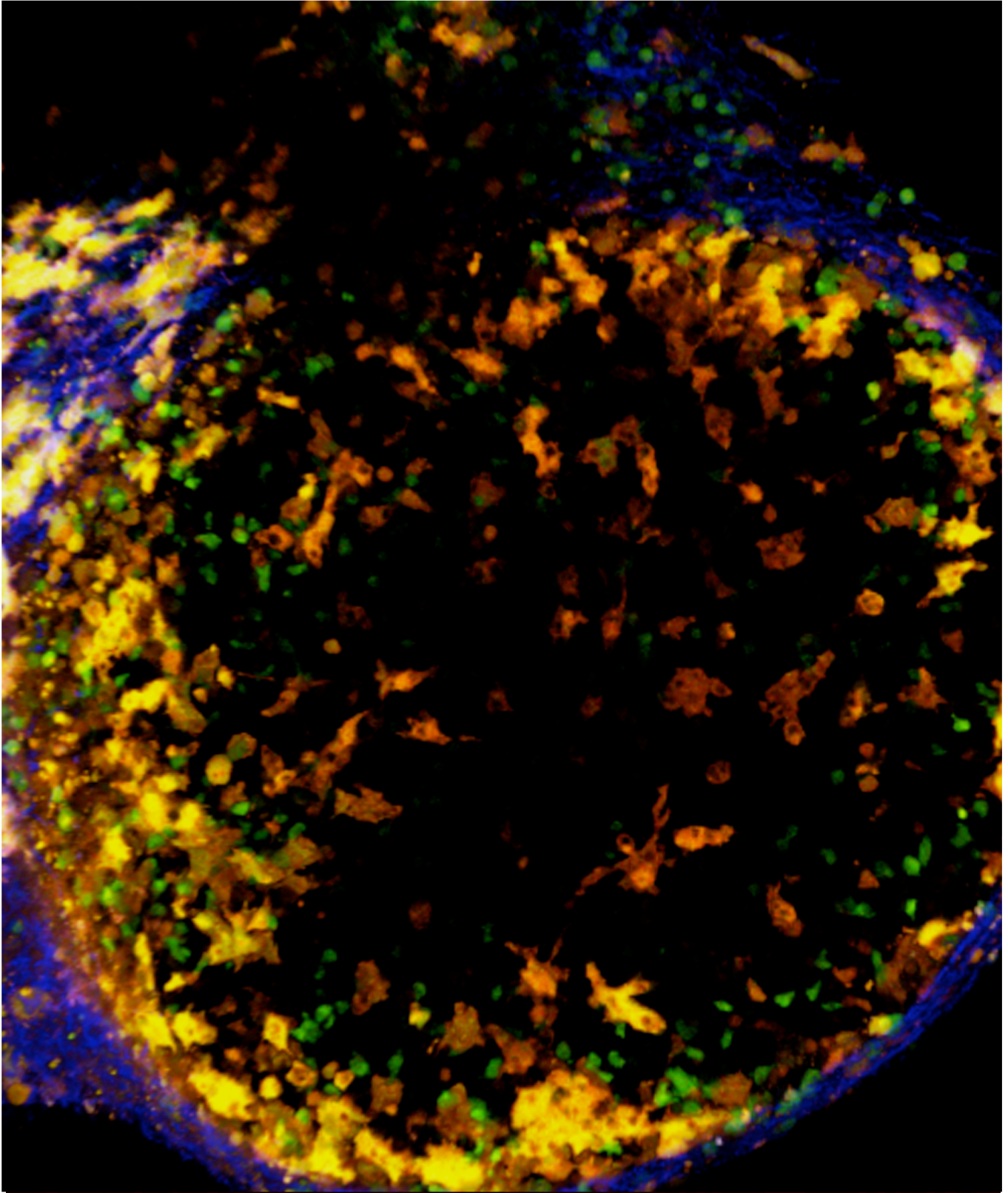


22st Annual Immunology Graduate Group Retreat



November 6-8, 2009
Willow Valley Resort and Conference Center
Lancaster, PA

The Immunology Graduate Group gratefully acknowledges the financial support of all our contributors for the 22nd 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 CA 09140 “Immunobiology of Normal and Neoplastic Lymphocytes” training grant

Institutes, Centers, Departments, and Divisions

Abramson Family Cancer Research Institute

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

Penn Center for Clinical Immunology

The Department of Microbiology

The Department of Pathology and Laboratory Medicine

The Department of Surgery

The Division of Cell Pathology, Children’s Hospital of Philadelphia

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Cover Photo

Localization of CD11cYFP dendritic cells and OT1GFP cells in the lymph node following infection with *Toxoplasma gondii*.

3-D projection of a z stack from the lymph node of a CD11cYFP mouse that was adoptively transferred with ovalbumin-specific CD8+ GFP+ T cells and infected with *T. gondii* expressing ovalbumin. An increased proportion of dendritic cells (yellow) and T cells (green) are associated with the sub-capsular region (capsule seen in blue) of the lymph node following infection. Also note the vacuolar structures within the dendritic cells, which are induced during infection. This image was acquired on a Leica SP5 2-photon microscope equipped with a picosecond laser and tunable internal detectors. Image provided by Chris Hunter and Beena John.

22nd Annual Immunology Retreat
Friday to Sunday, November 6-8, 2009
Willow Valley Resort and Conference Center
2416 Willow Street Pike, Lancaster, PA 17602-4898

Friday, November 6, 2009

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, Terrace Dining Room |
| 1:20-1:30 | Welcome, Steve Reiner, IGG Chair |
| 1:30-3:10 | Session I: Immune Response to Viral Infection
Session Chair: Michael Abt |
| 1:30-1:55 | Tony Barnitz, <i>Protein Kinase A phosphorylation activates HIV-1 Vpr cell cycle arrest</i> |
| 1:55-2:20 | Michael Abt, <i>Signals derived from intestinal bacteria augment anti-viral immunity</i> |
| 2:20-2:45 | Silke Jennrich, <i>The role of CCR7 in CD8 T cell egress from the lung during influenza A virus infection</i> |
| 2:45-3:10 | Alison Crawford, <i>Unravelling CD4 T cell exhaustion</i> |
| 3:10-3:30 | Break
1 st and 2 nd year students: Easel and posterboard set-up |
| 3:30-4:45 | Session II: Inflammation
Session Chair: Hilda Ramon |
| 3:30-3:55 | Hilda Ramon, <i>Ndfip1 Regulates T Cell-Mediated Gastrointestinal Inflammation and Susceptibility to Inflammatory Bowel Disease</i> |
| 3:55-4:20 | Donald Simons, <i>Inflammatory monocytes from arthritic mice are conditioned to drive efficient Th17 differentiation</i> |

4:20-4:45	Greg Sonnenberg, <i>Pathologic and protective functions of IL-22 in the lung are regulated by IL-17A</i>
4:45-6:00	Willow Valley registration and room check-in, Main Lobby <i>Please set up posters for the remainder of the conference in Statesman C/D</i>
5:30-7:00	Dinner Smorgasbord, Terrace Dining Room
7:00-8:35	Session III Session Chair: Jon Maltzman & Mike May
7:00-7:30	Lih-Ling Lin, Ph.D., Director, Inflammation Signaling Wyeth Research, Boston <i>"Development of kinase inhibitors for treating rheumatoid arthritis"</i>
7:30 – 7:35	Introduction to Keynote Speaker: Steve Reiner
7:35 – 8:35	Keynote Speaker, Fiona Powrie, Ph.D. Professor Sir Williams Dunn School of Pathology University of Oxford <i>"Gut reactions: Cellular and molecular pathways that contribute to intestinal homeostasis"</i>
8:45-12:00	Social, Statesman Hall A/B/C/D

Saturday, November 7, 2009

8:00-9:00 AM	Breakfast Smorgasbord, Terrace Dining Room
9:00-10:40	Session IV: All Things B Session Chair: Will Quinn
9:00-9:25	Radhika Goenka, <i>T Follicular Helper cells produce and sequester BLyS in the Germinal Center</i>
9:25-9:50	Marco Calamito, <i>γ-secretase regulates B cell activation and development independently of the Notch pathway"</i>
9:50-10:15	Will Quinn, <i>TRANCE expression marks long-lived plasma cells</i>

10:15-10:40	Alexandra Bortnik, <i>A novel feature of early antibody secreting cells</i>
10:40-10:55	Break
10:55-12:35	Session V: T cell development Session Chair: Dan Zlotoff
10:55-11:20	Dan Zlotoff, <i>CCR7 and CCR9 together recruit hematopoietic progenitors to the adult thymus</i>
11:20-11:45	Jeremiah Bell, <i>Notch Signaling Constrains the Myeloid Potential of Early Thymic Progenitors</i>
11:45-12:10	Brenna Brady, <i>Repetitive Genomic Sequences Partition Vβ Segments into Distinctly Regulated Genomic Units</i>
12:10-12:35	Anastasia Tikhonova, <i>MHC-independent specificity of QuadKO T cells is due to their alpha beta TCRs</i>
12:35-1:35 PM	Deli Lunch, Terrace A/B "To-Go" boxes available
12:35-3:30	Free time to explore Lancaster area
3:30-5:30	Poster Session, Statesman Room C/D
5:30-7:00	Dinner Smorgasbord, Terrace Dining Room
7:00-7:50	Session VI: Homeostasis Session Chair: Tao Zou
7:00-7:25	Tao Zou, <i>Dendritic cells drive regulatory T cell proliferation through antigen-dependent and -independent mechanisms</i>
7:25-7:50	Evann Corbo, <i>The role of SLP-76 in CD4 Memory Homeostasis</i>
7:50 – 8:00	Short Break
8:00 – 9:00	Session VII: Faculty Talks
8:00 – 8:30	Aimee Payne Assistant Professor of Dermatology <i>"Targeted therapy for autoantibody-mediated diseases"</i>

8:30 – 9:00 Taku Kambayashi
Assistant Professor of Pathology and Laboratory Medicine
"Modulation of antigen-specific T cell activation by mast cells"

9:00 Announcement of Awards for Best Oral Presentation and Best Poster

9.00-12:00 AM Party, Statesman Hall A/B/C/D

Sunday, November 8, 2009

8:00-11:00 AM Continental Breakfast, Statesman Hall, A/B

REMINDER: **Please check out of your room by 11 AM. Please take down your posters.**

END OF CONFERENCE

SAVE THE DATE
23rd Annual Immunology Graduate Group Retreat
November 5-7, 2010
Willow Valley Resort and Conference Center

Abstracts for Oral Presentations:

1. R. Anthony Barnitz, Fengyi Wan, Vinay Tripuraneni, Diane L. Bolton' and Michael J. Lenardo
"Protein Kinase A phosphorylation activates HIV-1 Vpr cell cycle arrest"
2. Michael Abt, Daniel Beiting, Dmytro Kobuley, Colby Zaph, John Wherry and David Artis
"Signals derived from intestinal bacteria augment anti-viral immunity"
3. Silke Jennrich, Florian Simon and Gudrun Debes
"The role of CCR7 in CD8 T cell egress from the lung during influenza A virus Infection"
4. Alison Crawford, and E John Wherry
"Unravelling CD4 T cell exhaustion"
5. Hilda E. Ramon, Christopher R. Riling, Baoli Yang, Hakon Hakonarson, and Paula M. Oliver
"Ndfip1 Regulates T Cell-Mediated Gastrointestinal Inflammation and Susceptibility to Inflammatory Bowel Disease"
6. Donald Simons, Alissa Basehoar, Lori Mroz, and Andrew Caton
"Inflammatory monocytes from arthritic mice are conditioned to drive efficient Th17 differentiation"
7. Gregory F. Sonnenberg, Meera G. Nair, Thomas J. Kirn, Colby Zaph, Lynette A. Fouser, and David Artis
"Pathologic and protective functions of IL-22 in the lung are regulated by IL-17A"
8. Radhika Goenka, Andrew H. Matthews, Jean L. Scholz, Patrick J. O'Neill, William Stohl, and Michael P. Cancro
"T Follicular Helper cells produce and sequester BlyS in the Germinal Center"
9. Marco Calamito, Bhaskar Srivastava, Matthew Thomas, Warren S. Pear, and David Allman
"γ-secretase regulates B cell activation and development independently of the Notch pathway"
10. William J Quinn III, William Stohl, Yongwon Choi and Michael P Cancro
"TRANCE expression marks long-lived plasma cells"
11. Alexandra Bortnick and David Allman
"A novel feature of early antibody secreting cells"

12. Daniel A. Zlotoff, Arivazhagan Sambandam, Theodore D. Logan, J. Jeremiah Bell, Benjamin A. Schwarz, and Avinash Bhandoola
“CCR7 and CCR9 together recruit hematopoietic progenitors to the adult thymus”

13. J. Jeremiah Bell, Warren S. Pear, and Avinash Bhandoola
“Notch Signaling Constrains the Myeloid Potential of Early Thymic Progenitors”

14. Brenna Brady and Craig Bassing
Repetitive Genomic Sequences Partition V β Segments into Distinctly Regulated Genomic Units

15. Anastasia Tikhonova, François Van Laethem, Leonid Pobezinsky, Terry I. Guinter, and Alfred Singer
“MHC-independent specificity of QuadKO T cells is due to their alpha beta TCRs”

16. Tao Zou, Andrew J. Caton, Gary A. Koretzky, and Taku Kambayashi
“Dendritic cells drive regulatory T cell proliferation through antigen-dependent and -independent mechanisms”

17. Evann Corbo, Karla Wiehagen, Michelle Schmidt, Eleni Argyropoulou, Nicholas Bushar, Donna Farber and Jonathan Maltzman
“The role of SLP-76 in CD4 Memory Homeostasis”

Abstracts for Posters:

- P1. Jill Angelosanto, Shawn Blackburn and E. John Wherry
“Progressive Loss of Memory Potential and Early Alterations in CD8⁺ T Cell Differentiation during Chronic Viral Infection”
- P2. Emily J. Chen, Dooyoung Lee, Daniel A. Hammer, Verena Niggli, and Janis K. Burkhardt
“Role of flotillins in uropod formation and T cell adhesion”
- P3. Anthony Chi, Alex Chavez, Avinash Bhandoola
“T lymphoid potential found in a subset of myeloid progenitors”
- P4. Maria L. Ciocca, John T. Chang, Leslie J. Berg, Gary A. Koretzky, Martha S. Jordan, & Steven L. Reiner
“Asymmetric Proteasome Segregation and Unequal Inheritance of a Fate Determinant During Cell Division”
- P5. Douglas V Dolfi, Antonio M Polley, Kenneth E Schmader, and E John Wherry
“Increased Tbet corresponds with increase in senescence marker CD57 in influenza virus-specific CD8⁺ T cells in aged individuals”
- P6. Scott M. Gordon, Caitlin Dejong, M. Ellen DeObaldia, Vikram R. Palanivel, and Steven L. Reiner
“A requirement for Eomesodermin in the development of natural killer cells”
- P7. Carolyn M. Gray and Michael May
“Identifying and Characterizing Novel IKK_α complexes”
- P8. Danielle Haney, Tedi Asher, David Ambrozak, David Price, Danny Douek, and Michael Betts
“Characteristics of Polyfunctional Human CD8 T cells”
- P9. Yi Hao, Patrick O'Neill, and Michael Cancro
“Aged B cells compete more effectively for survival *in vivo* and accumulate a unique B cell subset”
- P10. Kimberly A. Jordan & Christopher A. Hunter
“c-Rel is required for optimal development of a CD8⁺ effector T cell response to *Toxoplasma gondii*”
- P11. Charlly Kao, Michael Paley, Andrew Intlekofer, Steven L. Reiner, and E. John Wherry

“A Critical Role for T-bet in Preserving CD8 T Cell Function during Chronic Viral Infection”

P12. Dawson Knoblock, LiLi Tu, Olga Shestova, Rajan Jain, Stacey Rentschler, and Warren S. Pear

“Requirement of Notch1 transcriptional activation domain in leukemogenesis and development”

P13. T. Daniel Logan, Daniel A. Zlotoff, Pedro Cejas, and Avinash Bhandoola

“Isolation and characterization of thymic endothelial cells”

P14. Rebecca May and Taku Kambayashi

“SLP-76 is required for optimal NK cell activation and shaping of the Ly49 receptor repertoire”

P15. Shruti Naik, David Chou, Jason Hall, Nicolas Bouladoux, Yasmine Belkaid

“Skin commensal microflora regulate cutaneous immunity”

P16. Shaun O’Brien, Matthew Riese, and Gary Koretzky

“Cytokine production in CD8⁺ T cells deficient in DGKa or DGKz”

P17. Aisling C. O’Hara, Elia D. Tait, Jon S. Silver, Yasmine Belkaid, and Christopher Hunter

“The Immune Response to *Toxoplasma gondii* Regulates Regulatory T cells”

P18. Soyoung Oh, Andrew L. Rankin, Malinda Aitken, and Andrew J. Caton

“Effect of TCR specificity on the *in vivo* function of CD4⁺CD25⁺ regulatory T cells in an autoimmune setting”

P19. Michael Oropallo, Ann Marshak-Rothstein, and Michael Cancro

“The role of BLyS in the activation and survival of potentially pathogenic rheumatoid factor producing B cell”

P20. Olivia A Perng, Soyoung Oh, Donald Simons, Abby Liebow, Malinda Aitken, Lori Mroz, Alissa Basehoar, Christina Mergenthaler, and Andrew J Caton

“Factors Prompting the Development of Autoimmunity in Genetically Susceptible Mice”

P21. Jacqueline G. Perrigoue, Michael A. Paley, Eric Houpt, Pandelakis Koni and David Artis

“MHC class II-dependent effector synapses between intestinal epithelial cells and CD4⁺ T cells regulate infection-induced intestinal inflammation”

P22. Jennifer Reed and John Monroe
“TITLE miRNAs in B cell development”

P23. Steven Saenz, Mark Siracusa, Jacqueline Perrigoue, Taku Kambayashi,
Alison Budelsky and David Artis
“Investigating the functional biology of IL-17E”

P24. Keri B Sanborn, and Jordan S Orange
“Regulation of lytic granule-associated myosin IIA in NK cells during activation for cytotoxicity”

P25. Jonathan Silver, Jason Stumhofer, Matthias Ernst and Christopher Hunter
“In the absence of SOCS3, IL-6 blocks protective immunity to *T. gondii*”

P26. Sean Spencer, John Grainger, David Chou, Elizabeth Wohlfert, Jason Hall,
and Yasmine Belkaid
“Investigating the Role of Eosinophils in Intestinal Immune Homeostasis”

P27. Dil Afroz Sultana, Daniel A. Zlotoff and Avinash Bhandoola
“The Expression, Function and Regulation of P-selectin and its ligand PSGL1”

P28. Greta Weiss, Chiung-yu Huang, Marko Mircetic, Noah McKittrick, Amy
Baughman, Shanping Li, Safiatou Doumbo, Didier Doumtabe, Aissata Ongoiba,
Kassoum Kayentao, Boubacar Traore, Ogobara Doumbo, Susan K. Pierce, and
Peter Crompton
“Specific and bystander memory B cell and antibody responses to intense
seasonal *Plasmodium falciparum* malaria”

P29. Karla Wiehagen, Evann Corbo, Michelle Schmidt, Haina Shin, E. John
Wherry, and Jonathan S. Maltzman
“Continuous expression of SLP-76 is required for antigen specific CD8⁺ memory
T cell generation but not for persistence”

P30. Amaya I. Wolf, Krystyna Mozdzanowska, Laszlo Otvos, Jan Erikson
“An improved M2e-targeted peptide vaccine confers protection against influenza
virus infection”

Abstracts for Oral Presentations:

1

Protein Kinase A phosphorylation activates HIV-1 Vpr cell cycle arrest

R. Anthony Barnitz^{1,2}, Fengyi Wan¹, Vinay Tripuraneni¹, Diane L. Bolton¹, and Michael J. Lenardo¹

¹Laboratory of Immunology, NIAID, National Institutes of Health, ²Immunology Graduate Group, University of Pennsylvania

Infection with human immunodeficiency virus type 1 (HIV-1) causes an inexorable depletion of CD4⁺ T cells, and recent studies suggest that direct viral cytopathicity is a major factor. The 14 kD HIV-1 accessory protein Vpr contributes important to HIV-1-induced necrosis, which is correlated with cell cycle arrest. Vpr has also been shown to contribute to nuclear migration of the preintegration complex, and transactivation of the viral promoter. Phosphorylation of Vpr serine 79 (S79) is required to activate G₂M cell cycle blockade. Mutation of serine 79, S79A, attenuates both Vpr-mediated cell cycle arrest and nuclear import in macrophages, suggesting phosphorylation at this residue is critical for at least some of Vpr functions. However, the kinase responsible for phosphorylating Vpr remains unknown. Using bioinformatics tools, we found that serine 79 of Vpr is part of a putative phosphorylation site recognized by the cAMP-dependent Protein Kinase, PKA. We show that PKA interacts with Vpr by immunoprecipitation and FRET and directly phosphorylates S79. Inhibition of PKA activity during HIV-1 infection abolishes Vpr cell cycle arrest. These findings provide insight to the signaling event that activates Vpr cell cycle arrest, likely leading to the necrotic death of infected cells. Our findings also indicate that blocking the phosphorylation of Vpr by PKA, or the interaction between the two, may be targets for therapeutic intervention during HIV-1 infection.

2

Signals derived from intestinal bacteria augment anti-viral immunity

Michael Abt¹, Daniel Beiting², Dmytro Kobuley², Colby Zaph¹, John Wherry³ & David Artis¹

Departments of Pathobiology¹ & Biology², University of Pennsylvania, & The Wistar Institute³, Philadelphia, PA, 19104

Alterations in the composition of intestinal bacterial communities in humans are associated with enhanced susceptibility to multiple inflammatory diseases suggesting that signals derived from commensal bacteria may influence the development, function or homeostatic regulation of the immune system. Supporting this, germ-free mice exhibit reduced numbers of lymphocytes in the periphery and intestinal intraepithelial compartment. However, whether alterations in the acquisition or composition of commensal bacteria influence immunity to infection remains poorly defined. To test this, mice housed under conventional or germ-free conditions were infected i.p. with Lymphocytic Choriomeningitis Virus (LCMV Armstrong strain) and the development of antigen-specific CD8⁺ T cell responses were monitored. At day 7 post-infection, germ-free mice exhibited a significant reduction in the frequency and numbers of LCMV-specific CD8⁺ T cells in multiple tissues including the spleen and intestinal intraepithelial compartment. The diminished LCMV-specific CD8⁺ effector T cell response was not the result of inherent developmental defects in germ-free mice as depleting the intestinal bacteria in conventionally-housed mice via oral antibiotic treatment also resulted in a diminished LCMV-specific CD8⁺ T cell response following infection. Further investigation into the cause of the diminished CD8⁺ T cell response using transgenic p14 T cells revealed a delay in the activation and proliferation of p14 T cells in antibiotic treated

mice at days 3-4 following LCMV infection. This delay in the initiation of the adaptive immune response led to an impaired CD8⁺ T cell response resulting in delayed viral clearance. Taken together, these studies indicate that signals derived from intestinal bacteria can provide an adjuvant-like signal and aid in the rapid induction of the CD8⁺ T cell response required for immunity to systemic viral infection.

3

The role of CCR7 in CD8 T cell egress from the lung during influenza A virus infection

Silke Jennrich, Florian Simon and Gudrun Debes

Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine

Effector functions of T lymphocytes are closely linked to their migration *in vivo*. Memory/effector T lymphocytes efficiently enter sites of infection from blood and subsequently leave via the afferent lymph. While exit of resting T cells from non-inflamed extralymphoid tissue is regulated by their expression of the chemokine receptor CCR7, it is not known whether the receptor fulfills an equivalent role in effector T cell exit from acutely infected sites. Here, we analyzed CCR7 expression by *in vitro* polarized (CD8⁺) Tc1 cells and found that Tc1 cells express functional CCR7 while being cytotoxic *in vitro*. Importantly, upon adoptive transfer, *in vitro* polarized Tc1 cells were able to rescue mice infected with a lethal dose of influenza A virus. Transferred Tc1 cells did egress from infected lungs and could be recovered from draining lymph nodes. The pulmonary egress of Tc1 cells during acute infection required their expression of CCR7, demonstrating that egress from the inflamed lungs is an active, regulated process. Our data support a model in which T cell retention in and egress from infected tissue is controlled by the regulated expression of CCR7, thereby affecting local T cell effector function and efficiency of host defense.

4

Unravelling CD4 T cell exhaustion

Alison Crawford and E John Wherry

Wistar Institute

When an infection becomes chronic, antigen-specific CD8 T cells often become exhausted. This CD8 T cell exhaustion can have a major impact on the ability to clear the virus. Compared to CD8 T cells, however, there is relatively little information on exhaustion of virus-specific CD4 T cell responses. Thus, we investigated whether virus-specific CD4 T cells also become exhausted during chronic viral infection and how this exhaustion differed from CD8 T cells. We have performed a functional and phenotypical examination of LCMV-specific CD4 T cells during acute versus chronic infection with LCMV. The results show that while CD4 T cells do become exhausted, their exhaustion differs from CD8 T cells in terms of both differentiation pattern and function. Virus-specific CD4 T cells and CD8 T cells differentially express inhibitory receptors during chronic infection and the functional exhaustion of CD4 T cells in terms of cytokine production was not as absolute as CD8 T cells at the later stages of chronic infection. These studies help to unravel the complexity of CD4 T cell function during chronic infection.

5

Ndfip1 Regulates T Cell-Mediated Gastrointestinal Inflammation and Susceptibility to Inflammatory Bowel Disease

Hilda E. Ramon¹, Christopher R. Riling², Baoli Yang³, Hakon Hakonarson², and Paula M. Oliver²

¹ University of Pennsylvania, School of Medicine

² The Children's Hospital of Philadelphia ³ University of Iowa

Ndfip1 is an adaptor protein that regulates the function of the E3 ligase Itch, which induces the ubiquitination and subsequent degradation of JunB. JunB is a transcription factor that binds the IL-4 and IL-5 promoters thereby regulating the production of these T_H2 cytokines. Mice that are deficient in Ndfip1 or Itch develop T_H2-mediated inflammation in the skin and lungs and die prematurely. We now show that the inflammation in Ndfip1 deficient mice is not limited to the skin and lungs, but also present in the gastrointestinal tract. This inflammatory condition is primarily characterized by the infiltration of eosinophils and is accompanied by weight loss. T cells are both necessary and sufficient to drive disease in these mice. Furthermore, we have determined that Ndfip1^{-/-} T cells produce IL-5, a cytokine that causes Eosinophils to migrate out of the bone marrow and into tissues. The role of Ndfip1 in regulating Itch cannot completely account for this inflammatory phenotype given that Itch mutant mice develop a much less severe gastrointestinal inflammation. While these data show that Ndfip1 regulates gastrointestinal inflammation in mice, we now have evidence supporting a role for this adaptor protein in susceptibility to Inflammatory Bowel Disease in humans.

6

Inflammatory monocytes from arthritic mice are conditioned to drive efficient Th17 differentiation

Donald Simons, Alissa Basehoar, Lori Mroz, Andrew Caton
The Wistar Institute

Th17 cells are a recently identified subset of IL-17 secreting helper T cells that have been implicated in many types of autoimmune disease including rheumatoid arthritis. The signals that guide the development of Th17-mediated disease in vivo are not fully understood. Our lab has recently established a mouse model of arthritis in which self-antigen expressed by antigen presenting cells drives the differentiation and expansion of autoreactive Th17 cells. We have used this model to study the ability of specific APC subsets to deliver Th17-inductive signals, and determine how these signals are modulated by chronic inflammation in arthritis. Using FACS purified APCs from the spleens of healthy HA-transgenic mice to stimulate naïve HA-specific T cells in vitro, we found that both conventional dendritic cells (cDC) and inflammatory monocytes (iMO) are intrinsically capable of supporting Th17 differentiation. When arthritic APCs were used in this assay, we found that cDCs but not iMO had an enhanced capacity to generate Th17 cells; however, only the Th17 cells generated by arthritic iMO were able to expand in response to the Th17-trophic cytokine IL-23. This result may be due to decreased levels of the Th17-antagonistic cytokine IFN γ in the iMO-stimulated cultures, since arthritic iMO were uniquely deficient in the generation of IFN γ -secreting Th1 cells. Although IL-23 is dispensable for Th17 induction, T cell responsiveness to this cytokine is required for Th17-mediated pathology indicating that signals derived from iMO may play a central role in driving arthritis in this model.

7

Pathologic and protective functions of IL-22 in the lung are regulated by IL-17A

Gregory F. Sonnenberg^{1*}, Meera G. Nair^{1*}, Thomas J. Kirn¹, Colby Zaph¹, Lynette A. Fouser², and David Artis¹

¹Department of Pathobiology, University of Pennsylvania, Philadelphia, PA 19104, USA

²Wyeth Research-Inflammation, Cambridge, MA 02140, USA

Interleukin (IL-) 22 has both pro-inflammatory and anti-inflammatory properties depending on the context in which it is expressed. However, the factors that influence the functional outcomes of IL-22 expression remain poorly defined. We demonstrate that following exposure to bleomycin-induced airway inflammation, CD4⁺ T helper 17 (T_H17) cell-derived IL-17A and IL-22 are expressed in the lung. Bleomycin-induced disease was ameliorated in IL-22-deficient (*IL22*^{-/-}) mice or following administration of an anti-IL-22 neutralizing monoclonal antibody (mAb) to wild-type (WT) mice, indicating a pro-inflammatory role for IL-22 in the lung. To examine whether IL-17A influenced the pro-inflammatory properties of IL-22, bleomycin was administered to IL-17A^{-/-} mice. Despite elevated bleomycin-induced IL-22 production in the lung, IL-17A^{-/-} mice were protected from airway inflammation, suggesting a loss of the pro-inflammatory properties of IL-22. Indeed, delivery of anti-IL-22 mAb exacerbated bleomycin-induced airway inflammation in IL-17A^{-/-} mice, indicating that in the lung IL-22 is anti-inflammatory in the context of IL-17A deficiency. These data demonstrate that IL-17A is a regulator of IL-22 function in airway inflammation. Specifically, the presence or absence of IL-17A governs the pro-inflammatory and pathologic or anti-inflammatory and protective properties of IL-22 respectively.

8

T Follicular Helper cells produce and sequester BlyS in the Germinal Center

Radhika Goenka¹, Andrew H. Matthews¹, Jean L. Scholz¹, Patrick J. O'Neill, William Stohl², and Michael P. Cancro¹

¹Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; ²Keck School of Medicine, University of Southern California, Los Angeles, CA.

B Lymphocytes undergo selection for appropriate BCR specificity at two critical points. The first is during late development, where newly formed B cells with autoreactive or ineffective BCRs are eliminated before entering the primary pools. BlyS plays a key role in this process, such that the window of BCR signal strength required for survival is determined by BlyS availability. BlyS interacts with these cells via two receptors, TACI and BR3, both of which are expressed by emerging and mature primary B cells. Following antigen-driven activation, a second round of selection occurs during the germinal center (GC) reaction, where novel BCR specificities generated by somatic hypermutation are selected for enhanced antigen affinity and against self-reactivity. Whether BlyS plays a role in positive or negative selection in the GC remains unclear. Accordingly, we have used immunohistochemistry, confocal microscopy and flow-cytometric analyses to assess the distribution and availability of BlyS and its receptors within the GC. We find that in contrast to resting follicular B cells, GC B cells have down regulated TACI and lack surface bound BlyS, despite their continued expression of BR3 and ample BlyS reservoirs in the adjacent follicular regions. However, some BlyS staining is detected in the light zone of the GC, co-localized with B follicular helper T cells (T_{FH}). PCR and flow-cytometric analyses reveal that in contrast to resting naïve CD4⁺ T cells, T_{FH} produce BlyS, express TACI, and can bind BlyS. Ongoing

experiments using mixed bone marrow chimeras and BCR sequence analyses will assess how BLyS production and sequestration by T_{FH} influences selection within the GC.

9

“ γ -secretase regulates B cell activation and development independently of the Notch pathway”

Marco Calamito, Bhaskar Srivastava, Matthew Thomas, Warren S. Pear, and David Allman
University of Pennsylvania

The biochemical pathways underlying B cell receptor (BCR) function remain poorly understood. Here we show that Presenilin-1 and Presenilin-2 (PS1, PS2), the catalytic subunits of the γ -secretase protease, regulate B cell development and activation. Deletion of PS1 and PS2 had several effects on B cell development and function including loss of B1-B cells and abnormal BCR repertoire selection, as evidenced by a decrease in the percentage of Lambda light chain positive cells. We also demonstrate a role for PS1 and PS2 in normal proliferation, activation and calcium mobilization of B cells in response to BCR stimulation but not in response to Toll-like Receptor-agonists CpG and LPS. Strikingly, although presenilins are required for activation of the Notch pathway in B cells, genetic inhibition of Notch activation had no effect on these facets of B cell development or activation. These data reveal a novel, Notch independent, role for presenilins in BCR signaling and B cell function.

10

TRANCE expression marks long-lived plasma cells

William J Quinn III, William Stohl, Yongwon Choi and Michael P Cancro
University of Pennsylvania School of Medicine, Philadelphia PA

Plasma cells (PC) are the effectors of humoral immunity. Short-lived PC are generated rapidly after either thymus-independent (TI) or thymus-dependent (TD) immunization, yielding the low affinity antibody associated with early primary immune responses. In contrast, the lasting protective titers of isotype switched, high affinity antibodies associated with TD responses are maintained by long-lived PC. These cells persist indefinitely in the BM, suggesting that unique homing properties, survival requisites, and stromal interactions may underlie their protracted survival. Herein we show that TD PC express TNF-related activation-induced cytokine (TRANCE), whereas TI generated PC do not. TRANCE expression is important for both the establishment and maintenance of long-lived PCs, because disruption of TRANCE/TRANCE-receptor interactions leads to an 8-12 fold reduction in pre-existing long-lived plasma cell pools, and prevents the establishment of long-lived PC when administered upon initial immunization. Moreover, TRANCE expression enables TD PC to induce the production of APRIL in myeloid cells, which in turn fosters PC survival. Together, these findings reveal a novel circuit whereby TRANCE expressing PC both induce and maintain their own survival niche, and establish TRANCE as a definitive marker for long-lived PC and their progenitors.

11

A novel feature of early antibody secreting cells

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We are working to understand how and when antigen-activated B cells become competent to produce long-lived antibody secreting cells (ASCs) during the course of an immune response. One poorly understood characteristic of long-lived ASCs is their resistance to apoptosis in response to numerous extrinsic insults including ionizing radiation (IR). Here we show that resistance to radiation-induced apoptosis (RIA) occurs exceptionally early during the ASC differentiation program *in vivo*, even in response to T cell independent (TI) antigens which are thought to only generate short-lived ASCs. RIA resistance was cell intrinsic, not restricted to bone marrow resident ASCs, and unique to activated B cells within the ASC lineage, as germinal center B cells produced via T cell dependent (TD) immunization were not RIA-resistant. Furthermore, *in vitro* propagated ASCs and toll-like receptor activated B cells were also resistant to RIA even when B cells lacked the IRF4 transcription factor required for early ASC differentiation. These observations show that antigen-activated B cells generate RIA-resistant ASCs exceptionally early during TD and TI immune responses, and raise questions about the cellular and molecular features of short-lived versus long-lived ASCs.

12

CCR7 and CCR9 together recruit hematopoietic progenitors to the adult thymus

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T lymphopoiesis requires settling of the thymus by bone marrow derived precursors throughout adult life. Progenitor entry into the thymus is selective, but the molecular basis of this selectivity is incompletely understood. The chemokine receptor CCR9 has been demonstrated to be important in this process. However, progenitors lacking CCR9 can still enter the thymus, suggesting a role for additional molecules. Here we report that the chemokine receptor CCR7 is also required for efficient thymic settling. CCR7 is selectively expressed on bone marrow progenitors previously shown to have the capacity to settle the thymus, and CCR7^{-/-} progenitors are defective in settling the thymus. We further demonstrate that CCR7 sustains thymic settling in the absence of CCR9. Mice deficient for both CCR7 and CCR9 have severe reductions in the number of early thymic progenitors, and in competitive assays CCR7^{-/-}CCR9^{-/-} double knock-out progenitors are almost completely restricted from thymic settling. However, these mice possess near-normal thymic cellularity. Compensatory expansion of intrathymic populations can account for at least a part of this recovery. Together our results illustrate the critical role of chemokine receptor signaling in thymic settling and help to clarify the cellular identity of the physiologic thymic settling progenitors.

13

Notch Signaling Constrains the Myeloid Potential of Early Thymic Progenitors

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Notch is required for early T lineage development in the thymus; but whether Notch signaling is required for T lineage commitment, specification, or both is not clear. We

earlier showed that the majority of early T lineage progenitors (ETPs) within the thymus possess myeloid lineage potential. The myeloid potential of ETPs was most clearly evident when ETPs were removed from the thymus and assessed in the absence of Notch signals, indicating a role for Notch in the suppression of myeloid cell fates during early T cell development. To understand the importance of Notch-mediated suppression of myeloid fates within the thymus, we are investigating the consequences of interrupted Notch signaling in vivo. We find that expansion of myeloid populations, including granulocytes and macrophages but not dendritic cells, occurs in the thymus when Notch signals are interrupted. These results indicate that suppression of myeloid outcomes is one function of Notch signaling, suggesting a role for Notch in T lineage commitment. We are also defining the mechanism by which Notch constrains these myeloid potentials. We find that expression of the Notch target Hes1 results in downregulation of C/ebpa, a key myeloid transcriptional regulator. Our results suggest that Notch mediates suppression of the myeloid potentials of ETPs via its canonical target Hes1.

14

Repetitive Genomic Sequences Partition V β Segments into Distinctly Regulated Genomic Units

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The expression of a functional TCR α gene suppresses transcription and rearrangement of endogenous germline V β segments. Here we demonstrate that germline V β 10 segments residing immediately upstream of a pre-assembled V β DJ β 1.4C β 1 gene are transcribed and rearrange to DJ β 2 complexes in thymocytes. These assembled V β 10DJ β 2C β 2 genes are expressed within cell surface TCR α chains. Sense and anti-sense germline V β 10 transcription is silenced in mature $\alpha\beta$ T cells, coincident with an increase in CpG methylation of V β 10. Other V β segments are neither transcribed nor rearrange on this allele. Sequences among the transposons between V β 10 and upstream V β segments exhibit stable CpG methylation throughout development and mark a boundary between active and inactive germline V β segments. Our data suggest that these *cis* acting elements, which are conserved and interspersed throughout the V β cluster, suppress secondary V β rearrangements by choreographing the silencing of germline V β segments located upstream of actively transcribing V β DJ β C β genes.

15

MHC-independent specificity of QuadKO T cells is due to their alpha beta TCRs

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Alpha beta T cells generated in the thymus recognize only antigens presented by MHC or MHC-like molecules. Two models explaining this unique feature of alpha beta T-cells have been proposed. The first argues that MHC-specificity is a genetically encoded property of T cell receptor alpha and beta chains. The second postulates that pre-selection TCRs recognize both MHC-dependent as well as MHC-independent antigens, but that positive selection only rescues T cells bearing MHC-restricted TCRs. Using mice deficient in CD4 and CD8 co-receptors as well as deficient in MHC I and II (QuadKO), we have previously shown that MHC independent T cells can arise *in vivo*. We now document that the MHC-independent specificity of T cells from QuadKO mice is indeed due to MHC- independent alpha beta TCR. Following cloning of individual T cell

receptors from QuadKO T cells we retrovirally introduced them into TCR alpha beta negative cell line and conferred MHC-independent reactivity. These results demonstrate that T cells bearing MHC-independent TCRs can be selected in the thymus and support our contention that their selection is normally prevented by co-receptor sequestration of lck.

16

Dendritic cells drive regulatory T cell proliferation through antigen-dependent and -independent mechanisms

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Regulatory T cells (Treg) are a subset of T cells with suppressive function that protect the host from autoimmunity and prevent excessive immunopathology. A constant number of functional Treg must be present throughout life to provide continuous, protection for the host. Despite the intense study of this lineage, the mechanisms by which Treg are maintained at a steady state are still unclear. Here we investigated the role of dendritic cells (DC) in the control of Treg proliferation. We found that DC are uniquely able to drive polyclonal Treg to undergo spontaneous proliferation in splenocyte cultures. *In vivo* expansion of DC also resulted in polyclonal Treg expansion and an increased rate of Treg turnover, which was decreased by the deletion of the expanded DC. The expansion of Treg by DC involved interleukin-2 (IL-2) production from conventional CD4⁺ T cells through an MHC class II-dependent signal provided by DC. In the presence of IL-2, Treg proliferation occurred independent of MHC class II provided by DC, but still required DC absolutely. The MHC class II-independent signal provided by DC to Treg partially involved cell contact-dependent costimulatory signals. These data establish a role for DC in Treg homeostasis and provide a model for the complex interactions between DC, conventional CD4⁺ T cells, and Treg that are required to drive polyclonal Treg proliferation.

17

The role of SLP-76 in CD4 Memory Homeostasis

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CD4⁺ memory T cells are formed in response to infection or vaccination and provide protection to the host against re-infection. It is important to study the factors that regulate the maintenance of this population in order to understand how these cells are able to persist after pathogen clearance. A cardinal feature of memory T cells is long-term persistence resulting from a combination of enhanced survival and homeostatic turnover. Two pathways have been implicated in the regulation of memory cell persistence following antigen clearance: MHC:self-peptide T cell receptor (TCR) interactions and gamma chain cytokine signaling, such as IL-7. We have developed a temporally controlled system to study the requirements for tonic TCR signals utilizing Cre-mediated deletion of the SH2-domain-containing phosphoprotein of 76 kilodaltons (SLP-76) in memory T cells, as defined by high expression of the hyaluronic acid receptor CD44. SLP-76 conditional knockout (cKO) CD44^{hi} cells have a substantial defect in both

homeostatic turnover of the memory population and lymphopenia induced proliferation (LIP). To determine which SLP-76 dependent pathways are required for turnover of memory cells, we have attempted to complement the homeostatic proliferation defect by selective targeting of these pathways. Specifically, augmentation of the PI3K/AKT pathway by simultaneous deletion of PTEN and SLP-76 does not rescue homeostatic turnover or LIP when compared to SLP-76 cKO. Interestingly, complementation with mutated forms of SLP-76 can variably rescue homeostatic proliferation depending on the mutant form expressed suggesting a complex role for the N-terminal tyrosines of SLP-76. A better understanding of the pathways critical for memory CD4⁺ T cell homeostasis may allow for more rational drug and vaccine design in the future.

Abstracts for Posters:

P1

Progressive Loss of Memory Potential and Early Alterations in CD8⁺ T Cell Differentiation during Chronic Viral Infection

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Chronic viral infections are the product of a failed immune response, leading to persistent infection and T cell exhaustion. While some evidence suggests that T cell defects are progressive during chronic infection, two important questions remain: 1.) Is the progression of T cells towards exhaustion reversible, 2.) Do early events of chronic infection initiate exhaustion? To answer these questions, we first set up adoptive transfer systems to ask at what point in CD8⁺ T cell differentiation do cells commit to exhaustion and lose memory potential. Mice were infected with lymphocytic choriomeningitis virus (LCMV) using the Armstrong (Arm) strain (acute infection) or clone-13 (chronic infection). Virus specific CD8 T cells removed from the chronic infection at the effector phase (day 8 post-infection) and transferred into antigen free hosts recovered many, but not all, aspects of functional memory differentiation. If virus specific CD8 T cells were removed at day 15 or day 30 post-infection (p.i.) and transferred into an antigen free environment, however, these cells failed to regain normal memory differentiation. Conversely, cells primed in an acute environment were transferred into chronically infected hosts. When transferred from the effector stage of acute infection, CD8 T cells showed some signs of dysfunction in the chronically infected host, though not complete exhaustion. We next examined the early time points after either Arm or clone-13 infection to determine when differences in the CD8 T cell response begin. Activation and inhibitory receptor expression varied at day one p.i. and differences persisted until day four p.i. when the differentiation state of antigen-specific cells was similar between infections. Together these findings suggest that dysfunction due to chronic infection could begin with the initial response, but the exhausted differentiation pathway becomes progressively more permanent over time.

P2

Role of flotillins in uropod formation and T cell adhesion

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T cells change morphology in response to extracellular stimuli to form a protruding edge and a tail-like structure known as a uropod. These polarized cell structures have unique protein compositions, and segregation of their component proteins is necessary for proper T cell function. Proteins enriched in the uropod overlap with those enriched in the Distal Pole Complex (DPC), a protein complex formed distal to the site of TCR

engagement during T cell activation by antigen presenting cells. Ezrin and moesin are actin-binding proteins of the ERM family that organize cytoplasmic and transmembrane proteins to specific cortical membrane domains. Ezrin and moesin are enriched in both the DPC and the uropod, and were thought to be primary organizers of these structures. However, a recent publication by Rossy et al. (PLoS One, 2009 4:e5403) showed that the lipid-raft associated proteins flotillin 1 and 2 precede ezrin and moesin localization to the uropod and interact with an ERM binding protein, PSGL-1. We are therefore testing the hypothesis that flotillins are the primary organizers of the uropod, recruiting PSGL-1 and ERM proteins, which in turn recruit other DPC components. In addition, we are testing the function of flotillins and ERM proteins in controlling integrin-dependent T cell adhesion. Elucidating the role of flotillins may give insights into the fundamental mechanisms that control T cell polarity, as well as T cell migration and adhesion to the extracellular matrix.

P3

T lymphoid potential found in a subset of myeloid progenitors

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Common myeloid progenitors (CMPs) were first described as clonogenic progenitors for erythroid and myeloid cell lineages (Akashi, et al, *Nature*, 2000). However, we find that when co-cultured on OP9 stromal cells expressing Delta-like Notch ligands, populations of CMPs readily give rise to bona fide T cells in vitro, although less efficiently than conventional T cell progenitor populations. Limiting dilution assays established the precursor frequency in CMPs to be 1/28. This frequency is greatly increased when CMPs are transduced with ICN1. In addition, iv transfer of ICN1-transduced CMPs ultimately leads to T-cell acute lymphoblastic leukemia (T-ALL) in recipient mice. Not only do these results raise the possibility that myeloid progenitors could be the cellular origin in the pathogenesis of Notch1-meidated T-ALL, they also indicate that strong Notch1 signaling can impose the T cell fate on some myeloid progenitors, and suggest heterogeneity within CMPs. Indeed, "CMPs" can be further divided into subgroups, based on expression of CD150 that identifies myeloid (CD150⁻) and erythroid (CD150⁺) committed subsets (Pronk et al., *Cell Stem Cell*, 2007) and tyrosine kinase receptor Flt3. In agreement with a recent publication we find that the T potential of "CMPs" resides exclusively within the Flt3⁺CD150⁻ subset. We are currently further characterizing the lymphoid lineage potentials of the CD150⁻ myeloid progenitors, and pursuing possible mechanisms that result in loss of T and B lineage potential in myeloid differentiation.

P4

Asymmetric Proteasome Segregation and Unequal Inheritance of a Fate Determinant During Cell Division

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Development and homeostasis of multicellular organisms often necessitates sibling cells to adopt different fates. Two identically-born daughter cells can be made different through their subsequent encounters with extrinsic signals. By contrast, asymmetric cell division produces two daughter cells that have inherited an unequal share of fate-

determining molecules, making them different from their initiation. We previously offered evidence that a T lymphocyte dividing in response to a microbial challenge segregates fate determinants unequally between its daughter cells. One daughter cell appears to give rise to differentiated cells that provide acute function while the other daughter cell has stem cell-like characteristics. We now report a novel mechanism whereby the two daughter cells become differentially fated toward terminal differentiation versus self-renewal. A key transcriptional regulator that acts as a molecular switch between two opposing cell fates was found to be unequally inherited by daughter cells. Unexpectedly, the inequality in transmission of this key transcription factor appears to depend on segregation of proteasome to one pole of the dividing cell and localized degradation of the cell fate determinant as the cell is preparing to divide. We also found that asymmetry of the proteasome occurs during asymmetric cell division of the early *C. elegans* embryo, suggesting this may be an evolutionarily conserved mechanism. These results suggest that unequal destruction may provide a mechanism to generate disparity in the fate and function of the progeny of a stem cell.

P5

Increased Tbet corresponds with increase in senescence marker CD57 in influenza virus-specific CD8+ T cells in aged individuals

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Lack of protective immune response in aged individuals leads to significant mortality during yearly influenza epidemics. Understanding the underlying cause of immune dysfunction is essential to our knowledge of protective immunological memory and vaccine design. Trying to understand reasons for poor immunity to influenza virus in aged individuals despite repetitive exposure to influenza virus or vaccine we examined antigen-specific T cells from young of elderly humans using multiparameter flow cytometry. Although only minor differences were observed in the magnitude of influenza-specific CD4 or CD8 T cells responses in young and elderly individuals many phenotypic differences were observed. The transcription factor Tbet has recently been shown to regulate memory CD8 T cell responses and high expression of Tbet promotes the generation of senescent, terminally differentiated antiviral CD8 T cells. In elderly humans, increased Tbet was observed in both total CD8+ T cells and influenza-specific CD8+ T cells compared to influenza virus-specific CD8 T cells from young individuals. This increase in Tbet corresponded to an increased in the frequency of influenza-specific CD8+ T cells in aged individuals expressing the senescence associated molecule CD57 as compared to young. In addition to Tbet and CD57, the inhibitory receptors PD-1, 2B4, and Lag3 were all increased on total CD8+ T cells from aged individuals. However influenza-specific CD8+ T cells showed increases in 2B4 and Lag3, but decreases in PD-1 and CD160. The relationship between these inhibitory receptors, Tbet and CD57 is being examined. This data demonstrates distinct differences in influenza-specific CD8+ T cells in aged individuals and problems associated with optimal antiviral immune responses in the elderly could include senescence and altered expression of inhibitory receptors. These studies also identify potential targets for further study into the disparity in protection from severe influenza infection in aged individuals.

P6

A requirement for Eomesodermin in the development of natural killer cells

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Natural killer (NK) cells play a critical role in the rejection of viruses, tumors, and transplants. NK cell precursors develop from hematopoietic stem cells in the bone marrow and mature through distinct stages classified by acquisition of a battery of well-defined surface markers. The transcription factors that regulate development through these stages, however, remain to be elucidated. Eomesodermin (Eomes) is a T-box transcription factor whose expression is characteristic of lymphocytes, such as CD8 T cells and NK cells, that effect cell-mediated immunity. We now demonstrate that Eomes acts as a critical regulator of NK cell development. In mice with a hematopoietic-specific deletion of Eomes, maturing NK cells fail to express surface integrins known to mark the final stages of maturation in the bone marrow. They do possess a normal complement of inhibitory members of the Ly49 family of receptors involved in conveying self-tolerance. However, Eomes-deficient NK cells do not express the activating members of the Ly49 family, known to promote pro-inflammatory cytokine release and resistance against viral infection. Thus, Eomesodermin may coordinate a transcriptional program required for development of an optimally reactive and armed NK cell repertoire.

P7

Identifying and Characterizing Novel IKK_α complexes.

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The NF- κ B family of transcription factors includes five members, which homo- and hetero-dimerize to differentially regulate gene transcription. An immense amount of work has focused on understanding the mechanisms that control the classical NEMO- and IKK_α-dependent pathway of NF- κ B activation. An alternative non-canonical NEMO-independent pathway is less well understood, but is dependent on IKK_α alone; however, biochemical evidence supporting the existence of an IKK_α alone complex has yet to emerge. We therefore aim to biochemically characterize the IKK_α-alone complex and determine how this novel IKK species regulates non-canonical NF- κ B signaling. To accomplish this we have generated a Mouse Embryonic Fibroblast (MEF) cell line expressing a FLAG-tagged version of IKK_α. Introducing FLAG-IKK_α into IKK_α^{-/-} MEFs rescues both classical and non-canonical NF- κ B signaling, as evidenced by I κ B degradation and p100 processing into p52, respectively. Consistent with our previous studies, FLAG-IKK_α also rescues classical NF- κ B transcriptional activity in response to IL-1 and TNF α . The FLAG-tagged IKK_α immunoprecipitates IKK β and NEMO under basal conditions, and also traffics to the nucleus as documented with endogenous IKK_α. Fast protein liquid chromatography was used to separate complexes from reconstituted cells based on their molecular weight, and we have identified a complex containing IKK_α that is independent of NEMO and IKK β . We therefore aim to use these cells to partially purify and then isolate this IKK_α alone complex from fractions using anti-FLAG immunoprecipitation. This will allow us to identify novel IKK_α-associated proteins and further characterize the role of IKK_α alone complexes in regulating non-canonical as well as classical NF- κ B signaling.

P8

Characteristics of Polyfunctional Human CD8 T cells

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It has been shown that CD8 T cell polyfunctionality correlates with better immune protection during viral infection. However, the factors necessary to give rise to polyfunctional T cells have not been defined. Here we have examined the functional and

phenotypic properties of a polyfunctional human CD8 T cell population in two donors, specific for EBV and CMV. In both donors, polyfunctionality was defined by the ability of virus-specific T cells to produce IL-2, TNF, IFN γ and degranulate upon stimulation. EBV-specific cells were oligoclonal suggesting that polyfunctionality may not be restricted to a single clonal population within the total antigen-specific response. In the EBV-specific population, the responding cells were CD27^{low} CD45RO⁺ indicating an effector memory-like phenotype, whereas, the CMV-specific cells were CD27^{low}-CD45RO⁺ indicating an effector phenotype. Thus, neither clonality nor memory phenotype are predictive of the capacity to maintain polyfunctional properties, suggesting that other factors may define polyfunctional capability. Future studies will examine expression of several transcription factors associated with differential functional outcomes, including T-bet, eomesodermin, ROR γ t, etc. through transcriptional arrays and Quantigene analysis of EBV and CMV-specific T cell responses.

P9

Aged B cells compete more effectively for survival *in vivo* and accumulate a unique B cell subset.

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B cell generation and turnover rates change with age, altering homeostatic behaviour in mature peripheral populations. Consistent with these shifting homeostatic demands, follicular (FO) B cells in aged mice turn over more slowly than those in young mice. Why and how the aged B cells have longer lifespan remains unknown. Mounting evidence indicates that mature B cells compete for viability promoting resources to survive. Thus, age associated changes in turnover may indicate that B cells in aged mice represent a highly selected pool of cells with exceptional ability to compete for these resources. We have directly tested this idea by determining the competitive survival capacities of B cells from aged versus young mice. One week after adoptive transfer into replete recipients, we found more aged B cells survive than B cells from young donors. However, the numbers of aged and young donor B cells were similar one month after transfer. Interestingly, one month after transfer, we observed the expansion of an unusual B cell population – CD21^{int}/35^{int}CD23^{int}CD43^{int}AA4.1^{int}CD19⁺B220⁺. Analyses of intact C57BL/6 mice revealed that the pool of cells corresponding to this population gradually increases with age, expanding 5-fold by 22 months of age. These unusual mature B cells respond poorly to BCR, TLR and CD40 mediated stimulation, and display markedly less reliance on the survival factor BlyS. Together these findings reveal that primary B cell pools in aged individuals are enriched for highly fit competitors, and that a unique population with the characteristics of “exhausted” B cells accumulate with age.

P10

c-Rel is required for optimal development of a CD8⁺ effector T cell response to *Toxoplasma gondii*

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c-Rel is a member of the NF- κ B family of transcription factors, which are important in the regulation of innate and adaptive immunity. There remains an incomplete understanding of the role played by c-Rel in lymphocyte activation and effector function. We have used a replication-deficient strain of the obligate intracellular parasite *Toxoplasma gondii* to study the adaptive immune response in WT mice and mice deficient in the NF- κ B family member c-Rel. CD8⁺ T cells that produce the cytokine IFN- γ are critical in the immune response to *T. gondii*. Infection with CPS-OVA results in the expansion of endogenous

CD8⁺ T cells specific for an immunodominant epitope in ovalbumin that can be detected through the use of an H₂Kb-SIINFEKL OTI tetramer in both WT and c-Rel^{-/-} mice. While WT mice generated high numbers of these cells in the spleen and the peritoneal cavity, c-Rel^{-/-} mice generated significantly lower numbers of these cells 8 days following immunization, though the generation of OTI-specific cells could be rescued in the c-Rel^{-/-} mice through administration of IL-12p70. Further, at acute time-points the phenotype of these cells is largely similar to WT antigen-specific cells, with the exception of the NK cell activation marker KLRG1. Interestingly, at late time-points, a similar frequency of antigen-specific cells was found in WT and c-Rel^{-/-} mice. Two months after immunization, antigen-specific cells from c-Rel^{-/-} mice expressed higher levels of the IL-7Ra, a cytokine receptor that is important for the survival of memory CD8⁺ T cells. When immune WT and c-Rel^{-/-} mice were rechallenged with a high dose of CPS-OVA, the antigen-specific cells in c-Rel^{-/-} mice expanded more robustly than in WT mice. These results suggest that c-Rel is not required for the maintenance of an antigen-specific population or acquisition of a memory phenotype.

P11

A Critical Role for T-bet in Preserving CD8 T Cell Function during Chronic Viral Infection

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The T-box transcription factor, T-bet, regulates specific genes that are integral to CD8 T cell effector function such as interferon- γ , perforin, and granzyme B. In addition, T-bet has a more general role in specifying CD8 T cell differentiation fates during systemic, acute viral infections. We studied the role of T-bet during chronic infection in mice using the Clone-13 strain of lymphocytic choriomeningitis virus (LCMV). The expression of T-bet was inversely correlated with CD8 T cell exhaustion: subsets of cells expressing higher levels of the inhibitory receptor, PD-1, expressed noticeably less T-bet. Reducing T-bet expression by genetically ablating one allele of the T-bet gene, *tbx21*, favored the formation of more exhausted, PD-1^{hi} antigen-specific CD8 T cells and resulted in higher viral burden. When both *tbx21* alleles were ablated, mice exhibited sustained viremia and lost all antigen-specific CD8 T cells within 2 weeks following Clone-13 infection. Experiments with mixed chimeras demonstrated that these effects of T-bet on CD8 T cell differentiation are cell-intrinsic and independent of viral/antigen load. Also, ectopic overexpression of T-bet *in vitro* demonstrated that T-bet could directly repress PD-1 expression. Thus, T-bet appears to play a complex role during CD8 T cell differentiation, depending on the nature of the infection. During systemic, chronic viral infection, the loss of T-bet is a contributing factor that leads to exhaustion, in part through its regulation of PD-1.

P12

Requirement of Notch1 transcriptional activation domain in leukemogenesis and development

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Notch1 is a highly conserved transmembrane protein that regulates development and cell fate decisions in multicellular organisms. Following interaction with a Notch ligand, the intracellular portion of the Notch1 receptor (ICN1) is proteolytically cleaved and translocates to the nucleus to mediate transcription of Notch target genes. In addition to

mediating normal development, pathophysiologic Notch1 signaling has been implicated in T-cell lymphoblastic leukemias and lymphomas (T-ALL). ICN1 is composed of many functional domains. The N-terminus of Notch1 contains the RAM and ANK domains, both of which have been shown to be important for canonical Notch signaling. The C-terminus of ICN1 contains a PEST domain and a transcriptional activation domain, which has not been extensively characterized. Previous data from our lab has demonstrated that the Notch1 transcriptional activation domain (TAD) is essential for induction of T-cell leukemia by Notch1 in a bone marrow transplant model. To better understand the biological significance of the Notch1 TAD, our lab has generated a constitutive Notch1 TAD knockout mouse. In contrast to constitutive deletion of Notch1, mice lacking the Notch1 TAD survive until birth. Deletion of the Notch1 TAD does, however, result in early neonatal lethality that is associated with cardiac defects. Further investigation should yield important insight into the role of the Notch1 TAD in cardiac and T cell development.

P13

Isolation and characterization of thymic endothelial cells

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T cells are continuously produced throughout adult life. The thymus does not contain self-renewing stem cells, thereby requiring the importation of bone marrow-derived progenitors from the blood. The thymic endothelium expresses P-selectin, and this expression is important in the process of thymic settling. It is unknown whether P-selectin expression is unique to thymic endothelial cells, or whether it is ubiquitous among endothelial cell populations. Further, whether intrathymic mechanisms control expression of P-selectin on thymic endothelium remains unclear. By modifying existing endothelial cell isolation protocols, we consistently identified a distinct population of CD45⁻CD31⁺CD105⁺ thymic endothelial cells by flow cytometry. As previously shown, we confirmed that a subset of thymic endothelial cells expressed P-selectin; further, we established that P-selectin is not expressed by control lung endothelium. The fraction of P-selectin⁺ thymic endothelium varied among different mutant strains of mice, with the P-selectin⁺ fraction of thymic endothelium varying inversely with the number of early thymic progenitors. These data are consistent with a model in which thymic endothelial P-selectin expression is regulated by the number of intrathymic T cell progenitors in a feedback loop that maintains thymic homeostasis.

P14

SLP-76 is required for optimal NK cell activation and shaping of the Ly49 receptor repertoire

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Natural killer (NK) cells are innate immune cells that provide a critical line of defense against intracellular pathogens and tumors by displaying cytotoxicity and producing immune-activating cytokines. The proximal signaling pathways that lead to NK effector function are incompletely understood. The adaptor molecule SLP-76 is important in mediating signals in a variety of hematopoietic cell types. Thus, we examined whether SLP-76 is important in NK cell signaling downstream of the Ly49D activating receptor. SLP-76 was phosphorylated and clustered at the plasma membrane following Ly49D stimulation, suggesting that SLP-76 is activated downstream of the Ly49D receptor. SLP-76 was required for optimal signal transduction through Ly49D as NK cells from SLP-76-deficient mice exhibited diminished ERK and Akt phosphorylation compared to WT mice. This correlated with decreased IFN γ production and cytotoxicity by SLP-76-

deficient NK cells. NK cells from SLP-76-deficient mice appeared developmentally mature, however Ly49 family member inhibitory and activating receptors were expressed on a significantly lower proportion of NK cells from SLP-76-deficient compared to WT mice. Moreover, this selective developmental defect was NK cell-autonomous as WT/SLP-76 mixed bone marrow chimeras failed to correct the defect. However, this defect was not observed when SLP-76 was inducibly deleted in NK cells after full maturation. Despite normal development, these SLP-76-deficient NK cells still displayed defective IFN γ production and cytotoxicity. SLP-76 is vital for Ly49D-mediated NK cell signaling and in shaping of the NK cell Ly49 repertoire during development. These results demonstrate a critical role of SLP-76 in NK cell tolerance and activation.

P15

Skin commensal microflora regulate cutaneous immunity.

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Commensal microflora densely populate epithelial surfaces and play a crucial role in host protection. While the contribution of microbiota to intestinal immunity is the focus of several ongoing investigations, the contribution of skin resident microorganisms to cutaneous immunity has not yet been explored. To address this, we compared the immune cell composition of skin tissue between conventionally raised and germ free mice at steady state. In contrast to conventionally reared mice, germ free mice had reduced frequencies of gd T cells and increased frequencies of CD4⁺ Foxp3⁺ regulatory T cells. Further, IL-17A production by various immune cell populations was dramatically reduced. These data suggest that skin microflora or microbial byproducts influence cutaneous immune homeostasis. To extend these findings to the development and maintenance of cutaneous immune responses, we topically treated mice with antibiotics to alter skin flora and infected with the protozoan parasite *Leishmania major*. Skin tissue of topically antibiotic treated mice had increased frequencies of CD4⁺ Foxp3⁺ regulatory T cells and decreased frequencies of IFN γ ⁺ T effectors, indicating impaired immunity to *Leishmania major*. Consistent with this, treated mice had increased lesion size and parasite load. In contrast, orally antibiotic treated mice, which have reduced gut flora, did not exhibit diminished immune responses to *Leishmania major* or impaired parasite clearance. These data suggest that skin commensal flora act as natural adjuvants that are critical for mounting protective immune responses in the skin. Additionally, our observations offer new insights into the influence of distinct microbial niches on compartmentalization of tissue immunity.

P16

Cytokine production in CD8⁺ T cells deficient in DGKa or DGKz

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Diacylglycerol kinases (DGKs) function as a key control point of T cell receptor (TCR) signal transduction via the phosphorylation of diacylglycerol (DAG), which abrogates DAG-mediated ras signal transduction. Two isoforms of DGK are present in T cells, DGKa and DGKz. CD4⁺ T cells deficient in either of these kinases have been previously found to demonstrate an increased capacity for proliferation upon stimulation of the TCR, and a decreased requirement for co-stimulation in undergoing activation. The role of DGK deficiency in CD8⁺ T cells has been less thoroughly investigated. We evaluated the stimulative potential of CD8⁺ T cells from mice with germline deletions of DGKz or DGKa. We found that, in comparison with wild-type CD8⁺ T cells, CD8⁺ T cells deficient in DGKz and to a lesser extent DGKa, produce increased amounts of IFN γ and IL2 in

response to TCR stimulation, or stimulation with PMA and Ionomycin. Additionally, we found that DGKz-deficient TCR transgenic CD8+ T cells increase cytokine production in response to agonist peptide or IL2 alone, even in cells that express low levels of the activation marker CD44. These findings demonstrate an important role for DGKs in the regulation of cytokine production in CD8+ T cells. Future work will attempt to establish the biochemical events that account for our observations.

P17

The Immune Response to *Toxoplasma gondii* Regulates Regulatory T cells

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Regulatory CD4 T cells (Treg) are required to prevent autoimmunity and promote self-tolerance under homeostatic conditions. Treg cells also play a role in shaping the adaptive immune response during infection and there are examples where the ability of Tregs to dampen effector responses help to limit immune pathology, and/or promote pathogen survival. However, little is known about the homeostasis of these populations during infection. In the present study, oral infection with *T. gondii*, resulted in a profound depletion of Tregs. In order to investigate the mechanism of this change in the Treg population, we examined Treg survival and proliferation following challenge. These studies revealed that infection led to increased BrdU incorporation and Ki67 expression in Tregs suggesting that the loss of these cells was not due to a decrease in Treg proliferation. Transfer studies also indicated that this was not due to an infection-induced conversion of Tregs. Moreover, Tregs in the lamina propria, and Peyer's patches have decreased levels of the anti-apoptotic molecule Bcl-2 and increased levels of apoptosis based on Annexin V binding and live/dead staining compared to non-infected animals. Taken together, these findings suggest that the depletion of Treg cells during toxoplasmosis is due to increased apoptosis of these cells. We have shown that IL-6 and IL-27, cytokines induced during this infection, can limit the *in vitro* differentiation of adaptive Tregs and that IL-27 can also negatively regulate the production of IL-2, a key survival factor for Treg cells. These findings led to the idea that that infection induced changes in the levels of cytokines such as IL-2, IL-6, and IL-27 contribute to the cumulative loss of Tregs during infection. Indeed, natural and adaptive Tregs can respond to both IL-6 and IL-27 as measured by pSTAT1 and pSTAT3 staining. Additionally, preliminary data suggests there is enhanced survival of Tregs in the absence of IL-6 or IL-27 signals during infection. Thus, we propose that infection with *T. gondii* could potentially block both natural Treg survival and adaptive Treg differentiation and this may be an important step in allowing the development of a potent protective T cell response.

P18

Effect of TCR specificity on the *in vivo* function of CD4⁺CD25⁺ regulatory T cells in an autoimmune setting

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We are studying how specificity for self peptides affects the ability of CD4⁺CD25⁺ regulatory T cells (T_{regs}) to regulate autoimmunity. To address this we are using transgenic mice that coexpress influenza virus hemagglutinin (HA) targeted to antigen presenting cells (APCs) and a transgenic TCR (TS1) with high reactivity for the HA. Approximately 80 percent of TS1xHACII develop spontaneous autoimmune arthritis by 12 weeks of age. When on a RAG^{-/-} background, these mice still develop arthritis, identifying the HA-peptide recognized by the transgenic TCR as the target antigen that drives disease. It is also notable that in intact animals, arthritis develops despite the

presence of Foxp3⁺CD4⁺CD25⁺ T cells, including a population that is HA-specific. To further understand T_{reg} function in this system, we sorted CD4⁺CD25⁺ T cells from different sources and transferred the cells into pre-arthritic mice. We have found that polyclonal T_{reg} populations from BALB/c or HAcII mice are able to prevent arthritis in TS1xHAcII mice. Remarkably, T_{regs} from TS1xHA28 mice, which are enriched in specificity for the target antigen that drives disease, fail to prevent arthritis. However, these T_{regs} can suppress an influenza antibody response, indicating that they are functional in other *in vivo* settings. Ongoing studies suggest that potent antigenic stimulation by HA-expressing APCs induces downregulation of Foxp3 in HA-specific T_{regs}, which may account for a loss of function and/or conversion of these cells into a pathogenic population.

P19

The role of BLyS in the activation and survival of potentially pathogenic rheumatoid factor producing B cell

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Rheumatoid factor (RF) autoantibodies are associated with Systemic Lupus Erythematosus and other humoral autoimmune disorders. Previous research using AM14 mice, which have a transgene encoding a B cell antigen receptor (BCR) typical of pathogenic RF producing B cells, has demonstrated that these cells are induced to proliferate by chromatin immune complexes. This work revealed a combined requisite for BCR and Toll-like receptor (TLR) signaling in the onset of RF production. Although these studies revealed a key mechanistic link between common autoimmune BCR specificities and TLRs, they focused on induced proliferation rather than cell survival or differentiation. In collaborative studies with the Marshak-Rothstein laboratory, we have recently found that although AM14 B cells stimulated with chromatin immune complexes indeed divide, they die rapidly after proliferating. This cell death seems specific to stimulation with the immune complex, as the majority of cell death after stimulation with anti-IgM or CPG occurs in the undivided, rather than activated cells. Moreover, we have found that addition of the survival cytokine BLyS (B lymphocyte stimulator) rescues survival of AM14 B cells proliferating in response to chromatin immune complexes. We believe our observations may link the immune complex mediated activation of RF-producing B cells with the finding that elevated BLyS levels are often seen in SLE.

P20

MHC class II-dependent effector synapses between intestinal epithelial cells and CD4⁺ T cells regulate infection-induced intestinal inflammation

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Despite recent advances, the regulation of immune responses at the site of inflammation remains incompletely understood. This regulation is critical at mucosal surfaces, such as the intestine, in order to maintain the epithelial barrier. Previous studies have shown that intestinal epithelial cells (IECs) play an active role in antigen sampling and possess the molecular machinery required for antigen processing and presentation via MHC class II. However, the functional consequences of antigen presentation by IEC are unknown. Here, we generate mice with a lineage-specific deletion of MHC class II within IECs (*iab*^{-IEC}) to directly interrogate the functional consequences of IEC-intrinsic MHC class II expression on both tolerance and immunity within the gastrointestinal tract. While dispensable for the generation or maintenance of oral tolerance, MHC class II expression by IECs appears to be required to limit inflammation in a model of acute

colitis using infection with the enteric pathogen *Citrobacter rodentium*. Following infection with *Citrobacter*, *iab*^{IEC} mice exhibit increased production of IFN- γ , TNF- α and IL-17 and enhanced intestinal inflammation compared to their littermate controls. *in vitro*, IECs inhibited proliferation and cytokine production in activated CD4⁺ T cells in a contact-dependent manner. Taken together, these results demonstrate a previously unrecognized role for cognate IEC-CD4⁺ T cell interactions in limiting infection-induced intestinal inflammation.

P21

Factors Prompting the Development of Autoimmunity in Genetically Susceptible Mice

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Environmental factors such as infectious agents have been implicated as a possible trigger for the development of autoimmune diseases due to the observation that genetics alone do not predict susceptibility. Other possible factors important in dictating disease development may be stochastic differences between individuals (e.g. immune repertoire formation). To investigate these possible autoimmune susceptibility factors, our lab utilizes a spontaneous, low penetrance mouse model of autoimmune arthritis. In this model, interactions between hemagglutinin (HA)-expressing antigen presenting cells (APCs) and cross-reactive CD4⁺ T cells that recognize the S1 peptide of HA results in ~30% of the genetically susceptible mice developing arthritis. To test the possibility that the cascade of events following a viral infection (e.g. APC activation, induction of inflammatory cytokines) can increase the penetrance or severity of arthritis, these genetically susceptible mice were locally injected with influenza virus (which express HA) and tracked longitudinally. Moreover, we have examined these immunized mice for changes in the percentages and characteristics of certain cellular subsets (such as Th lineages, Tregs and APCs) and levels of inflammatory cytokines to determine if these changes correlate with the presentation of disease. These studies aim to elucidate the mechanisms that can dictate the development of autoimmune arthritis among genetically susceptible individuals.

P22

miRNAs in B cell development

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microRNAs (miRNAs) are small (20-22nt), non-protein coding RNAs that regulate gene expression by blocking translation and/or mRNA degradation. Experimental evidence supports a role for miRNAs in cancer and metastasis, and more specifically in the regulation of many biological processes including cell survival, proliferation and apoptosis. miRNAs also function in hematopoiesis and in the development of immune responses. In B cells, miRNAs regulate genes involved in the pro to pre B cell transition and also function in germinal center reactions. However, the importance of miRNAs in transitional, marginal zone and mature B cells has not been studied. We have sought to define and understand the importance of miRNA-mediated regulation of gene expression in these cells. miRNA profiles have demonstrated that transitional B cells express higher levels of several miRNAs, including miR-322, miR-351, miR-363 and miR-503. In order to identify genes regulated by these differentially expressed miRNAs, we are comparing gene expression patterns in these B cell subsets with computationally predicted miRNA targets. We will then experimentally validate the miRNA-mediated repression of gene

expression and examine the functional significance of this regulation. Our findings will contribute to understanding mechanisms regulating B cell development and immune homeostasis.

P23

Investigating the functional biology of IL-17E

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Recent studies have demonstrated key functions of interleukin (IL) -17E in Th2 cytokine-mediated host protective immunity and allergic airway inflammation, however the cellular mechanisms through which IL-17E drives such responses remains unclear. Here we show that IL-17E promotes the accumulation of a lineage negative (Lin^{neg}) cell population defined by expression of Sca-1 and intermediate expression of c-kit (c-kit^{int}) in the gut-associated lymphoid tissue (GALT). Further, utilizing IL-4/eGFP reporter mice, both a GFP^{neg} and a GFP^{pos} population were observed. While the c-kit^{int}-GFP^{pos} cells were capable of differentiating into mast cells, the IL-17E-elicited c-kit^{int}-GFP^{neg} cell population exhibited multi-potent capacity, giving rise to cells of monocyte/macrophage or granulocyte lineages. Progeny from c-kit^{int}-GFP^{neg}, but not c-kit^{int}-GFP^{pos}, cells were competent antigen presenting cells that could promote CD4⁺ Th2 cell differentiation. The ability of IL-17E to induce the emergence of a MPP^{type2} cell population identifies a link between the IL-17 cytokine family and peripheral hematopoiesis and suggests a previously unrecognized innate immune pathway that promotes Th2 cytokine responses at mucosal sites.

P24

Regulation of lytic granule-associated myosin IIA in NK cells during activation for cytotoxicity

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NK cells kill infected or transformed target cells via formation of an actin-rich immunological synapse (IS) and directed secretion of lytic granules containing granzymes and perforin. Following polarization of lytic granules, they must traverse the actin-rich IS and fuse with the NK cell membrane in order for directed secretion of their contents to occur. We have recently defined a critical role for lytic granule-associated myosin IIA in enabling the interaction of granules with actin and their transit through the actin-rich IS to the synaptic membrane. In order to determine how myosin IIA is regulated in NK cells, we examined the phosphorylation of myosin IIA in resting and activated NK cells. Myosin IIA is phosphorylated in resting NK cells, and this phosphorylation decreases upon activation. This pattern is altered by a truncation mutation affecting the C-terminus of myosin IIA. Further studies will define the regulation of granule-associated myosin IIA specifically, as well as the functional significance of this regulation.

P25

In the absence of SOCS3, IL-6 blocks protective immunity to *T. gondii*

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The IL-6/gp130 family of cytokines is composed of cytokines, including IL-6 and IL-27, that use gp130 as part of their receptor complex and play important roles in resistance to many pathogens. Signaling through gp130 activates the Jak/STAT pathway to mediate downstream transcriptional effects as well as the Suppressor of Cytokine Signaling 3 (SOCS3) which limits gp130 signaling by directly binding to the receptor. GP130 Y757F mice express a mutant form of this receptor, which is unable to interact with SOCS3 and show sustained activation of STAT1 and STAT3 in response to gp130 signals. Gp130 Y757F mice infected with *Toxoplasma* are acutely susceptible to infection due to high parasite burdens and decreased production of IFN γ by NK cells and IL-12 by dendritic cells and macrophages *in vivo*. *In vitro*, accessory cell populations from these mice produce less IL-12 and cannot control parasite replication, and IL-6 exacerbates both these phenotypes. Administration of IL-6 blocking antibodies during infection restores production of IL-12 and results in decreased parasite burdens. Collectively, these studies demonstrate that control of gp130-mediated signaling is critical in resistance to *Toxoplasma gondii* and highlight the importance of appropriate regulation of the anti-inflammatory properties of IL-6.

P26

Investigating the Role of Eosinophils in Intestinal Immune Homeostasis

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Eosinophils are multifunctional granulocytes capable of secreting a wide array of cytokines allowing them to modulate the regulatory environment of tissues they occupy. In steady state conditions eosinophils are particularly abundant in lamina propria (Lp) of the intestinal tract. Thus, we sought to examine the role of eosinophils in intestinal immune homeostasis. In naïve mice we found that eosinophils compose a significant proportion of total cellularity in the Lp (>15%) and are distributed throughout the Lp with an increased density in the villi of the small intestine. Eosinophils expressed MHCII at intermediate levels when isolated from the mesenteric lymph nodes and Spleen, but no MHCII expression was detected in eosinophils isolated from the small intestine Lp (siLp). We then examined cells isolated from the Lp in Δ dblGATA mice lacking eosinophils. In the absence of eosinophils there was a decreased proportion of both ROR γ t expressing and IL-17A producing CD4⁺ T cells and a concomitant increase in IFN- γ producing CD4⁺ T cells from the siLp. Additionally, there was a dramatic reduction of CD103 expression among CD4⁺ T-cells in the siLp of Δ dblGATA mice. These data suggest a potential disruption of TGF- β signaling in the absence of eosinophils. All together, our results suggest that eosinophils may play an important role in modulating the intestinal immune environment and may contribute to intestinal immune homeostasis.

P27

The Expression, Function and Regulation of P-selectin and its ligand PSGL1

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T cells develop in the thymus from bone marrow (BM)-derived hematopoietic progenitors that settle the thymus via the blood. The molecular basis for progenitor entry into the thymus is poorly understood, but p-selectin and P-selectin glycoprotein ligand 1 (PSGL1) are suggested to be involved. We investigated the expression of PSGL1 on progenitors in bone marrow. Our preliminary results demonstrate that PSGL1 expression is low on hematopoietic stem cells and downstream multipotential progenitors, but becomes highly expressed on lymphoid-primed multipotent progenitor (LMPP) in the BM. These data

indicate that P selectin binding is developmentally regulated. Using competitive thymus reconstitution assays we confirmed that PSGL1 is a functionally important component of the thymic homing process. Our current results are focused on understanding the mechanism by which PSGL1 is selectively upregulated on thymic homing progenitors.

P28

Specific and bystander memory B cell and antibody responses to intense seasonal *Plasmodium falciparum* malaria

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In contrast to the host immune response to many other pathogens, immunity to malaria is slow to develop. As humoral responses are known to be critical to blood stage immunity, we conducted a one-year longitudinal cohort study investigating the mechanisms underlying the acquisition and maintenance of MBC and Ab to two vaccine-candidate blood stage antigens, AMA1 and MSP1. In cross-sectional analysis prior to the start of the transmission season, AMA1 and MSP1 specific MBC and Ab increased with age and cumulative exposure, both in magnitude and number of individuals positive. AMA1 and MSP1 specific MBC and Ab significantly increased during convalescence after the first episode of malaria for each child, then fell to just above the baseline by one year. Thus MBC to *Pf* are generated at a slow rate with incremental increases each malaria season, but seem to be maintained normally. Addressing the longstanding question of bystander activation to acute malaria, tetanus-specific MBC do increase slightly but significantly at convalescence and this level is maintained 6 months later, lending evidence to the hypothesis of MBC maintenance via polyclonal activation. However, there was no increase in the tetanus-specific Ab levels, indicating that MBC and plasma cell replication are regulated independently and Ab is not secreted in response to non-specific stimulation, as might be predicted in the interest of efficiency of a targeted humoral response. Addressing the question of a possible mechanism for the delay in acquisition of *Pf*-specific MBC, we observed that individuals in our cohort study had a significantly expanded atypical MBC population previously described in untreated HIV patients. Numbers of atypical MBCs were higher in children with chronic asymptomatic *Pf* infections compared with uninfected children, suggesting that the chronic presence of the parasite may drive expansion of this population. Understanding the development of *Pf*-specific classical MBC and the origin and function of the atypical MBCs could be critical in designing an effective malaria vaccine.

P29

Continuous expression of SLP-76 is required for antigen specific CD8⁺ memory T cell generation but not for persistence

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Signal transduction pathways initiated by the T cell receptor (TCR) and cytokine receptors are critical in T lymphocyte activation, differentiation and homeostasis. The requirement for tonic TCR signaling in development and naïve T cell maintenance is clear, but controversial in the memory T cell compartment. The SH2-domain-containing phosphoprotein of 76 kilodaltons (SLP-76) adaptor protein is critical for proximal pre-

TCR and TCR-generated signaling. To assess signaling requirements in memory formation and homeostasis, tonic and activating TCR signals are interrupted using temporally-mediated conditional deletion of SLP-76 following infection with lymphocytic choriomeningitis virus. The data are consistent with a model in which TCR-mediated signals are necessary for naïve T cell maintenance and normal contraction after infection, but are not required to mediate long-term memory T cell homeostasis.

P30

An improved M2e-targeted peptide vaccine confers protection against influenza virus infection

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The influenza virus A matrix protein 2 ectodomain (M2e) sequence has remained highly conserved among various human influenza A virus strains and is therefore a promising target for a protective vaccine. Based on previous work using a synthetic M2e-based multi-antigenic peptide vaccine (Mozdzanowska et al., Vaccine 2003; Virology Journal 2007), we generated a novel peptide and investigated its efficacy in inducing an anti-M2e antibody (Ab) response and its ability to confer protection against viral challenge.

The new peptide construct containing four M2e-peptide sequences coupled to T helper epitopes from the *Plasmodium falciparum* CS protein and the hepatitis B virus antigen was administered together with adjuvants intranasally and subcutaneously into mice as described (Mozdzanowska et al., Virology Journal 2007). In contrast to its predecessor peptide, we found that vaccination induced much higher anti-M2e serum Ab titers against peptide and native M2e. This correlated with a large number of M2-specific Ab-secreting cells in lungs and bone marrow. Moreover, the serum of vaccinated mice was also crossreactive against the influenza virus subtype A/FM (H1N1), which contains a variant M2e-sequence different in 3 amino acid (aa) positions. Importantly, this new peptide vaccine regimen showed significant protection with remarkably reduced viral titers in lungs of mice after challenge with influenza A strains X31 (H3N2), PR/8 (H1N1) and two mutant viruses, termed P10H and P10L (Zharikova et al., JVirol. 2005), with M2e-mutations at aa position 10 that are also found in recent H5, H7 and H9 virus strains. In conclusion, our studies show promising results towards the further development of a potential “universal” influenza vaccine with M2e as a target.