The Immunology Graduate Group gratefully acknowledges the financial support of all our contributors for the 21st Annual Retreat:

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The Immunology Graduate Group extends its special thanks to Wyeth Research for its sponsorship of the Best Oral Presentation and Best Poster Presentation Awards.

Cover Photo
Asymmetric cell division of a T lymphocyte. Three-dimensional confocal microscopic image of an activated CD8+ T cell undergoing cytokinesis. Anti-beta-tubulin staining is red, DNA staining is blue, and anti-PAR3 staining is green. Note asymmetric segregation of PAR3 into the daughter cells. Unequal inheritance of proteins between daughter T cells may contribute to essential fate diversity during the immune response. Image provided by Steve Reiner and John Chang.

21st Annual Immunology Retreat
Friday to Sunday, November 14-16, 2008
Willow Valley Resort and Conference Center
2416 Willow Street Pike, Lancaster, PA 17602-4898

Friday, November 14, 2008

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.

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
1:30-3:30	**Session I – Statesman Hall A/B/C/D**
1:30-2:45	**Part I: Hematopoiesis and Development** **Session Chair: Marisa Juntilla**
1:30-1:55	Marisa Juntilla, *Hematopoietic stem cells require Akt1 and Akt2 for long-term self-renewal*
1:55-2:20	Andrea Carpenter, *An epigenetic component to TCRb allelic exclusion*
2:20-2:45	Anthony Pajerowski, *Severe block in hematopoiesis in the absence of NKAP*
2:45-3:05	Break 1st and 2nd year students: Easel and posterboard set-up
3:05-4:45	**Part II: B Cell Development and Homeostasis** **Session Chair: Marco Calamito**
3:05-3:30	Marco Calamito, *Requirement for AKT1 and AKT2 in the development of marginal zone B-cells*
3:30-3:55	Jennifer Reed, *MicroRNA profiles in peripheral B cell subsets*
3:55-4:20	Daniel Northrup, *Pax5 induction of CD19 expression is EBF independent*
4:20-6:00	Willow Valley registration and room check-in, Main Lobby

Please set up posters for the remainder of the conference in Statesman C/D

5:30-7:00 **Dinner Smorgasbord, Terrace Dining Room**

7:00-10:00 **Session II, Statesman Hall A/B/C/D**
Session Chair: David Artis

7:00-8:00 Introduction to Keynote Speaker: Steve Reiner
Keynote Speaker, **Marc K. Jenkins, Ph.D.**
Distinguished McKnight University Professor
Department of Microbiology, and Center for Immunology
University of Minnesota
Tracking antigen-specific helper T cells after vaccination

8:00-8:20 Break

8:20-8:50 Cara Williams, Respiratory Diseases, Wyeth. Boston
Cytosolic Phospholipase A2 Antagonism in the treatment of respiratory disease: From Bench to Bedside

8:50-9:20 Gudrun Debes
Pathobiology, Vet School, University of Pennsylvania
Mechanisms of lymphocyte recirculation: T cell exit from peripheral tissues

9:20-9:50 Hui Hu
Immunology Program, The Wistar Institute
Essential roles of Foxp1 in the immune system

10:00-12:00 **Social, Statesman Hall A/B/C/D**

Saturday, November 15, 2008

8:00-9:00 AM **Breakfast Smorgasbord, Terrace Dining Room**

9:00-12:00 PM **Session III, Statesman Hall A/B/C/D**

9:00-10:15 **Part I: Signaling in the Immune System**
Session Chair: Jennifer Smith-Garvin

9:00-9:25 Jennifer Smith-Garvin, *Distinguishing functional and developmental requirements for SLP-76 phosphotyrosines*

9:25-9:50	John Chang, *Different by destruction: Unequal inheritance of the transcription factor T-bet as a mechanism to diversify daughter T cell fates*
9:50-10:15	Keri Sanborn, *Myosin IIA associates with NK cell lytic granules to enable their function at the immunological synapse*
10:15-10:25	Break
10:25-12:05	**Part II: Regulation and function of $CD8^+$ T cell responses to infection** **Session Chair: Haina Shin**
10:25-10:50	Haina Shin, *Role of blimp-1 in T cell dysfunction during chronic viral infection*
10:50-11:15	Kim Jordan, *C-Rel is required for optimal development of a CD8 T cell response to* Toxoplasma gondii
11:15-11:40	Charlly Kao, *Novel roles for T-bet in the maintenance and differentiation of antigen-specific CD8 T Cells during chronic viral infections*
11:40-12:05	Adam Hersberger, *HIV-specific CD8 T cells from elite controllers rapidly upregulate perforin after stimulation*
12:05-1:00 PM	**Deli Lunch, Terrace A/B "To-Go" boxes available**
12:05-2:30	**Free time to explore Lancaster area**
2:30-3:30	**Trainees meet the editors** **Doug Braaten, *Nature Immunology*** **Peter Lee, *Immunity*** **Heather Van Epps, *Journal of Experimental Medicine***
3:30-5:30	**Poster Session, Statesman Room C/D**
5:30-7:00	**Dinner Smorgasbord, Terrace Dining Room**
7:00-10:00 PM	**Session IV, Statesman Hall A/B**
7:00-8:15	**Part I: $CD4^+$ T cell regulation of immunity and inflammation** **Session Chair: Jonathan Silver**
7:00-7:25	Jonathan Silver, *IL-6 is required for the generation of T follicular helper cells during* Toxoplasma gondii *infection*

7:25-7:50	Jackie Perrigoue, *Delineating requirements for antigen presentation in the intestine*
7:50-8:15	Steven Saenz, *Investigating the functional biology of IL-25 during helminth infection*
8:15-8:30	Break
8:30-9:45	**Part II: Immuno-regulation** **Session Chair: Jason Hall**
8:30-8:55	Jason Hall, *Signaling via vitamin A mediates intestinal immune homeostasis and amplifies inflammatory responses following oral challenge*
8:55-9:20	Soyoung Oh, *Role of TCR specificity on in vivo function of $CD4^+CD25^+$ regulatory T cells in an autoimmune setting*
9:20-9:45	Betsy Taylor, *TSLP influences peripheral dendritic cell and T helper cell responses in murine models of intestinal infection and inflammatory bowel disease*
9:45-12:00 AM	**Party, Statesman Hall A/B/C/D**
10:00	**Announcement of Wyeth Awards for Best Oral Presentation and Best Poster**

Sunday, November 16, 2008

9:00-11:00 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

22nd Annual Immunology Graduate Group Retreat
November 6-8, 2009
Willow Valley Resort and Conference Center

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

Abstracts for Oral Presentations:

1. Marisa M. Juntilla, Vineet Patil, Rohan Joshi, Gary A. Koretzky
"Hematopoietic Stem Cells Require Akt1 and Akt2 for Long-Term Self-Renewal"

2. Andrea C. Carpenter and Craig H. Bassing, PhD.
"An Epigenetic Component to TCRb Allelic Exclusion"

3. Tony Pajerowski, Haig Aghahanian, Michael J. Shapiro and Virginia Smith Shapiro
"Severe block in hematopoiesis in the absence of NKAP"

4. Marco Calamito, Marisa Juntilla, Matthew Thomas, Dan Northrup, Alex Bortnic, Morris Birnbaum, Gary Koretzky and David Allman
"Requirement for AKT1 and AKT2 in the development of marginal zone B-cells"

5. Jennifer Reed, John Monroe
"microRNA profiles in peripheral B cell subsets"

6. Dan Northrup, Matt Thomas, David Allman
"Pax5 induction of CD19 expression is EBF independent"

7. Jennifer E. Smith-Garvin, Jeremy Burns, Eric J Brown, Martha S. Jordan, Gary A. Koretzky
"Distinguishing Functional and Developmental Requirements for SLP-76 Phosphotyrosines in T cells"

8. John T. Chang, Jiyeon Kim, Ichiko Kinjyo, Vikram Palanivel, Caitlin Dejong, Courtney McClurkin, Michael Paley, Leslie Berg, Martha Jordan, Gary Koretzky, and Steven L. Reiner
"Different by Destruction: Unequal Inheritance of the Transcription Factor T-bet as a Mechanism to Diversify Daughter T Cell Fates"

9. Keri B Sanborn, Gregory Rak, Saumya Y Maru, Pinaki P Banerjee, Jordan S Orange
"Myosin IIA associates with NK cell lytic granules to enable their function at the immunological synapse"

10. Haina Shin, Charlly Kao, Shawn D. Blackburn, and E. John Wherry
"Role of Blimp-1 in T cell dysfunction during chronic viral infection"

11. Charlly Kao, Andrew Intlekofer, Steven L. Reiner, and E. J. Wherry
"Novel Roles for T-bet in the Maintenance and Differentiation of Antigen-Specific CD8 T Cells during Chronic Viral Infections"

12. Kimberly A. Jordan, Florence Dzierszinski, David S. Roos, Hsiou-Chi Liou & Christopher A. Hunter

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

13. Hersperger AR, Sheth P, Shin LYY, Kaul R, and Betts MR
"HIV-specific CD8 T cells from Elite Controllers Rapidly Upregulate Perforin After Stimulation"

14. Jonathan Silver, Jason Stumhofer and Christopher Hunter
"IL-6 is required for the generation of T follicular helper cells during *Toxoplasma gondii* infection"

15. Jacqueline Perrigoue, Eric Allenspach, Terri Laufer, and David Artis
"Delineating requirements for antigen presentation in the intestine"

16. S. A. Saenz, C. Zaph, M. Mohrs, A. Budelsky, D. Artis
"Investigating the functional biology of IL-25 during helminth infection"

17. Jason A. Hall, Cheng-Ming Sun, Elizabeth Wohlfert, Robin Kastenmeyer, Yasmine Belkaid
"Signaling via vitamin A mediates intestinal immune homeostasis and amplifies inflammatory responses following oral challenge"

18. Soyoung Oh, Andrew L. Rankin, Malinda Aitken, Andrew J. Caton
"Role of TCR specificity on in vivo function of $CD4^+CD25^+$ regulatory T cells in an autoimmune setting"

19. Betsy C. Taylor, Colby Zaph, Amy E. Troy, Yurong Du, Katherine Guild, Michael R. Comeau, and David Artis
"TSLP influences peripheral dendritic cell and T helper cell responses in murine models of intestinal infection and inflammatory bowel disease"

Abstracts for Posters:

P1. Michael Abt, Daniel Beiting, Dymtro Kobuley Colby Zaph, Yimin Yu,
John Wherry & David Artis
"The Influence of commensal bacteria in the virus-specific CD8+ T cell responses"

P2. Robert A. Barnitz and Michael J. Lenardo
"HIV-1 infection sensitizes CD4+ T cells to cell death induced by TNF-alpha"

P3. Alexandra Bortnick and David Allman.
"TLR9 activity promotes radioresistance and early plasma cell differentiation in naïve B cells"

P4. Erica L. Carpenter and Robert H. Vonderheide
"Activation of B cells via CD40 and TLR9 in patients with cancer"

P5. Esteban Carrizosa, Shuixing Li, and Janis K. Burkhardt
"Relationship between HS1 actin binding and productive T cell activation"

P6. Anthony Chi, Arivu Sambandam, Alex Chavez, and Avinash Bhandoola
"Common Myeloid Progenitor possess latent T lineage potential"

P7. SL Colpitts, NM Dalton, PM Gray, and P Scott
"Concomitant immunity to *Leishmania major* is supported by a population of Th1-polarized IL7R^{high} CD4^{+} T cells"

P8. Evann Corbo, Michelle Schmidt, Jill Angelosanto, Irene Chernova, Joeseph Kissil, and Jonathan Maltzman
"The Role of Merlin in T Cells"

P9. Priya Dedhia, Karen Keeshan, Maria Vega, Sacha Uljon, Lanwei Xu, Candice Romany, Olga Shestova, Stephen Blacklow, and Warren Pear.
"Trib1, Trib2, but not Trib3 degrade C/EBP α and induce acute myelogenous leukemia."

P10. Danielle Haney and Michael Betts
"Detection of Membrane Bound TNFα (mTNF) on the Surface of Antigen-Specific CD8 T cells"

P11. Haitao Hu, Drew Weissman
"Immunopathogenesis of HIV: regulatory T cells and Envelope induced inhibition of CD4+ T cell immunity"

P12. Martha S. Jordan, Jeremy C. Burns, Rebecca Baker, Evan Corbo, Christine Anterasian, Gary A. Koretzky
"The SH2 domain of SLP-76 is required for integrin and NFkB activation following TCR stimulation"

P13. Ji-Yeon Kim, John T. Chang, Ichiko Kinjyo, Courtney McClurkin, Caitlin Dejong, Leslie Berg, Martha Jordan, Gary Koretzky, and Steven L. Reiner
"Unequal T Cell Inheritance of the Transcription Factor T-bet Is Mediated through Sequential Phosphorylation, Ubiquitination and Mitotic Degradation Initiated by Antigen Receptor Signaling"

P14. Michelle Kinder, Suresh Shelat, Mondira Kundu, Liang Zhao, Ellen Puré
"12/15-Lipoxygenase is a critical regulator of hematopoietic stem cell function"

P15. Dawson Knoblock, Yumi Ohtani, Terry Fang, Cristina Del Bianco, Stephen Blacklow, Warren Pear
"Cooperation between Notch and Gata3 in the regulation of Th2 differentiation"

P16. Lenox LE, Jordan MJ, Prieto C, Cholapranee A, Koretzky GA, Nichols KE
"The N-terminal tyrosines of SLP-76 are required for optimal integrin and FcγR-dependent PMN functions"

P17. Adeeb Rahman & Laurence Turka
"MyD88 controls efficiency of hematopoietic reconstitution following bone marrow transplantation"

P18. Hilda E. Ramon, Christopher Riling and Paula M. Oliver
"The Role of Ndfip1 in T Cell Activation in a Model of Inflammatory Bowel Disease"

P19. Meredith H. Shaffer, Renell S. Dupree, Ichiko Saotome, Andrea I. McClatchey, and Janis K. Burkhardt
"Ezrin and moesin are required for β1, but not β2, integrin-dependant T cell adhesion"

P20. Sean Spencer, Nicolas Bouladoux, Jason Hall, Cheng-Ming Sun, Sylvia Bolland, Yasmine Belkaid
"A Complex Role for TLR7 in Autoimmunity and Immune Regulation"

P21. Laura Simon Treml and Michael P. Cancro
"Loss of TACI reduces B cell fitness for the marginal zone"

P22. Karla Wiehagen, Michelle Schmidt, E. John Wherry, Jonathan S. Maltzman
"Memory T cell maintenance does not depend on SLP-76 mediated signaling"

Abstracts for Oral Presentations:

1

Hematopoietic Stem Cells Require Akt1 and Akt2 for Long-Term Self-Renewal

Marisa M. Juntilla, Vineet Patil, Rohan Joshi, Gary A. Koretzky
Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine

Murine hematopoietic stem cells (HSCs) rely on components of the Akt signaling pathway, such as FOXO family members and PTEN, for efficient self-renewal and continued survival. However, it is unknown whether Akt is also required for murine HSC function. We hypothesized that Akt would be required for HSC self-renewal, and that the absence of Akt would lead to hematopoietic failure resulting in developmental defects in multiple lineages. To address the effect of Akt loss in HSCs we used competitive and non-competitive murine fetal liver- bone marrow chimeras. In short-term assays, Akt1-/-Akt2-/- fetal liver cells reconstituted the LSK compartment of an irradiated host as well or better than wildtype cells, although failed to generate wildtype levels of more differentiated cells in multiple lineages. When placed in a competitive environment, Akt1-/-Akt2-/- HSCs were outcompeted by wildtype HSCs in serial bone marrow transplant assays, indicating a requirement for Akt1 and Akt2 in the maintainance of long-term hematopoietic stem cells. Akt1-/-Akt2-/- LSKs tend to remain in the G0 phase of the cell cycle compared to wildtype LSKs, suggesting the failure in serial transplant assays may be due to increased quiesence in the absence of Akt1 and Akt2. Additionally, the intracellular content of reactive oxygen species (ROS) in HSCs is dependent on Akt signaling because Akt1-/-Akt2-/- HSCs have decreased ROS levels. Furthermore, pharmacologic augmentation of ROS in the absence of Akt1 and Akt2 results in an exit from quiescence and rescue of differentiation both *in vivo* and *in vitro*. Together, these data implicate Akt1 and Akt2 as critical regulators of long-term HSC function and suggest that defective ROS homeostasis may contribute to failed hematopoiesis.

2

An Epigenetic Component to TCRb Allelic Exclusion

Andrea C. Carpenter and Craig H. Bassing, PhD.
Immunology Graduate Group and Abramson Family Cancer Research Institute of the University of Pennsylvania; Pathology and Laboratory Medicine Department of the Children's Hospital of Philadelphia

The majority of B and T lymphocytes express cell surface immunoglobulin (Ig) or T cell receptor (TCR) proteins from only a single allele. Such allelic exclusion of antigen receptors is thought to ensure their proper selection and, thereby, prevent auto-immunity. Despite intense efforts, the molecular mechanisms that enforce Ig and TCRb allelic exclusion remain elusive. The assembly and expression of TCRb chains from an allele signals TCRb-mediated feedback inhibition that prevents the further assembly of TCRb variable region exons on the other allele in 90% of T cells. The remaining 10% of T cells contain two functional Tcrb rearrangements. This minority population genetically has the capacity to express two T cell receptors. However, the expression of both TCRb is not detectable, suggesting another molecular component contributes to enforcement of TCRb allelic exclusion. We set out to elucidate potential mechanisms that regulate cell-surface Tcrb expression post-recombination, such as chromosome accessibility, transcription, translation, and pairing with the pre-Ta chain. To this end, we used mouse models containing two distinct pre-assembled Tcrb exons. When mice genetically harbor two unique pre-assembled exons, their T cells phenotypically have two

different surface TCRb expression patterns, one where the T cells only express one of the pre-assembled exons and another where T cells express both Tcrb exons. Therefore, we reasoned we could study the population maintaining allelic exclision to gain insight into additional mechanisms by which TCRb allelic exclusion is enforced. We investigated the nature of the T lineage cells that express only one TCRb exon and have determined that the level of regulation is mono-allelic TCRb transcription very early in development. In whole, our work indicates an epigenetic component contributes to enforcement of TCRb allelic exclusion and raises the intriguing possibility that dysfunction of the T cell epigenome might contribute to development of auto-immunity. The elucidation of these epigenetic regulatory mechanisms and their contribution to human health and disease awaits further investigation.

3

Severe block in hematopoiesis in the absence of NKAP

Tony Pajerowski, Haig Aghahanian, Michael J. Shapiro and Virginia Smith Shapiro
Department of Immunology, Mayo Clinic and the Department of Pathology and Laboratory Medicine, University of Pennsylvania

During hematopoiesis, progressive differentiation of long-term, multipotent, self-renewing hematopoietic stem cells (HSC) results in all the cellular components of blood. Many lineage fate decisions result from alterations in gene expression, arising from coordinated regulation by transcriptional activators, transcriptional repressors and chromatin remodelers. Here, we have identified a novel regulator of hematopoiesis, NKAP. Initially, we isolated NKAP on a genetic complementation screen to rescue the T cell activation defect in a Jurkat mutant cell line. Subsequent biochemical analysis of NKAP function demonstrated that it functions as a transcriptional repressor, and is a novel component of the Notch corepressor complex. To understand the function of NKAP in hematopoietic development, NKAP was conditionally deleted in HSC by use of a vav-cre transgenic line. Strikingly, we found that the loss of NKAP abrogated the T and B cell development. In addition, blocks were also observed in the myeloid, megakaryocyte and erythroid lineages. Thus, NKAP is among a unique group of transcriptional regulators regulate the development of multiple lineages.

4

Requirement for AKT1 and AKT2 in the development of marginal zone B-cells

Marco Calamito, Marisa Juntilla, Matthew Thomas, Dan Northrup, Alex Bortnic, Morris Birnbaum, Gary Koretzky and David Allman
IGG

Although the AKT family of serine/threonine kinases is key to cellular metabolism and survival in a wide variety of cell types, the role of the three AKT isoforms (AKT1-3) in B cell development has not been tested. To address this issue, we established chimeras with fetal liver (FL) progenitors possessing null mutations in AKT1 and AKT2. Strikingly, loss of AKT1 and AKT2 function led to a selective loss of marginal zone (MZ) B cells, a unique mature B cell subset required for host defense against blood-born pathogens. Surprisingly, the development of many other B-lineage cells including B-lineage precursors in the marrow and transitional and follicular B cells in the spleen was largely normal. Double chimeras established by mixing AKT 1/2 -deficient and wild type progenitors yielded similar results, although under these more stringent competitive conditions additional peripheral B cell subsets including follicular B cells were significantly reduced. Interestingly, enforced expression of Bcl-xL, an anti-apoptotic member of

the Bcl-2 family, in AKT1-/- AKT2-/- progenitors failed to rescue MZ B-cell development, suggesting that AKT1/2 derived signals provide survival independent functions for MZ B cells. Interestingly, unlike chimeras in which an $AKT1^{-/-}$ $AKT2^{-/-}$ genotype resulted in the dramatic loss of MZ B cells, frequencies of MZ B cells were only marginally reduced in unmanipulated $AKT1^{-/-}$ or $AKT2^{-/-}$ single knockout adults. Together these data show that AKT1 and AKT2 work in concert to promote MZ B cell development, and suggest that MZ B cells have unique metabolic requirements.

5

microRNA profiles in peripheral B cell subsets

Jennifer Reed, John Monroe
University of Pennsylvania, Genentech

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 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 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 contrast, the miRNA expression profiles between mature and marginal zone B cells were very similar. 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.

6

Pax5 induction of CD19 expression is EBF independent

Dan Northrup, Matt Thomas, David Allman

Recently we have described the role of EBF in forcing B lineage commitment at the expense of myeloid development. Furthermore we have demonstrated that B lineage fate is imposed in the absence of Pax5, when EBF was ectopically expressed. These progenitors are arrested at an early pre-pro B stage. Although Pax5 regulates many genes associated with B lineage development it cannot promote B cell fate from EBF deficient progenitors. We sought to address which targets of Pax5 would be EBF dependent. Therefore we forced expression of Pax5 in an $EBF^{-/-}$ progenitor line, as had been done before. In contrast to week long assays of lineage development we interrogated the cell lines at early time points. In contrast to what had been published we found that Pax5 was capable of inducing expression of CD19 and repressing flt3 in the absence of EBF. However after approximately 3 days of culture these cells were lost. By adding both EBF and Pax5 into the cell line we were able to sustain CD19 expressing cells. These results suggest that Pax5 is capable to activate parts of the B cell program in the

absence of EBF, but that EBF is critical to the survival of Pax5 expressing cells. The targets of EBF that regulate cell survival or proliferation will be an area of future focus.

7

Distinguishing Functional and Developmental Requirements for SLP-76 Phosphotyrosines in T cells

Jennifer E. Smith-Garvin[1,2], Jeremy Burns[1,2], Eric J Brown[1], Martha S. Jordan[1,2], Gary A. Koretzky[1,2,3]
[1]Department of Cancer Biology AFCRI, [2]Department of Pathology and Laboratory Medicine UPenn School of Medicine, [3]Department of Medicine UPenn School of Medicine.

SLP-76 is an adaptor protein required for T-cell development and function. The N-terminus of SLP-76 contains three critical tyrosines (residues 112,128 and 145) that are phosphorylated upon TCR stimulation. Two genomic knock-in (KI) mice were generated that harbor Y to F mutations at positions 112 and 128 (Y112/128F) or 145 (Y145F). Both strains of mice, but especially the Y145F, show striking defects in both positive and negative selection consistent with decreased pre-TCR signals. T cells and thymocytes from both KI mice show markedly diminished abilities to signal through their TCR and to form conjugates with APCs *in vitro.* To determine the contribution of abnormal T cell selection to the dysfunction observed in peripheral KI T cells our lab has generated conditional KI (cKI) mice whereby T cells develop normally in the presence of a floxed WT SLP-76 allele and a Y145F or Y112/128F SLP-76 allele. Using tamoxifen inducible Cre activity, WT SLP-76 expression is deleted in the T cells leaving expression of only mutated SLP-76 protein. Cre active cKI T cells expressing either SLP-76 mutant have normal numbers and distributions of peripheral CD4 and CD8 T cell subsets. However, the cKI T cells down-regulate CD5 expression and show defective signaling events downstream of the TCR that are similar or more severe than those observed in KI T cells. These data demonstrate that the functional defects are independent of the developmental defects. Prelimary data suggest that KI mice are able to mount a near normal immune response to infection, despite their abnormal T cell development and *in vitro* T cell function. To determine if this is the result of a compensatory selection mechanism during KI T cell development, studies are underway to compare the ability cKI T and KI T cells to respond to infections *in vivo.*

8

Different by Destruction: Unequal Inheritance of the Transcription Factor T-bet as a Mechanism to Diversify Daughter T Cell Fates

John T. Chang, Jiyeon Kim, Ichiko Kinjyo, Vikram Palanivel, Caitlin Dejong, Courtney McClurkin, Michael Paley, Leslie Berg, Martha Jordan, Gary Koretzky, and Steven L. Reiner
Abramson Family Cancer Research Institute and Department of Medicine, University of Pennsylvania, Philadelphia, PA

We have previously suggested that asymmetric cell division might represent a mechanism to ensure that appropriate diversity of cell fate arises from the descendants of a single lymphocyte during the immune response. We have begun to explore which determinants might specify distinct cell fates in the first two daughter T cells. We now show that the fate-determining transcription factor T-bet is asymmetrically inherited by dividing CD4+ and CD8+ T cells recruited into an immune response. T-bet is induced in interphase T cells within hours of activation. During mitosis, T-bet undergoes proteosome-dependent degradation. Mitotic

destruction is mediated by T cell receptor-induced tyrosine phosphorylation of T-bet. Unequal inheritance of T-bet is associated with asymmetric segregation of the proteasomal degradative machinery during mitosis and cytokinesis. Mutations of T-bet at the critical tyrosine and those disabling the T cell receptor-associated kinase, ITK, both result in symmetric inheritance of T-bet without affecting asymmetry of the proteasome. These results suggest that two experimentally distinct mechanisms promote the unequal inheritance of T-bet by initial daughter T cells – one signal that targets T cell for mitotic destruction and another signal that renders inequality in the inheritance of the cellular machinery that destroys T-bet. These findings offer a new framework for understanding how signaling to a single T lymphocyte can result in unequal fate determination of its daughter cells.

9

Myosin IIA associates with NK cell lytic granules to enable their function at the immunological synapse

Keri B Sanborn,[1] Gregory Rak,[2] Saumya Y Maru,[2] Pinaki P Banerjee,[2] Jordan S Orange[1,2]
[1] Immunology Graduate Group, University of Pennsylvania School of Medicine
[2] Joseph Stokes Jr. Research Institute of the Children's Hospital of Philadelphia

NK cell cytotoxicity requires the formation of an actin-rich immunological synapse (IS) with a target cell and the polarization of lytic granules containing granzymes and perforin toward the IS. Following polarization of lytic granules, they traverse through the actin-rich IS to join the NK cell membrane in order for directed secretion of their contents to occur. We examined the role of myosin IIA as a candidate for facilitating this pre-final step in lytic NK cell IS function. We found that myosin IIA was constitutively associated with lytic granules from human NK cells. Myosin IIA-associated lytic granules adhered to F-actin, and this interaction was sensitive to the presence of ATP. In NK cells from patients with a heterozygous nonsense mutation in myosin IIA, NK cell cytotoxicity, lytic granule penetration into F-actin at the IS, and interaction of isolated granules with F-actin were all decreased. Similarly, inhibition of myosin function also diminished the approach of lytic granules to and their dynamics at the IS. Thus, NK cell lytic granule-associated myosin IIA enables their interaction with actin and final transit through the actin-rich IS to the synaptic membrane, and can be defective in the context of naturally occurring human myosin IIA mutation.

10

Role of Blimp-1 in T cell dysfunction during chronic viral infection

Haina Shin, Charlly Kao, Shawn D. Blackburn, and E. John Wherry
University of Pennsylvania/The Wistar Institute

Memory CD8 T cells are a critical component of long-term protective immunity. After an acute infection, antigen-specific memory CD8 T cells differentiate in the absence of antigen and inflammation, acquiring distinct phenotypic and functional properties that allow them to confer long-term protection. During chronic viral infection, however, antigen-specific CD8 T cell become functionally exhausted and memory differentiation becomes altered. In the presence of persistent antigen and inflammation, antigen-specific CD8 T cells upregulate a host of inhibitory markers and fail to acquire the phenotypic and functional properties associated with normal memory CD8 T cells. To determine the molecular mechanism behind these events, we have focused our experiments on Blimp-1, a transcriptional repressor normally associated with the terminal differentiation of plasma cells. Recent studies have shown Blimp-1 to have an

important role in T cell homeostasis, and we have found that Blimp-1 is highly overexpressed in antigen-specific CD8 T cells during chronic viral infection. Our experiments show that conditional deletion of Blimp-1 can lead to increased formation of memory-like antigen-specific CD8 T cells at the expense of terminally differentiated effector CD8 T cells. Also, despite high viral titers, antigen-specific CD8 T cells lacking Blimp-1 also exhibit a broader range of cytokine production and have a greatly decreased expression of inhibitory markers on a per cell basis. These experiments suggest an important role for Blimp-1 in regulating both functional exhaustion and memory development during chronic viral infection, and provide insight into how alterations in transcriptional profile of exhausted, antigen-specific CD8 T cells may regulate multiple aspects of T cell dysfunction during chronic viral infection.

11

Novel Roles for T-bet in the Maintenance and Differentiation of Antigen-Specific CD8 T Cells during Chronic Viral Infections

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The T-box transcription factor, T-bet, has a well-established role as a master regulator of CD4 T cell commitment to the T_H1 lineage; however, its importance in the regulation of CD8 differentiation has only recently begun to be elucidated. T-bet regulates the expression of the cytokine, interferon-g, and other genes integral to CD8 effector function, such as granzyme and perforin. During a systemic, acute viral infection, the quantitative levels of T-bet expression, in response to inflammatory stimuli, dictates the ability of CD8 T cells to form either long-term memory cells or shorter-lived effectors. We studied the expression and function of T-bet in mice infected with the chronic Clone-13 strain of lymphocytic choriomeningitis virus (LCMV) and compared the response to that observed with the acute Armstrong strain. Surprisingly, we found that expression of T-bet is noticeably lower in the subset of CD8 T-cells expressing high levels of the inhibitory surface receptor, PD-1, which has been shown to be specifically upregulated during chronic infection and contributes, at least in part, to the dysfunction observed on “exhausted” CD8 T cells. Also, T-bet-deficient mice exhibit higher viral burden and lose their virus-specific CD8 T cells within 2-3 weeks following infection with Clone-13. The T-bet$^{-/-}$ CD8s express higher levels of both PD-1 and another inhibitory receptor, Lag-3, which has also been implicated in CD8 exhaustion. Thus, T-bet appears to play a complex role during CD8 differentiation, depending on the nature of the infection. During systemic, chronic viral infection, the loss of T-bet may be a contributing factor that leads to exhaustion, in part through its regulation of PD-1 and other inhibitory receptors.

12

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

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c-Rel is a member of the NF-κB family of transcription factors, which are important in the regulation of innate and adaptive immunity. 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-κB family member c-Rel. WT mice immunized against T. gondii will have a protective secondary response, while c-Rel deficient mice remain highly susceptible. Since $CD8^+$ T cells that produce the cytokine IFN-γ are critical for the control of *T. gondii*, we focused on the development of the $CD8^+$ adaptive immune response to immunization. Infection with CPS-OVA results in the expansion of endogenous $CD8^+$ T cells specific for an immunodominant epitope in ovalbumin that can be detected in both WT and c-$Rel^{-/-}$ mice, though numbers and frequencies of these antigen-specific cells are significantly lower in c-$Rel^{-/-}$ mice.

Since c-Rel is expressed in all hematopoietic cells, an adoptive transfer system was employed to examine the requirement for T cell-intrinsic requirements for c-Rel. Therefore, c-$Rel^{-/-}$ OTI mice were generated to examine the phenotype and function of antigen-specific c-$Rel^{-/-}$ T cells following infection in a WT recipient. This approach revealed that c-$Rel^{-/-}$ OTI cells expanded to a similar extent as WT OTI cells at early time-points, but later following infection c-$Rel^{-/-}$ OTI cells did not survive, indicating an intrinsic requirement for c-Rel for T cell survival. The reciprocal adoptive transfer experiments demonstrated that c-Rel is required in the APC compartment for T cells to make IFN-γ in response to protein but not peptide antigen.

13

HIV-specific CD8 T cells from Elite Controllers Rapidly Upregulate Perforin After Stimulation

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Evidence suggests that CD8 T cells are important to the control of HIV replication, but the mechanism by which this occurs is unclear. In fact, few differences in CD8 T cell cytotoxic function have been observed to date in HIV-infected subjects that control viral replication compared to individuals that do not have a favorable disease course. We have recently discovered the novel ability of human CD8 T cells to rapidly upregulate perforin following antigen-specific stimulation. Using polychromatic flow cytometry, we measured perforin upregulation, cytokine production and degranulation following stimulation of *ex vivo* CD8 T cells from HIV-infected elite controllers, viremic controllers and chronically infected individuals. We observe that on average 40% of the CD8 T cell response in elite controllers upregulates perforin following HIV-specific stimulation versus about 15% in the other cohorts. However, there is no difference in the proportion of the response that produces IFN-g. Additionally, the cells that upregulate perforin are of largely an effector/effector memory phenotype. The rapid perforin upregulation displayed by CD8 T cells in elite controllers may contribute to the superior control of HIV replication in these subjects.

14

IL-6 is required for the generation of T follicular helper cells during *Toxoplasma gondii* infection

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IL-6 is a pleiotropic cytokine that was originally identified as a B cell growth factor. Mice deficient in IL-6 were previously demonstrated to be susceptible to infection with the intracellular parasite, *Toxoplasma gondii*. Despite the appreciation that IL-6 is important during toxoplasmosis, the mechanism underlying the susceptibility remains unclear. However, both IL-6 KO and B cell deficient mice infected with toxoplasma develop similar pathologies, including higher parasite burdens, inflammation and necrosis in the liver and central nervous system. In order to assess whether or not there was a humoral defect in the IL-6 KO we measured serum Immunoglobulin during the course of infection with *Toxoplasma gondii*. IL-6 KO mice have less parasite-specific IgG2a when compared to WT and fewer PNA+ Germinal Center B cells. The deficiency of GC B cells is not due to diminished expansion of the total B cell population during the course of infection, suggesting a germinal center-specific defect. Previous studies have demonstrated the importance of CD4+ T cells in maintaining germinal center reactions. More specifically a subset of CD4 T cells that express the chemokine receptor CXCR5, called T Follicular helper cells (T_{FH}), were identified as having a primary role in maintaining germinal center reactions. IL-6 KO mice infected with toxoplasma have a 50-60% reduction in T_{FH} cells during the course of infection in LN and Spleen. These deficits correlate with reduced production of the cytokine IL-21, a known T_{FH} differentiation factor. Infected IL-6 KO that were given 2e6 T_{FH} cells intravenously have more parasite-specific IgG2a and fewer areas of necrosis in the liver. Collectively, these data suggest that the T_{FH} cell defect underlies the humoral defects in infected IL-6 KO mice and that these cells are required for production of antigen-specific immunoglobulins. T cell help for B cells is a fundamental aspect of immunity, and our work has revealed a novel role for IL-6 in the generation of T_{FH} cells.

15

Delineating requirements for antigen presentation in the intestine

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Intestinal dendritic cells (DC) have been implicated as critical mediators of both adaptive tolerance and inflammation in the gastrointestinal tract. However, their role in the priming and differentiation of $CD4^+$ T cells following infection with enteric pathogens remains largely unclear. To test whether DC-restricted expression of MHC class II is sufficient to promote protective $CD4^+$ T cell responses in the intestine, mice in which I-$A\beta^b$ expression is restricted to $CD11c^+$ DCs (CD11c/$A\beta^b$ mice) were infected with the intestinal parasite *Trichuris*, immunity to which is dependent on $CD4^+$ Th2 cells. Following infection, littermate control mice exhibited Th2 cytokine-dependent goblet cell hyperplasia and eradication of *Trichuris*. In contrast while CD11c/$A\beta^b$ mice promoted the efficient expansion of $CD4^+$ T cells, they developed persistent infection associated with reduced Th2 cytokine production, increased IFN-γ expression, and pathogen-induced intestinal inflammation. These data suggest that in addition to conventional DCs, another population of MHC class II expressing cells is required for Th2 cytokine-mediated immunity in the intestine. Utilizing MHC class II reporter mice, we identify non-professional antigen presenting cells (APC) present in the intestine early during *Trichuris* infection with the potential to influence parasite-specific $CD4^+$ Th2 responses. Determining the functional significance of these APC in regulating $CD4^+$ T cell responses may have implications for the design and delivery of mucosal vaccines and treatment of T cell-mediated inflammatory diseases.

16

Investigating the functional biology of IL-25 during helminth infection.

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Recent studies have demonstrated key functions of interleukin (IL) -25 in T_H2 cytokine-mediated host protective immunity and exacerbation of allergic airway inflammation, however the cell lineages targeted by IL-25 to elicit such responses remains unclear. We show that IL-25 acts through an innate cell mechanism to drive T_H2 cytokine responses. Utilizing IL-4/eGFP reporter mice that express green fluorescent protein (GFP) under the IL-4 loci, we demonstrated that IL-25 specifically induced a c-kit$^+$ cell population in the mesenteric lymph nodes. This population was also present in the Peyer's patches, cecal patch, and peritoneum of treated mice. Despite displaying a cell morphology similar to classical mast cells, the IL-25-induced c-kit$^+$ cell population lacked expression of FcεR1 and surface bound IgE. Additionally, IL-25 elicited a T_H2 cytokine response and induced this c-kit$^+$ cell population independently of IL-3 and SCF signaling, pathways known to regulate mast cell development. Furthermore, we demonstrated that in mice deficient in the receptor for IL-25 or IL-17A, IL-17Rb and IL-17Ra respectively, the IL-25-mediated induction of this c-kit$^+$ population was lost. Thus, these data suggest that IL-25 acts through a mast-cell independent mechanism to promote T_H2 cytokine responses, which is dependent on both IL-17Ra and IL-17Rb.

17

Signaling via vitamin A mediates intestinal immune homeostasis and amplifies inflammatory responses following oral challenge

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The gastrointestinal tract and its associated lymphoid tissues (GALT) has evolved a dense network of regulatory mechanisms for prevention of spontaneous responses to commensal bacteria and food protein that come into ritual contact with its surfaces. Several of these mechanisms hinge on external cues derived from diet. Humans with Vitamin A deficiency tend to suffer from chronic diarrhea and are more susceptible to gastrointestinal infection. This vitamin, exclusively obtained through diet, exerts its role on the GALT immune system via the metabolite, retinoic acid (RA). RA secreted by GALT dendritic cells (DC), promotes lymphocyte migration into the small intestine. We and other labs have also proposed that RA is critical for the GALT's unique capacity to induce Foxp3+ regulatory T cells (Treg) from naïve precursors *in vivo*. To further address the influence of vitamin A derived signals on the function of the GALT immune system *in vivo*, we generated vitamin A deficient (VAD) mice through diet depletion. In this study, we demonstrate that VAD mice have an impaired ability to generate Treg within the GALT. Unexpectedly, we observe an increase in their frequencies of endogenous Treg that is quite dramatic within the small intestinal lamina propria (Lp). Complementing this augmentation, CD4+ effector T cell proliferation is significantly reduced. Moreover, the IL17+ effector population is virtually absent. Upon oral challenge with mucosal vaccine or Toxoplasma gondii, VAD mice mount markedly impaired immune responses accompanied by poor expansion of Lp effector CD4+ T cells. Preliminary evidence suggests that impaired responses in VAD mice can be rescued with vitamin A sufficient Lp antigen presenting cells. Together these data put vitamin

A at the crossroads of intestinal immune homeostasis and inflammation and suggest that its metabolites negatively regulate the threshold for intestinal immune responses.

18

Role of TCR specificity on 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 with either high (TS1) or low (TS1(SW)) reactivity for the HA. TS1xHACII and TS1(SW)xHACII, yield a high and low penetrance model of spontaneous autoimmune arthritis respectively. When on a $RAG^{-/-}$ background, these mice still develop arthritis, identifying the HA-peptide recognized by the transgenic TCRs 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, not only fail to prevent arthritis in the high penetrance setting but also exacerbate disease in the low penetrance model. However, these T_{regs} can suppress an influenza B cell 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.

19

TSLP influences peripheral dendritic cell and T helper cell responses in murine models of intestinal infection and inflammatory bowel disease

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Intestinal epithelial cells (IECs) produce thymic stromal lymphopoietin (TSLP), however the *in vivo* influence of TSLP-TSLP receptor (TSLPR) interactions on immunity and inflammation in the intestine remains unclear. Here we show that TSLP-TSLPR interactions are critical for immunity to the intestinal pathogen *Trichuris*. Monoclonal antibody-mediated neutralization of TSLP or deletion of the TSLPR in normally resistant mice resulted in defective expression of T_H2 cytokines and persistent infection. Susceptibility was accompanied by elevated expression of IL-12/23p40, IFN-γ and IL-17A, and development of severe intestinal inflammation. Critically, neutralization of IFN-γ in *Trichuris*-infected $TSLPR^{-/-}$ mice restored T_H2 cytokine responses and resulted in worm expulsion, providing the first demonstration of TSLPR-independent pathways for T_H2 cytokine production and suggesting the primary role for TSLP in the intestine may be to limit expression of proinflammatory cytokines. Supporting this hypothesis, $TSLPR^{-/-}$ mice displayed elevated production of IL-12/23p40 and IFN-γ and developed heightened intestinal

inflammation upon dextran sodium sulfate-induced colitis, demonstrating a previously unrecognized immunoregulatory role for TSLP in a murine model of inflammatory bowel disease. Collectively, these data suggest that rather than directly promoting expression of T_H2 cytokines, the dominant functions of TSLP in the intestine are to limit the expression of proinflammatory cytokines and prevent inflammation.

Abstracts for Posters:

P1

The Influence of commensal bacteria in the virus-specific CD8+ T cell responses

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Alterations in the composition of intestinal commensal bacteria in humans are associated with enhanced susceptibility to multiple inflammatory diseases suggesting that signals derived from commensal bacteria may influence the development and/or function of the immune system. Supporting this, germ-free or gnotobiotic mice exhibit reduced numbers of lymphocytes in the periphery, lamina propria 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 gnotobiotic conditions were infected with Lymphocytic Choriomeningitis Virus (LCMV). At day 7 post-infection, gnotobiotic mice exhibited a significant reduction in the frequency and numbers of LCMV-specific $CD8^+$ T cells in multiple tissues including the spleen and intraepithelial compartment. Furthermore, LCMV specific $CD8^+$ T cells from gnotobiotic mice were less capable of producing IFN-γ following LCMV peptide stimulation. In addition, at day 36 post-infection, when conventionally-housed mice exhibit viral clearance and establishment of LCMV-specific memory $CD8^+$ T cells, gnotobiotic mice had a significantly diminished population of LCMV-specific memory $CD8^+$ T cells. Impaired LCMV-specific $CD8^+$ effector and memory T cell responses were not the result of inherent defects in gnotobiotic mice as oral administration of a cocktail of antibiotics to conventionally-housed mice also displayed defective LCMV-specific $CD8^+$ T cell response following infection. Collectively, these data suggest an integral role of commensal bacteria in influencing virus-specific $CD8^+$ T cell responses in the periphery.

P2

HIV-1 infection sensitizes CD4+ T cells to cell death induced by TNF-alpha

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AIDS results from a dramatic loss of CD4+ T-lymphocytes in HIV-infected individuals. HIV cytopathicity directly contributes to the initial depletion of infected CD4+ T cells, particularly in the gastrointestinal tract. Infected individuals also exhibit elevated levels of Tumor Necrosis Factor-alpha (TNF), a potent pro-inflammatory cytokine capable of inducing cell death. We investigated the effect of TNF on HIV-induced cytopathicity using an in vitro infection model of the Jurkat T cell line. We found that addition of recombinant TNF drastically increased cell death of HIV-infected Jurkat cells, without affecting the viability of uninfected cells. In contrast,

cells infected with a control lentivirus that only expresses GFP do not die after TNF treatment, indicating that sensitizing T cells to TNF-induced death is a specific property of HIV. Infection with a non-cytopathic, attenuated mutant of HIV still sensitizes cells to TNF-induced death, indicating death occurs independently of TNF-enhanced viral gene transcription. HIV infection does not alter cell surface expression of TNF receptor I or II, suggesting that HIV instead influences TNFR signaling pathways intracellularly. Finally, we demonstrated that cytopathicity triggered by HIV infection of primary CD4+ T cells was reduced upon treatment with the TNF antagonist Enbrel, indicating endogenous TNF production contributes to HIV-induced cell death during active infection. Further work is aimed at identifying the component of HIV that sensitizes T cells to TNF-induced death, and how that component interacts biochemically with the TNFR signaling pathway. Elucidating the molecular determinants of HIV-induced T cell cytopathicity will hopefully improve treatment strategies during the critical early stages of HIV infection.

P3

TLR9 activity promotes radioresistance and early plasma cell differentiation in naïve B cells

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Antibody-secreting plasma cells are rare among lymphocytes in that they are resistant to radiation-induced apoptosis (RIA). However the mechanism underlying plasma cell radioresistance is unknown. Similarly, how B cells avoid apoptosis due to double stranded DNA breaks that occur upon antibody class switch recombination (CSR) is unanswered. To address these questions we asked whether *in vitro* stimulation of naïve B cells with mitogens known to induce early plasma cell differentiation and CSR confer resistance to RIA. We find that stimulation with the Toll-like Receptor (TLR)-4 agonist LPS promotes resistance to p53-dependent RIA. Strikingly, stimulation with CpG oligonucelotides, a chief TLR9 agonist, for as little as one hour also induces robust protection from RIA. Further, CpG stimulation of naïve B cells initiates molecular events associated with very early plasma cell differentiation and CSR. These include decreased transcripts for the B cell transcription factor Pax5 but increased transcripts for the master plasma cell transcription factor Blimp-1 and Activation Induced Deaminase (AID), a key enzyme required for CSR. Moreover, CpG stimulation induces CSR specifically to IgG2b and IgG3 isotypes. We conclude that TLR4 and TLR9 activity are sufficient to initiate a very early plasma cell differentiation program that includes resistance to p53-dependent RIA. Future studies will include experiments to address how TLR-signaling abrogates p53-dependent apoptosis in primary B cells.

P4

Activation of B cells via CD40 and TLR9 in patients with cancer

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We are presently focused on understanding whether and how activation of B cells in melanoma patients can enhance anti-tumor responses. It has been shown that, beyond dendritic cells, B cells can function as antigen presenting cells to drive T cell responses. However, the activation state of a B cell can influence whether its encounter with a T cell results in activation or tolerization. This is particularly relevant in cancer in which immune-mediated anti-tumor responses can be limited by tolerized T cells. CD40 and TLR9 are two key pathways of B cell

activation, and both are being actively targeted in cancer clinical trials to induce anti-tumor cellular immunity. However, synergy of these APC activation pathways is poorly understood, particularly in human B cells. Therefore, our work specifically focuses on the stimulation of purified human B cells *in vitro* using CD40 and TLR agonists, individually or in combination, in order to determine signals that optimally activate B cells as APC. Our experimental system includes purifying B cells from whole blood obtained from normal donors or melanoma patients. These cells are evaluated using six-color flow cytometry both prior to and after in vitro stimulation. In addition, the proliferative response and amount and type of cytokines secreted are also measured post-stimulation. In our work so far, we have found multiple deficits in the ability of the melanoma patient B cells to respond to stimulation as compared to that of the normal donors. This has included differences in the upregulation of cell surface activation markers, proliferation, and cytokine secretion. Current experiments explore whether antigen-loaded CD40/TLR9 activated B cells from both healthy volunteers and patients with melanoma can optimally induce effective T cell responses against tumors.

P5

Relationship between HS1 actin binding and productive T cell activation

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Productive T cell activation is requires dynamic cytoskeletal rearrangements leading to net actin polymerization at the immunological synapse (IS). Among the proteins that mediate these events is the cytoskeletal adaptor protein HS1. We have shown that T cells lacking HS1 fail to stabilize actin rich structures and do not produce IL-2 efficiently. Phosphorylation of HS1 at two key regulatory tyrosines promotes the recruitment of HS1 to the IS through a pathway involving the binding of the Tec family kinase Itk and also allows interaction with many other components of the T cell signaling machinery. We are now beginning a set of structure-function studies aimed at understanding how actin binding relates to productive T cell signaling. To that end, we are exploiting a mutant form of HS1 described in a lupus patient. This variant promotes activation-induced cell death in B cells more efficiently than wild-type HS1. This form of the molecule lacks a fragment of the F-actin binding region, and we have confirmed that this results in diminished binding to actin *in vitro*. We are in the process of analyzing its effect in T cells. Because the SH3 domain of HS1 might also regulate interactions with the actin cytoskeleton, we are also analyzing the effect of mutations that affect SH3 domain function.

P6

Common Myeloid Progenitor possess latent T lineage potential

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Notch signaling is an evolutionarily conserved mechanism, involved in numerous cellular activities, including lineage differentiation. Of the four mammalian Notch receptors (Notch1 – 4), signaling through Notch1 has been shown to be critical for T cell lineage development. While Notch1-deficient progenitor cells fail to generate thymocytes, ectopic expression of Notch1 signaling can promote extrathymic T lineage development in the bone marrow. We observe that as common myeloid progenitors (CMPs) differentiate from multipotent progenitors (MPPs) towards myeloerythroid lineages, the expression of Notch1 and Lunatic fringe, a positive Notch1

signaling regulator, is down-regulated. However, CMPs, unlike their downstream progenitors, retain the capability of responding to supra-physiological levels of Notch1 signaling, achieved by *in vitro* co-culture of OP9-DL1 or OP9-DL4, and give rise to the T lineage cells. Furthermore, the majority of CMPs can respond to ICN1, a mimic for constitutively active Notch1 signaling, on a cellular basis. While overexpression of ICN1 in CMPs drastically increases its efficiency to generate T cells in thymus, intravenous transfer of ICN1-overexpressing CMPs can lead to the development of T cell leukemia (T-ALL). Not only does this study demonstrate the plasticity between the T and myeloid cell fate decision, it also highlights Notch1 and Lunatic fringe as crucial determinants for T lineage differentiation. Furthermore, it suggests that CMPs should be considered as a possible candidate for the search of T leukemic stem cells.

P7

Concomitant immunity to *Leishmania major* is supported by a population of Th1-polarized IL7R^{high} CD4^{+} T cells

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Infection with the intracellular protozoan parasite *Leishmania major* induces a state of concomitant immunity where secondary immunity is dependent upon the persistence of the original pathogen. Little is known about the factors that mediate concomitant immunity. Our lab identified two populations of cells that are generated following *L. major* infection, termed central memory T (T_{cm}) cells and effector T (T_{eff}) cells based on their high and low expression levels of the lymph node-homing molecule CD62L, respectively. To test the capacity of these cells to be long-lived, we used flow cytometry to determine expression of the IL7R, which is known to promote T cell survival in the presence of its cognate cytokine IL-7. We found that CD4^{+} T_{cm} cells maintained following a primary infection express high levels of the IL7R. Interestingly, we found that even though the IL7R was downregulated on the majority of activated cells early following infection, a significant population of IL7R expressing cells was apparent by 2 weeks post infection that included both CD62L^{high} and CD62L^{low} cells. This led us to ask if Th1 polarized T_{eff} cells could also be IL7R^{high}. Indeed, we used 2 complimentary approaches and found cells that had upregulated the Th1-promoting transcription factor T-bet and also cells that had transcribed the locus for IFNγ expressing high levels of the IL7R. We believe that these cell populations that are pre-disposed towards the production of Th1 cytokines are important in maintaining concomitant immunity and provide a novel target cell population for future attempts at vaccine design.

P8

The Role of Merlin in T Cells

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Regulation of the actin cytoskeleton plays an important role in multiple aspects of T cell function, including development, localization, and activation. Ezrin, moesin, and radixin (ERM) proteins are key regulators of these filaments. Merlin, encoded by the NF2 gene, is an ERM family member. Merlin has been extensively studied in non-hemopetic cells and functions in the regulation of cell size, polarity, division, motility, adhesion, and endosome trafficking. Similar to other ERM proteins, merlin has been shown to link the actin cytoskeleton with the proteins in the

cell membrane, and to stabilize actin filaments. Here we show that merlin is expressed in both developing thymocytes and peripheral T cells. Using a loss of function approach we sought to determine the role of merlin in T cell development and activation. Since merlin -/- mice are embryonic lethal we used conditional gene deletion in the T cell lineage by interbreeding flox-NF2 mice with LCK-Cre or CD4-Cre transgenics. Preliminary experiments show that merlin is not necessary for thymocyte development, and that mice lacking the merlin protein have normal percentages and numbers T lymphocytes in their periphery. *In vitro* studies show that merlin is not required for TCR mediated upregulation of CD69 and CD25. Further studies are ongoing to elucidate other roles merlin may be playing in these cells.

P9

Trib1, Trib2, but not Trib3 degrade C/EBP α and induce acute myelogenous leukemia.

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Trib1, Trib2, and Trib3 are mammalian homologues of the Tribbles protein family, an evolutionarily conserved group of proteins that can mediate proteasome-dependent degradation. Evidence suggests that these proteins function as adapters, where they recruit E3 ligases and enhance ubiquination of the target protein in order to promote its degradation. To date, increased Trib1 and Trib2 mRNA expression has been shown to correlate with acute myelogenous leukemia (AML) in humans and induces AML in mice; whereas Trib3 has not been associated with AML. In order to understand the effects of Trib family members in hematopoietic cells, we reconstituted mice with hematopoietic progenitors retrovirally expressing Trib1, Trib2, or Trib3. Trib1 and Trib2 mice developed AML whereas Trib3 mice did not. Our previous data suggested that Trib2-mediated degradation of the transcription factor, C/EBP α, is important for leukemogenesis. We now show that Trib1, like Trib2, strongly binds C/EBP α and induces its degradation. In contrast, Trib3 weakly binds C/EBP α and fails to induce its degradation. Consistent with the ability to strongly bind and degrade C/EBP α, Trib1 and Trib2, but not Trib3, block differentiation of myeloid cells. We are currently mapping the structural domains that account for the differences between Trib1/Trib2 and Trib3 in leukemogenesis. Together, our results strengthen the correlation between Trib-induced C/EBP α degradation and induction of AML. Furthermore, our data show that different Tribbles family members have distinct targets and understanding this specificity may provide opportunities to therapeutically target Tribbles.

P10

Detection of Membrane Bound TNFα (mTNF) on the Surface of Antigen-Specific CD8 T cells

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TNFα has two biologically active forms: an initial membrane associated form (mTNF) and a soluble form (sTNF), the latter created by the TNFα Converting Enzyme (TACE) cleavage of

mTNF. Due to neonatal lethality of TACE knockout murine systems, it has been difficult to identify the function of mTNF. To define the potential role of TNFα in the human CD8 T cell response, we have developed an assay that utilizes a TACE inhibitor to prevent mTNF cleavage from the cell surface of activated cells. TAPI-0, an inhibitor of human TACE, was added to peptide or superantigen stimulated human PBMC to prevent cleavage of mTNF. TAPI-0 inhibited TNFa release from stimulated cells, as confirmed by TNFα ELISA. We then tested whether we could detect mTNF by flow cytometric analysis. Human PBMCs were stimulated for varying periods of time in the presence of TAPI-0, and mTNF was examined on both $CD4^+$ and $CD8^+$ T cells. TNFα was readily detectable on the surface of activated ($CD69^+$) T cells in the presence of TAPI-0 at levels comparable to those found in TNFα -intracellular cytokine staining controls. This procedure is highly amenable to live cell sorting, as responding cells need not be permeablized or fixed prior to detection and isolation. Thus, this novel procedure can be used to define the role of mTNF in general immune function and various disease states.

P11

Immunopathogenesis of HIV: regulatory T cells and Envelope induced inhibition of CD4+ T cell immunity

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HIV Envelope glycoprotein (Env) induces inhibition of bystander CD4+ T cell activation as a mechanism for immunopathogenesis. Recently CD4+CD25+FoxP3 regulatory T cells (Treg) have been implicated to inhibit both HIV-specific CD4+ and CD8+ T cell immunity. Considering the potential interaction of CD4+ Treg cells with Env during the antigen specific immune response, we explored in the first part the role of Treg cells in Env induced suppression of CD4+ T cell immunity *in vitro* using coculture of human PBMC with Env either expressed on cells or present on free HIV virons. We found that Env exposure induces neither the increased frequency nor more activated phenotype of Treg cells. Depletion of Treg cells fails to overcome Env induced inhibition of CD4+ T cell activation. Importantly, Env exposure does not change the functional activity of natural as well as activation-induced Treg cells. These data argue against the direct role of Treg cells in *in vivo* immunosuppression by HIV Env. In the second part, we then investigated the virus specific immune response in chronic HIV infection by establishing a chronic SIV macaque infection model in the presence of viral suppression by anti-retroviral therapy (ART). Interestingly, poly-chromatic flow cytometry analysis showed that SIV Gag vaccine induces a rapid secondary T cell responses at Day 3 after vaccination, suggesting the maintained, at least partially, viral specific cellular immunity in the context of pharmaceutic control of viral replication. Next research will focus on the phenotypic and functional analysis for Treg cells in this model.
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P12

The SH2 domain of SLP-76 is required for integrin and NFkB activation following TCR stimulation

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SLP-76 is an adaptor protein that is essential for T cell development, as mice deficient for SLP-76 have no mature T cells. SLP-76 has multiple functional domains that serve as docking sites for effector molecules that are important for optimal TCR signaling. A single SH2 domain lies within the C-terminal region of SLP-76 that inducibly associates with the adaptor ADAP and the serine-threonine kinase, HPK1. SLP-76 deficient cell lines and primary T cells expressing an R->K mutation (RK) in the SH2 domain of SLP-76 are defective in the upregulation of early activation markers and T cell proliferation but, Ca2+ flux, CD3 clustering, and ERK phosphorylation following TCR stimulation are normal. To identify the biochemical mechanisms underlying these functional defects, we investigated whether mutation of the SLP-76 SH2 domain effects inside-out-signaling to integrins and activation of NFκB, pathways supported by ADAP and HPK1 (the proteins that associate with the SH2 domain of SLP-76). Using SLP-76 deficient cell lines reconstituted with an RK SH2 mutation and floxed SLP-76 mice expressing CD4 cre and a SLP-76 RK transgene, we demonstrate that the SH2 domain of SLP-76 is important for TCR-induced adhesion to ICAM-1, TCR induced conjugate formation, and NFκB signaling downstream of the TCR. Additionally, in vivo analysis of floxed SLP-76, RK transgenic mice that also express a TCR transgene clearly demonstrate that the SH2 domain of SLP-76 is required for proper positive selection, a role not previously attributed to this domain.

P13

Unequal T Cell Inheritance of the Transcription Factor T-bet Is Mediated through Sequential Phosphorylation, Ubiquitination and Mitotic Degradation Initiated by Antigen Receptor Signaling

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The T-box transcription factor T-bet has been shown to be a critical regulator of Type 1 immunity in both CD4+ and CD8+ T cells. We have recently observed that the amount of T-bet becomes markedly reduced, as well as asymmetrically distributed, in T cells preparing for division. Using immunoprecipitation and Western blotting, we now show that T-bet is induced in interphase T cells after activation. In addition, T-bet undergoes antigen receptor-induced tyrosine phosphorylation. To explore the apparent cell cycle-dependent degradation of T-bet, we synchronized activated T cells in G2/prometaphase using nocodazole. After nocodazole arrest, T cells were released into metaphase and T-bet levels were assessed kinetically by immunoblotting. T-bet appears to be degraded in mitosis within 30 minutes of release from nocodazole. Using immunoprecipitation of T-bet followed by immunoblotting with an anti-ubiquitin antibody, we detected a poly-ubiquitinated form of T-bet at the time of its degradation. Ubiquitination and degradation of T-bet was dependent on the proteasome, as the addition of the proteasomal inhibitor MG-132 enhanced the levels of poly-ubiquitinated T-bet and prevented its degradation. We also found that antigen receptor-induced phosphorylation of T-bet at a specific tyrosine residue is necessary for its subsequent ubiquitination, degradation, and asymmetric localization. Asymmetric distribution of T-bet during mitosis and cytokinesis appears to be related to the asymmetric segregation of proteasomal components. These findings suggest that antigen-induced T cell signaling is not solely aimed at altering transcription of an activated, interphase cell. Signaling to a T cell preparing for division can also modify preformed cellular components in a way that will ultimately render unequal transcriptional programs between its daughter cells.

P14

12/15-Lipoxygenase is a critical regulator of hematopoietic stem cell function

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Fatty acid metabolism is a critical regulator of immune cell function but its role in hematopoiesis is largely unknown. We have previously demonstrated that 12/15-Lipoxygenase (12/15-LOX) functions as a suppressor of leukemia, which is a result of dysregulated hematopoiesis. In this study, we show that 12/15-LOX deficient mice, Alox15, have multiple hematopoietic abnormalities including defects in lymphoid, erythroid and myeloid cell development. However, the most profound defect is in hematopoietic stem cell (HSC) function. Alox15 HSC exhibit a competitive defect during bone marrow reconstitution of lethally irradiated mice. Moreover, 12/15-LOX promotes HSC quiescence and self-renewal. These processes are likely governed through 12/15-LOX regulation of the transcription factor ICSBP/IRF-8 and β-catenin signaling. The understanding of 12/15-LOX and fatty acid metabolism in hematopoietic stem cell function has implications for hematopoiesis, aging and leukemogenesis.

P15

Cooperation between Notch and Gata3 in the regulation of Th2 differentiation

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Helper T cells differentiate into effector lineages that mediate immunity to different classes of pathogens following antigenic stimulation. This differentiation is initiated by signals from antigen presenting cells that induce lineage specific transcription factors. The Th1, Th2, Th17, and Treg lineages are induced by Tbet, Gata3, ROR-gT, and FoxP3, respectively. One pathway that has emerged as an important regulator of helper T cell differentiation is the Notch signaling pathway. Notch signaling has an established role in regulating cell fate decisions and has recently been implicated in Th2 differentiation. In addition to being required for optimal Th2 responses, Notch was found to directly regulate transcription of the Th2 cytokine, IL4, and the Th2 specific transcription factor, Gata3. We have also shown that Notch may cooperate with Gata3 to enhance Gata3 and IL4 transcription. Recent data from our lab suggests that the cooperation between Notch and Gata3 may be important for optimal binding of these factors to target DNA, providing a potential mechanism for the synergy between Notch and Gata3.

P16

The N-terminal tyrosines of SLP-76 are required for optimal integrin and FcγR-dependent PMN functions

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Neutrophils (PMN) eliminate pathogens with a repertoire of antimicrobial functions; however, uncontrolled PMN activity damages host tissue and is associated with inflammatory disease. The adaptor SLP-76 is a critical mediator of Fc-gamma receptor (FcγR) and integrin induced PMN activation. FcγR and integrin engagement results in phosphorylation of SLP-76, as well as its movement from the cytosol to the plasma membrane, where it organizes signaling complexes that activate downstream pathways. To address how the N-terminal tyrosines of SLP-76 propagate signals in PMN, we generated knock-in mice expressing tyrosine to phenyalanine mutations in residue 145 (Y145F) or 112 and 128 (Y112/128F), the amino acids thought to mediate interaction of Btk/Tec kinases or Vav and Nck with SLP-76, respectively. Based on the known roles of these putative binding partners, we hypothesized that these Y→F mutations would impair the ability of SLP-76 to support integrin and FcgR-induced functions. PMN from both strains of mice exhibit reduced FcγR- and integrin-dependent ROI production, spreading and degranulation. Interestingly, FcγR-dependent Ca2+ flux and spreading was more profoundly diminished in the Y145F PMN than the Y112/128F suggesting that Y145 is the single most critical tyrosine for FcγR-dependent function. Analysis of mice harboring one Y145F and one Y112/128F allele reveal that these mutations do not significantly complement each other when present in trans, suggesting a lack of cooperativity between two or more mutant SLP-76 molecules during FcγR- or integrin associated signaling in PMN. To determine whether the observed *in vitro* functional defects have relevance to PMN activity *in vivo*, we will test the tyrosine mutants in animal models of inflammation which require PMN for disease pathology. Collectively, these data will increase our understanding of how SLP-76 functions in PMN and may provide insights into novel pathways to be targeted in the treatment of inflammatory disease.

P17

MyD88 controls efficiency of hematopoietic reconstitution following bone marrow transplantation

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MyD88 is an adaptor protein that is required for signaling through most TLRs as well as the IL-1R family. While these pathways are primarily considered to play a role in mature APCs of the innate immune system, TLRs are expressed on multiple hematopoietic cell lineages. Hematopoietic stem and progenitor cells have been shown to express TLRs and treatment with TLR ligands can influence their differentiation. However, it is unclear whether these pathways play a role during normal hematopoietic development in the absence of exogenous TLR ligands.

Competitive bone marrow transplant experiments in lethally irradiated mice show that WT cells display a significant advantage over MyD88-/- cells in the reconstituted myeloid and lymphoid compartments. We investigate whether impaired differentiation of MyD88-/- progenitor cells or reduced survival of mature cells account for this failure to effectively compete with WT cells. We also examine which MyD88-dependent receptor pathways are potentially involved in hematopoietic reconstitution following bone marrow transplantation.

P18

The Role of Ndfip1 in T Cell Activation in a Model of Inflammatory Bowel Disease

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Nedd4 family interacting protein 1 (Ndfip1) is known to play an important role in T cell function. Mice deficient in Ndfip1 develop an inflammatory condition in the skin and lungs. Further analysis has shown that Ndfip1 KO mice also develop an inflammatory bowel disease, which is characterized by infiltration of eosinophils along the entire digestive track. The onset of disease is accompanied by weight loss. We recently found that Ndfip1-/- T cells are both necessary and sufficient to induce pathogenesis of this disease. Thus, we hypothesize that IL-5 secretion by CD4 T cells mediates the recruitment of eosinophils to the digestive track. Currently, experiments are underway to test this hypothesis. Ndfip1-/- T cells have been shown to have a bias towards T_H2 differentiation, which can be explained by the finding that Ndfip1 binds Itch and promotes the degradation of JunB. However, we believe that Ndfip1 has roles other than regulating Itch. We will show data supporting this hypothesis. Additionally we will show that Ndfip1-/- T cells have increased proliferation compared to WT cells in response TCR stimulation. This data supports that Ndfip1 regulates T cell activation through an Itch-independent mechanism.

P19

Ezrin and moesin are required for β1, but not β2, integrin-dependant T cell adhesion

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The highly homologous ERM proteins link transmembrane and cytoplasmic proteins to the actin cytoskeleton. The two ERMs expressed in T cells, ezrin and moesin, are implicated in promoting T cell activation and polarity, but their function in this context is poorly understood. We conducted an analysis of ezrin and moesin function in T cell migration using conditional ezrin knockout T cells in combination with siRNA-mediated suppression of moesin. T cells lacking both ezrin and moesin showed reduced migration to CCL19 and impaired homotypic adhesion in vitro. In addition, ezrin and moesin were required for uropod formation and activation-induced adhesion in response to fibronectin, a β1 integrin ligand. Despite lacking uropods, ezrin and moesin-deficient cells still exhibited a polarized localization of the uropod markers plectin and myosin, suggesting these cells have maintained overall polarity but lacked the actinomyosin contractility required to form a uropod. However, myosin was not dysregulated, as myosin phosphorylation remained intact in these cells. Surprisingly, ezrin and moesin were not required for uropod formation or adhesion to ICAM-1, a β2 integrin ligand. Taken together, these data show that ezrin and moesin are required for specific aspects of T cell trafficking, including β1 integrin-dependant adhesion. In addition, T cells must possess different pathways leading to the activation of β1 versus β2 integrins.

P20

A Complex Role for TLR7 in Autoimmunity and Immune Regulation

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Toll-like receptors (TLRs) are key structures in sensing and responding to microbial threats. Recent evidence suggests that TLRs also play a significant role in autoimmunity through cross-recognition of self molecules. TLR7, which is activated by viral ssRNA, may also recognize host mRNA. We are investigating a transgenic mouse model with a 4-16 fold increase in TLR7 mRNA. In this model, increased expression of TLR7 alone is sufficient to induce a lupus-like syndrome in mice with spontaneous autoimmunity, systemic inflammation and premature death. Previous studies suggest that the initiation of self antibody production in this model is largely driven by TLR mediated activation of self reactive B cells. Conversely, increased TLR7 expression may serve a protective role by promoting an increase in Foxp3+ T regulatory cells (Treg). Prior to the onset of autoimmunity, we observe a large increase of Tregs in the spleen, lamina propria, mesynteric lymph nodes and thymus. This suggests that the onset of autoimmunity may be delayed due to the influence of Tregs. Thus, increased TLR7 expression in this model may promote autoimmunity in B cells while also serving as a positive signal for Treg induction. In vitro studies support the notion that TLR7 is a positive signal for T regulatory priming by dendritic cells (DC). In culture, MLN DCs induce significantly more Foxp3+ Tregs in the presence of a TLR7 agonist and TGFb, than with TGFb alone. Contrary to this, we previously found that TLR9 signaling negatively influences Treg induction. We are currently investigating the apparently antagonistic roles of TLR7 and TLR9 signaling on Treg homeostasis and also the ability of Tregs to control autoimmunity in this model.

P21

Loss of TACI reduces B cell fitness for the marginal zone

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The BLyS family of receptors and ligands plays a pivotal role in B cell survival and differentiation. This group includes two cytokines, BLyS (also called BAFF) and APRIL, which can interact with three receptors: BLyS receptor 3 (BR3), transmembrane activator calcium modulator and cyclophilin ligand interactor (TACI), and B cell maturation antigen (BCMA). Each of the receptors appears to play a different role in determining cell fate. TACI was originally thought to act as a negative regulator of B cell survival. The original reports described that TACI knockout mice have increased B cell numbers and develop lymphoproliferative disease as they age. We show here that the increased B cell numbers occur in all peripheral compartments, including the marginal zone. These mice also have increased serum BLyS levels, suggesting that TACI may act merely as a sink for soluble BLyS. However, in the competitive environment of a mixed marrow chimera, while the TACI deficient cells contribute an increased percentage to transitional and follicular B cells, they contribute a reduced percent to the marginal zone and this defect worsens over time. Marginal zone cells are known to be heavily BLyS dependent, however, these mice have normal to elevated serum BLyS concentrations. These results indicate a novel role for TACI in either the entry or maintenance of cells within the marginal zone.

P22

Memory T cell maintenance does not depend on SLP-76 mediated signaling

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The memory T cell population is responsible for maintenance of long-term resistance to pathogen-specific re-infection. While the requirement of cytokine generated signals for persistence of memory T cells is clear, the need for persistent signaling through the T cell receptor (TCR) is less well understood. Normally, memory T cells persist in vivo due to a combination of long-term survival and ongoing homeostatic turnover. It has previously been shown that populations of CD44hi, CD62Llo memory T cells require TCR signals for turnover not for survival. These "tonic" TCR-generated signals required for turnover in memory T cells are likely weaker than activating TCR signals, and generated by interaction with MHC:self-peptide. The SH2-domain-containing phosphoprotein of 76 kilodaltons (SLP-76) adaptor protein is critical for proximal TCR-generated signaling. To assess long-term T cell survival in the absence of tonic TCR signals, we ablate TCR signals through conditional deletion of SLP-76 in antigen-specific memory T cells. Specifically, mice were infected with lymphocytic choriomeningitis virus (LCMV) and allowed to eradicate the infection. After lymphocyte contraction and the development of LCMV-specific memory T cell populations, Cre activity was induced. LCMV-specific memory T cells, as assessed by LCMV-GP33:MHC tetramer staining, underwent efficient Cre-mediated deletion based on YFP expression from a Cre reporter. Despite loss of SLP-76 mediated signals, a LCMV antigen-specific memory population persists in peripheral blood out to eight weeks after SLP-76 deletion. These data are consistent with a model in which cytokine signals can mediate memory T cell survival in the absence of any TCR-mediated signals.