CONFERENCE SCHEDULE

All Sessions: Merrill Hall

Saturday, January 26th		
8:00 PM	***The Dan H. Campbell Memorial Lecture***	
9:00–11:00 PM	***Reception in the Nautilus Room***	
Sunday, January27th		
8:30–12:00 Noon	**Session I**	**Lymphocyte development and differentiation**
4:00– 6:00 PM	**Poster Session**	***Fred Farr Forum and Kiln Rooms***
7:30–10:00 PM	**Session II**	**Pattern recognition and other innate immune strategies**
10:00–11:00 PM	***Reception***	***Fred Farr Forum and Kiln Rooms***
Monday, January 28th		
8:30–12:00 Noon	**Session III**	**The immune response to infection**
4:00– 6:00 PM	**Poster Session**	**Fred Farr Forum and Kiln Rooms**
4:00- 6:00 PM	**Oral Poster Presentation Session, Merrill Hall, *sponsored by The American Association of Immunologists***	
7:30–10:00 PM	**Session IV**	**Intestinal immunity and host-commensal interactions**
10:00–11:00 PM	***Reception***	***Fred Farr Forum and Kiln Rooms***
Tuesday, January 29st		
8:30–12:00 Noon	**Session V**	**Breaking tolerance: autoimmunity and inflammatory disease**

CONFERENCE PROGRAM

SESSION I

Sunday Morning **Lymphocyte development and differentiation**

8:30-12:00 Noon ***Chairperson: Kristin A. Hogquist***

Speakers:

Kristin A. Hogquist
University of Minnesota, Minneapolis, Minnesota
"**Self-recognition in T cell development"**

Albert Bendelac
University of Chicago, Chicago, Illinois
"**Differentiation of Bcl6 and PLZF expressing lineages"**

Paul M. Allen
Washington University in Saint Louis, St. Louis, Missouri
"A voltage-gated sodium channel is essential for positive selection of $CD4^+$ T cells"

Chandrashekhar Pasare
University of Texas Southwestern at Dallas, Dallas, Texas
"Priming micro-environments, cytokine cues and T cell differentiation"

Two Short Presentations chosen from abstracts

Sunday Afternoon
4:00 – 6:00 PM

POSTER SESSION and informal discussion groups.

SESSION II

Sunday Evening
7:30–10:00

Pattern Recognition and other Innate Immune Strategies
Chairperson: Gregory M. Barton

Speakers:

Gregory M. Barton
University of California, Berkeley, Berkeley, California
"Regulation of endosomal Toll-like receptors"

Zhijian (James) Chen
University of Texas Southwestern at Dallas, Dallas, Texas
"Innate immune sensing and signaling of cytosolic DNA through a cyclic dinucleotide pathway

Jessica A. Hamerman
Benaroya Research Institute at Virginia Mason, Seattle, Washington
"TLRs, interferons and myelopoiesis"

Daniel Kastner
National Human Genome Research Institute, Bethesda, Maryland
"Horror autoinflammaticus: The expanding spectrum of systemic autoinflammatory disease"

SESSION III

Monday Morning
8:30-12:00 Noon

The Immune Response to Infection
Chairperson: Denise Monack

Speakers:

Denise Monack
Stanford University, Stanford, California
"Persistent *Salmonella* infections require a regulator of host fatty acid metabolism"

Daniel L. Barber
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland
"Mechanisms of Mycobacteria-associated Immune Reconstitution Inflammatory Syndrome"

John Harty
University of Iowa, Iowa City, Iowa
"Inflammatory cues optimize CD8 T cell responses"

Bali Pulendran
Emory University, Atlanta, Georgia
"Systems vaccinology: enabling rational vaccine design with systems biology"

Two Short Presentations chosen from Abstracts

Monday Afternoon

4:00 – 6:00 PM **Oral Poster Presentations (Merrill Hall). Sponsored by AAI.**

4:00 – 6:00 PM **POSTER SESSION**

SESSION IV

Monday Evening

7:30 -10:00 PM

Intestinal Immunity and Host-Commensal Interactions

Chairperson: Lora Hooper

Speakers:

Lora Hooper
University of Texas Southwestern at Dallas, Dallas, Texas
"Sensing of environmental signals by the intestinal epithelium"

Chyi-Song Hsieh
Washington University in Saint Louis, St. Louis, Missouri
"Education of the immune system by commensal microbiota"

Tanya Golovkina
University of Chicago, Chicago, Illinois
"Retroviruses and commensal microbiota"

Marco Colonna
Washington University in Saint Louis, St. Louis, Missouri
"Innate Lymphoid Cells in Mucosal Immunity"

Awards Presentations to Postdoctoral, Graduate and Young Investigators.
Ray Owen Poster Awards; Council Poster Awards
Ray Owen Young Investigator Awards; Presentation Awards; Travel Awards
(Sponsored by AAI, BioLegend and MCI Council)

SESSION V

Tuesday Morning

8:30-12:00 Noon

Breaking tolerance: autoimmunity and inflammatory disease

Chairperson: Alexander Y. Rudensky

Speakers:

Alexander Y. Rudensky
Memorial Sloan-Kettering Cancer Center, New York City, New York
"Regulatory T cells control NK cells by limiting IL-2-mediated T cell help"

Mark S. Anderson
University of California, San Francisco, San Francisco, California
"Control of immune tolerance by Aire-expressing cells"

Philip Greenberg
University of Washington, Seattle, Washington
"Therapeutic targeting of tumor antigens that are self-proteins with T cells"

Robert L. Coffman
Dynavax Technologies, Berkeley, California
"TLR recognition of DNA and RNA in autoimmunity and inflammation"

Self-recognition in T cell development

Kris Hogquist, Gretta Stritesky, Keli Holzapfel, and You-Jeong Lee

Center for Immunology, University of Minnesota

Minneapolis, Minnesota

The healthy adaptive immune system is responsive to foreign antigens, and tolerant to self. However, several types of T cells have, and even require, substantial self-reactivity for their particular functions in immunity: nTreg cells, iNKT cells, and nIELs. We used a Nur77GFP reporter mouse to understand the self-reactivity of various T cell populations, define the relative rates of positive and negative selection, and examine the consequences of NKT cell self-reactivity in particular.

1. Moran, A.E., Holzapfel K.L. Xing, Y., Cunningham, N.R., Maltzman, J.S., Punt, J., and **K.A. Hogquist** T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. *J. Exp. Med.* 208:1279 (2011).
2. Weinreich, M.A., Odumade, O.A., Jameson S.C., and **K.A. Hogquist**. PLZF+ T cells regulate memory-like CD8 T cell development. *Nature Immunology*, 11:709 (2010).

Differentiation of Bcl6 and PLZF expressing lineages

Albert Bendelac
University of Chicago, Howard Hughes Medical Institute
Chicago, Illinois

The BTB-ZF transcription factors Bcl6 and PLZF critically regulate major lymphoid lineages, including germinal center B cells (GCB) and follicular helper T cells (Tfh) [1], and NKT cells [2]. We have shown that both Bcl6 and PLZF bound the E3 ubiquitin ligase cullin 3 and transported it to target genes where they were associated with a large complex made of histone-modifying proteins [3]. Proteomic analysis indicated that this transport was associated with changes of ubiquitination in histones and the genome organizers SATB1 and LaminB1. Conditional ablation of cullin 3 resulted in selective alterations of the transcriptional programs associated with Bcl6 and PLZF. Surprisingly, while germinal center B cells and NKT cells were absent, follicular helper T cell responses were massively exaggerated. Our current studies suggest that Bcl6-cullin 3 complexes mediate a negative feedback loop that limits Tfh responses.

References

1 Crotty, S. Follicular helper CD4 T cells (TFH). *Annual review of immunology* **29**, 621-663, doi:10.1146/annurev-immunol-031210-101400 (2011).

2 Seiler, M. P. *et al.* Elevated and sustained expression of the transcription factors Egr1 and Egr2 controls NKT lineage differentiation in response to TCR signaling. *Nature immunology* **13**, 264-271, doi:10.1038/ni.2230 (2012).

3 Mathew, R. *et al.* BTB-ZF factors recruit the E3 ligase cullin 3 to regulate lymphoid effector programs. *Nature* **491**, 618-621, doi:10.1038/nature11548 (2012).

A voltage-gated sodium channel is essential for positive selection of $CD4^+$ T cells

Paul M. Allen
Department of Pathology and Immunology
Washington University School of Medicine
St. Louis, MO 63110

Positive selection of T cells requires sustained Ca^{2+} influx induced by weak peptide/MHC ligands. The molecular mechanism governing sustained Ca^{2+} entry into double positive (DP) thymocytes during positive selection remains poorly defined. Our lab has previously identified an endogenous positively selecting self peptide, gp250, which induces positive selection *in vitro* of AND TCR. To determine if gp250 was the dominant positive selecting ligand of AND T cells, we introduced the gp250 deficiency onto the AND TCR transgenic H-2^k background to generate a mouse that genetically lacked the protein gp250. Deficiency of gp250 significantly impaired the positive selection of AND CD4 T cells in thymus. Intrathymically injected gp250 peptide to the gp250 deficient mouse restored positive selection of AND CD4 T cells, indicating the impaired positive selection resulted from gp250 deficiency. These results show that gp250/I-E^k, despite its weak interaction with the AND TCR, functions essentially as the dominant selecting ligand for AND TCR. With the novel system of naturally occurring positive selection gp250 and AND TCR, we wanted to characterize molecular mechanism of positive selection signals. We found gp250/I-E^k induced a sustained Ca^{2+} signal and in vitro positive selection of AND thymocytes, whereas the MCC agonist induced a transient Ca^{2+} signal and negative selection. Comparison of transcripts of AND DP thymocytes stimulated with gp250/I-E^k with those stimulated with MCC/I-E^k identified several candidate genes that might function specifically in positive selection. Through analysis of these candidates, we made the novel observation that DP thymocytes expressed a VGSC (composed of a pore-forming SCN5A subunit and a regulatory SCN4B subunit). The expression of both subunits was precisely correlated with β selection and positive selection, and the expression was exclusively maintained by positive selection signals. We hypothesized positive selection signals through T cell receptor activate VGSCs, depolarizing the membrane and activating some type of voltage-gated Ca^{2+} channel (CaV) to induce sustained Ca^{2+} flux. Pharmacological inhibition of the VGSC pore impaired *in vitro* positive selection and diminished DP thymocytes to induce Ca^{2+} influx in respond to positive selection signals. Peripheral AND $CD4^+$ T cells transfected with the VGSC, which they normally do not express, gained the ability to respond to positively selecting ligands, directly demonstrating that expression of the VGSC was responsible for increased sensitivity to weak TCR ligands. In lentiviral bone marrow reconstitution, shRNA knockdown of scn5a specifically inhibited thymic selection of $CD4^+$ T cells. This novel finding of an active VGSC in thymocytes provides a mechanism by which a weak positive selecting signal can induce the sustained Ca^{2+} signals required for $CD4^+$ T cell development.

Lo, W-L., N.J. Felix, J.J. Walters, H. Rohrs, M.L. Gross, and P.M. Allen. 2009. An endogenous peptide positively selects and augments the activation and survival of peripheral $CD4^+$ T cells. Nat. Immunol. 10:1155-1161.

Morris, G.P. and P.M. Allen. 2012. How the TCR balances sensitivity and specificity for the recognition of self and pathogens**.** Nat Immunol. 13:121-128

Lo, W.L., D.L. Donermeyer, and P.M. Allen. 2012. A voltage-gated sodium channel is essential for the positive selection of $CD4^+$ T cells. Nat. Immunol. 13:880-887.

Priming Micro-environments, Cytokine cues and T cell differentiation

Chandrashekhar Pasare
Department of Immunology
University of Texas Southwestern Medical Center
Dallas, Texas

Activation of pattern recognition receptors on dendritic cells (DCs) leads to their maturation, which is critical for activation of naïve CD4 T cells. Activated DCs also secrete several pro-inflammatory cytokines, including Interleukin (IL)-12, IL-6 and IL-1, all of which play an important role in activation and differentiation of various CD4 T cell lineages. The cytokine IL-12 plays a major role in differentiation of Th1 lineage cells whereas a combination of IL-6 and Transforming growth factor-beta (TGF-beta) has been demonstrated to control and guide Th17 lineage differentiation. Our recent studies have discovered that cytokine requirements for Th17 polarization depend entirely on the site of priming. While IL-6 plays a critical role in Th17 lineage priming in mucosal tissues such as the lamina propria of the gut and the lungs, it is not required for Th17 priming in the spleen. However, IL-1R mediated MyD88 dependent signaling in CD4 T cells plays an irreplaceable role in Th17 priming in all tissues. Importantly, we find that DC populations resident in the spleen and lamina propria guide IL-6 independent and dependent pathways of Th17 differentiation, respectively. While $CD103^{hi}$ DCs are absent in the spleen, they are present in mucosal tissues and the skin and play an important role in regulating Th17 differentiation. Our studies have revealed that $CD103^{hi}$ DCs impose the requirement of IL-6 for Th17 priming in both lamina propria of the gut as well as the skin draining lymph nodes. Additionally, we have also discovered that the function of $CD103^{hi}$ DCs can be perturbed by gut microflora that can lead to IL-6 independent priming of Th17 cells in the intestines. These results reveal fundamental differences by which systemic and mucosal immune systems regulate Th17 cell lineage differentiation[1]. Further work will focus on the dynamic regulation of $CD103^{+}$ DC by the intestinal microbiota, and the role of $CD103^{+}$ DCs in controlling intestinal immune homeostasis.

References

1. Hu, W., Troutman, T.D., Edukulla, R., and **Pasare, C.** (2011). Priming microenvironments dictate cytokine requirements for T helper 17 cell lineage commitment. **Immunity** *35*, 1010-1022.

Regulation of endosomal Toll-like receptors

Gregory M. Barton, Bettina Lee, Zachary Newman, Joanne Moon,
Jeffrey Shu, Randy Schekman
Department of Molecular & Cell Biology
University of California, Berkeley

The use of nucleic acids as a signature of infectious non-self by Toll-like receptors (TLRs) 7 and 9 exposes the host to potential self recognition and autoimmunity. We have proposed that tolerance to self nucleic acids requires the restriction of receptor activation to endolysosomes via receptor proteolysis, and we are studying how the localization of endosomal TLRs is regulated. Our recent studies indicate that the trafficking of individual TLRs is differentially controlled by the multi-pass transmembrane protein, Unc93b1. Such differential regulation may explain how specific TLRs make distinct contributions to autoimmune diseases.

References:

1. Ewald SE, Lee BL, Lau L, Wickliffe KE, Shi G-P, Chapman HA, and Barton GM. The ectodomain of Toll-like receptor 9 is cleaved to generate a functional receptor. *Nature* (2008) 456:658-662.
2. Ewald SE, Engel A, Lee J, Wang M, Bogyo M, Barton GM. Nucleic acid recognition by Toll-like receptors is coupled to stepwise processing by cathepsins and asparagine endopeptidase. *Journal of Experimental Medicine* (2011) 208:643-651.
3. Mouchess ML, Arpaia N, Souza G, Barbalat R, Ewald SE, Lau L, Barton GM. Transmembrane mutations in Toll-like Receptor 9 bypass the requirement for ectodomain proteolysis and induce fatal inflammation. *Immunity*, (2011) 35:1-12.

Innate immune sensing and signaling of cytosolic DNA through a cyclic dinucleotide pathway

Zhijian 'James' Chen

Department of Molecular Biology
University of Texas Southwestern Medical Center, Dallas, Texas

The inappropriate presence of DNA in the cytosol is a danger signal that alerts the host of potential microbial invasion and triggers innate immune responses including the production of type-I interferons. Under certain pathological conditions, self DNA, including those derived from retroelements, could also trigger autoimmune responses from the cytosol, resulting in human diseases such as lupus. Cytosolic DNA induces interferons through a signaling pathway that involves the adaptor protein STING, the kinases IKK and TBK1, and the transcription factors NF-κB and IRF3. How DNA is detected in the cytosol and how DNA triggers STING activation remain poorly understood. Through a biochemical approach, we identified a cyclic GMP-AMP synthase (cGAS) that catalyzes the synthesis of cyclic GMP-AMP (cGAMP) in a manner that depends on the binding of cGAS to DNA. cGAMP binds to and activates STING, leading to the induction of interferons and other cytokines. Our results not only reveal cGAS as a cytosolic DNA sensor that triggers the type-I interferon pathway, but also uncover a cyclic dinucleotide signaling pathway that was previously not known to exist in metazoa. The finding that cGAMP functions as a second messenger in the cytosolic DNA pathway provides a new signaling mechanism in innate immunity. Furthermore, our work suggests that cGAS is an attractive therapeutic target for the treatment of autoimmune diseases.

References:

1) Sun, L., Wu, J., Du, F., Chen, X., and Chen, Z.J. (2012). Cyclic GMP-AMP Synthase Is a Cytosolic DNA Sensor That Activates the Type I Interferon Pathway. ***Science***. (in press)

2) Wu, J., Sun, L., Chen, X., Du, F., Shi, H., Chen, C., and Chen, Z.J. (2012). Cyclic GMP-AMP Is an Endogenous Second Messenger in Innate Immune Signaling by Cytosolic DNA. ***Science***. (in press)

3) Tanaka, Y., and Chen, Z.J. (2012). STING Specifies IRF3 Phosphorylation by TBK1 in the Cytosolic DNA Signaling Pathway. ***Science Signaling*** *5*, ra20.

4) Chiu, Y.H., Macmillan, J.B., and Chen, Z.J. (2009). RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. ***Cell*** *138*, 576-591.

TLRs, interferons and myelopoiesis

Matthew B. Buechler[*†], Thomas H. Teal[‡], Keith B. Elkon[*‡] and **Jessica A. Hamerman**[*†]

[*]Immunology Program, Benaroya Research Institute at Virginia Mason, Seattle, WA 98101
[†]Department of Immunology, University of Washington, Seattle, Washington
[‡]Division of Rheumatology, University of Washington, Seattle, Washington

During infection and inflammation, myeloid cell output from the bone marrow increases in a process called emergency myelopoiesis. To examine the determinants of this process, we examined mice overexpressing TLR7 (TLR7.1 mice), a model of SLE pathogenesis with peripheral myeloid expansion. We show that TLR7.1 mice have a dramatic expansion of splenic cells that derive from granulocyte/macrophage progenitors (GMP) compared to WT mice. In the bone marrow, TLR7.1 mice exhibited hallmarks of emergency myelopoiesis and contained a discrete population of Sca-1^{+} GMP, termed emergency GMP (eGMP). The emergency myelopoiesis and peripheral myeloid expansion in TLR7.1 mice was dependent on type I IFN signaling. TLR7.1 plasmacytoid DC were cell-intrinsically activated by TLR7 overexpression and constitutively produced type I IFN directly ex vivo. In wild-type mice, acute TLR7 agonist administration and infection with the RNA virus LCMV also drove type I IFN-dependent eGMP generation. eGMP are more proliferative in vivo and are superior myeloid progenitors than classical Sca-1^{-} GMP. Therefore, type I IFN can act upon myeloid progenitors to promote the development of eGMP, which leads to an expansion of their progeny in the periphery.

Buechler, MB, Teal, TH, Elkon, KB, Hamerman, JA. Cutting Edge: Type I IFN drives emergency myelopoiesis and peripheral myeloid expansion during chronic Toll-like receptor 7 signaling. *Journal of Immunology* (2013) 190 (3).

Horror Autoinflammaticus: The Expanding Spectrum of Systemic Autoinflammatory Disease

Dan Kastner, M.D., Ph.D.
Scientific Director, National Human Genome Research Institute

The genetic and genomic analysis of inherited disorders of inflammation has had a major impact on our understanding of innate immunity in man. In this lecture I will focus on three topics. The first involves the study of a number of Mendelian disorders that we now know are caused by mutations in genes involved in IL-1β activation. These illnesses include familial Mediterranean fever (FMF), the cryopyrin-associated periodic syndromes (CAPS, which include familial cold autoinflammatory syndrome, Muckle-Wells syndrome, and neonatal-onset multisystem inflammatory disease), the deficiency of the IL-1 receptor antagonist (DIRA), and the syndrome of pyogenic arthritis with pyoderma gangrenosum and acne (PAPA). Although they are phenotypically distinct, excessive IL-1β signaling plays an important role in the pathogenesis of each of these illnesses, as evidenced by the effectiveness of therapies targeting this cytokine, and there is an emerging body of data implicating IL-1β in the pathogenesis of other more common, genetically complex illnesses. Recent work from our laboratory implicates intracellular Ca^{2+} and cAMP in the regulation of IL-1β activation. The second topic that I will discuss concerns the investigation of two related phenotypes, each of which is caused by mutations in *PLCG2*. One of these illnesses, now denoted PLAID (phospholipase $C\gamma_2$-associated antibody deficiency and immune dysregulation), was discovered through classical genetic analysis, and is characterized by cold-induced urticaria, with varying degrees of both autoimmunity and immunodeficiency. It is caused by dominantly inherited genomic deletions in the autoinhibitory domain of *PLCG2,* leading to constitutive enzyme activation but paradoxically reduced signaling in leukocyte subsets. The related phenotype, APLAID (autoinflammatory PLAID), was discovered by whole-exome sequencing, and is caused by the S707Y missense change in the same autoinhibitory domain disrupted in PLAID. Finally, I will review recent genome-wide association studies our laboratory has performed in Behçet's disease. In addition to confirming the long-recognized association with *HLA-B*51,* GWAS establishes an association of Behçet's disease with common variants in several other non-MHC loci, including *IL10, IL23R, CCR1, KLRC4, ERAP1,* and *STAT4*, while deep resequencing indicates a role for rare variants in some of these loci, and in others regulating the innate immune system.

Persistent *Salmonella* infections require a regulator of host fatty acid metabolism

Denise M. Monack, Ph.D.
Department of Microbiology and Immunology
Stanford University, Stanford, California

The global burden of disease caused by intracellular pathogens remains one of the largest challenges facing the international biomedical community. Millions of human cases of salmonellosis are reported worldwide every year, resulting in huge economic costs to society and thousands of deaths. We have shown previously in a mouse typhoid model that *Salmonella* persists in macrophages within systemic tissues of chronically infected mice. However, very little is known about the physiological state of the macrophages that harbor intracellular *Salmonella* in chronic carriers. We show that *Salmonella* preferentially associates with anti-inflammatory/M2 macrophages at later stages of infection. In addition, we show that a host transcriptional factor that plays a role in sustaining fatty acid metabolism in M2 macrophages, is upregulated in *Salmonella*-infected macrophages. In the absence of this regulator *Salmonella* is unable to replicate in macrophages and we link this to availability of glucose. Altogether, our work describes the molecular mechanisms underlying a bacterial pathogen's dependence on macrophage metabolism for chronic carriage.

References:

Monack, D.M., D.M. Bouley, and S. Falkow. 2004. *Salmonella typhimurium* persists within macrophages in the mesenteric lymph nodes of chronically infected *Nramp1*$^{+/+}$ mice and can be reactivated by IFNγ neutralization. J. Exp. Med. 199:231-241.

Lawley, T.D., K. Chan, L.J. Thompson, C.C. Kim, G.R. Govoni, and **D.M. Monack**. 2006. Genome-wide screen for *Salmonella* genes required for long-term systemic infection of the mouse. PLoS Pathog. Feb;2(2):e11. Epub Feb 24.

Lawley, T.D., D.M. Bouley, Y.E. Hoy, C. Gerke, D.A. Relman and **D.M. Monack**. 2007. Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and the indigenous intestinal microbiota. Infect. Immun. 76(1):403-16.

Arpaia, N., J. Godec, L. Lau, K.E. Sivick, L.M. McLaughlin, M.B. Jones, T. Drachev, S.N. Peterson, **D.M. Monack**, and G.M. Barton. 2011. TLR signaling is required for Salmonella typhimurium virulence.Cell. 144(5):675-88.

Mechanisms of Mycobacteria-associated Immune Reconstitution Inflammatory Syndrome

Daniel L. Barber
Laboratory of Parasitic Diseases
National Institutes of Health, National Institute of Allergy and Infectious Diseases
Bethesda, Maryland

Antiretroviral therapy significantly extends the survival of HIV infected individuals by restoring host protective immunity. However, instead of the expected improvement in symptoms, some HIV patients experience a rapid deterioration within the first few weeks after the initiation of antiviral therapy. This adverse outcome of ART, referred to as Immune Reconstitution Inflammatory Syndrome (IRIS) has become a major problem in the clinical management of the HIV pandemic, and the only available treatment strategy is to immunosuppress these HIV patients with corticosteroids. Little is understood about its root causes or the mechanisms of pathogenesis, but patients with severe lymphopenia, concurrent opportunistic infection (OI), and the most robust CD4 T cell reconstitution following ART are the most susceptible to developing IRIS. In fact, it is thought that the reconstituting CD4 T cells specific for the OI mount dysregulated responses that drive the pathology of IRIS. To investigate the basic mechanisms of disease, we have established a robust murine model of experimentally inducible IRIS that recapitulates the restoration of CD4 T cell responses in T cell deficient hosts with an established OI. Although CD4 T cells are critical for control of mycobacteria, adoptive transfer of CD4 T cells into T cell deficient mice harboring a chronic *Mycobacterium avium* infection leads to a fatal wasting disease, and most mice succumb within 3 weeks of T cell transfer. Using this model we investigate the mechanisms of immune reconstitution disease with the goal of developing novel therapeutic strategies that allow for the safe restoration of immunity in HIV infected individuals.

References:

1. Barber, D. L. *et al.* Th1-driven immune reconstitution disease in Mycobacterium avium-infected mice. *Blood* **116**, 3485–3493 (2010).
2. Barber, D. L., Andrade, B. B., Sereti, I. & Sher, A. Immune reconstitution inflammatory syndrome: the trouble with immunity when you had none. *Nat. Rev. Microbiol.* **10**, 150–156 (2012).

Inflammatory cues optimize CD8 T cell responses

Gabriel Starbeck-Miller, Hai-Hui Xue and John T. Harty
Department of Microbiology, Pathology and Interdisciplinary Program in Immunology
University of Iowa, Iowa City, Iowa

Naïve CD8 T-cell activation requires TCR ligation and costimulation to induce cellular division. However, optimal accumulation of activated CD8 T-cells requires direct inflammatory cytokine signaling (–signal 3" cytokine). While *in vitro* studies suggest –signal 3" cytokines enhance T-cell survival and not division, the mechanisms underlying optimal accumulation of CD8 T-cells *in vivo* are unknown. In particular, it is unclear how inflammatory cytokines, which are transiently produced early after infection, affect T-cell accumulation at the peak of the response that occurs many days later. Here, we show that direct exposure of CD8 T-cells to –signal 3" cytokines early during the immune response maintains surface expression of CD25, the high affinity IL-2 receptor. This fosters sustained division of activated CD8 T cells in response to endogenous IL-2, through activation of the PI3K/Akt pathway and expression of FoxM1, a positive regulator of cell-cycle progression genes. Thus, –signal 3" cytokines optimize effector CD8 T-cell accumulation through a temporally orchestrated sequence of cytokine signals that sustain division.

Relevant citations

1. Badovinac, V.P., K.A.N. Messingham, A. Jabbari, J.S. Haring and **J. T. Harty** (2005) Accelerated generation of memory CD8+ T cells and prime-boost response after dend cell vaccination. **Nature Medicine. 11:** 748-756**.**
2. Haring, J.S., V.P. Badovinac and **J.T. Harty** (2006) Inflaming the CD8+ T cell respor **Immunity. 25:**19-29.
3. Pham, N.-L. L., V.P. Badovinac and **J. T. Harty** (2009) A default pathway of memory CD8 T cell differentiation after dendritic cell immunization is deflected by encounter with inflammatory cytokines during antigen-driven proliferation. **Journal of Immunology. 183:** 2337-2348. PMCID: PMC2786780
4. Pham, N.-L.L., V.P. Badovinac and **J.T. Harty** (2010) Differential role of –signal 3" inflammatory cytokines in regulating CD8 T cell expansion and differentiation. **Frontiers in Immunology.** Front. Immun. doi: 10.3389/fimmu.2011.00004**.**

Systems Vaccinology: enabling rational vaccine design with systems biology

Bali Pulendran
Emory University, Atlanta, Georgia

Despite their great success, we understand little about how effective vaccines stimulate protective immune responses. Two recent developments promise to yield such understanding: the appreciation of the crucial role of the innate immune system in sensing microorganisms and tuning immune responses, and advances in systems biology. In this presentation, I will discuss how these developments are yielding insights into the mechanism of some of the most successful vaccines ever developed. Furthermore, such developments promise to address a major challenge\ in vaccinology: that the efficacy of a vaccine can only be ascertained retrospectively, upon infection. The identification of molecular signatures induced rapidly after vaccination, which correlate with and predict the later development of protective immune responses, would represent a strategy to prospectively determine vaccine efficacy. Such a strategy would be particularly useful when evaluating the efficacy or immunogenicity of untested vaccines, or inidentifying individuals with sub-optimal responses amongst high risk populations, such as infants or the elderly. We have recently used a systems biology approach to identify early gene signatures that correlate with, and predict the later immune responses in humans vaccinated with the live attenuated yellow fever vaccine YF-17D, or with the influenza vaccines. I will review these studies, and discuss their broader implications for vaccinology.

References

1. Systems vaccinology. Pulendran B, Li S, Nakaya HI. *Immunity*. 2010 Oct 29;33(4):516-29.
2. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, Kennedy K, Wu H, Bennouna S, Oluoch H, Miller J, Vencio RZ, Mulligan M, Aderem A, Ahmed R, Pulendran B. *Nat Immunol.* 2009 Jan;10(1):116-25. Epub 2008 Nov 23.
3. Immunological mechanisms of vaccination. Pulendran B, Ahmed R. *Nat Immunol.* 2011 Jun;12(6):509-17.
4. Systems biology of vaccination for seasonal influenza in humans. Nakaya HI, Wrammert J, Lee EK, Racioppi L, Marie-Kunze S, Haining WN, Means AR, Kasturi SP, Khan N, Li GM, McCausland M, Kanchan V, Kokko KE, Li S, Elbein R, Mehta AK, Aderem A, Subbarao K, Ahmed R, Pulendran B. *Nat Immunol.* 2011 Jul 10;12(8):786-95. doi: 10.1038/ni.2067

Sensing of environmental signals by the intestinal epithelium

Lora V. Hooper
Department of Immunology
The University of Texas Southwestern Medical Center
Dallas, Texas

The mammalian intestine is home to ~100 trillion bacteria that perform important metabolic functions for their hosts. Intestinal epithelial cells are an important physical interface between host tissues and the vast populations of luminal bacteria, and play a central role in directing immune responses to the microbiota. We are investigating how intestinal epithelial cells sense environmental signals such as the microbiota, nutrients, and light, and how they integrate these signals to modulate immune responses that protect against bacterial invasion of mucosal tissues. We have shown that intestinal bacteria direct expression of key antimicrobial proteins that are essential for maintaining physical separation between the microbiota and the small intestinal epithelial surface. In addition, we are currently investigating how light signals and the circadian clock transcriptional network impact the development of epithelial immune responses to the intestinal microbiota, and how these influences impact the development of adaptive immunity to the microbiota. Together, our findings set the stage for a detailed understanding of how intestinal epithelial cells integrate environmental signals to direct innate and adaptive immunity to the microbiota.

References

1. Cash, H. L., Whitham, C. V., Behrendt, C. L., and Hooper, L. V. (2006) Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 313, 1126-1130.

2. Vaishnava, S., Behrendt, C. L., Ismail, A.S., Eckmann, L., and Hooper, L. V. (2008) Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proc. Natl. Acad. Sci. USA* 105, 20858-20863.

3. Vaishnava, S., Yamamoto, M., Severson, K.M., Ruhn, K.A., Yu, X., Koren, O., Ley, R., Wakeland, E.K., and Hooper, L.V. (2011) The antibacterial lectin RegIIIγ promotes the spatial segregation of microbiota and host in the intestine. *Science* 334, 255-258.

4. Hooper, L.V., Littman, D.R., Macpherson, A.J. (2012) Interactions between the microbiota and the immune system. *Science* 336, 1268-1273.

Education of the Immune System by Commensal Microbiota

Chyi-Song Hsieh. Katherine Nutsch, Sindhuja Rao, Stephanie Lathrop
Dept. of Medicine, Div. of Rheumatology, Washington University, St. Louis, MO, USA 63110

T cell tolerance to self originates via an orchestrated process in which T cells capable of causing autoimmunity are eliminated or differentiated into Foxp3$^+$ regulatory T (Treg) cells at an early stage in their development before they are released as mature cells with pathogenic capacity. However, the gut represents a unique problem for the immune system as it harbors a large number of commensal microbes in constant contact with the body, and yet inappropriate immune responses to these bacteria may lead to inflammatory bowel disease. Our studies suggest that the process of tolerance in the gut is dependent on the peripheral differentiation of naïve T cells into Foxp3+ induced Treg (iTreg) cells in response to commensal bacterial antigens. Based on these observations, we proposed that the immune system is educated such that it becomes tolerant to the non-self antigens derived from commensal bacteria. We have subsequently generated transgenic mice that express iTreg TCRs that are dependent on specific commensal microbiota. We found that naïve iTreg TCR transgenic cells rapidly and efficiently differentiate into Foxp3+ cells after transfer into normal congenic hosts, with up to 50% Foxp3+ cells observed within 1 week, and 80%+ by 3 weeks. Interestingly, the newly developed Foxp3+ cells appear to have undergone the greatest number of divisions, suggesting that Treg cell differentiation is an active process, rather than simply the avoidance of other effector T cell fates. Consistent with the notion of microbial antigen recognition, this process is inhibited by broad spectrum antibiotics. While nTreg TCR transgenic cells also undergo peripheral conversion, only the iTreg TCR transgenic cells traffic to the colon. Thus, commensal bacteria can efficiently direct the differentiation of naïve T cells to become tolerogenic Foxp3+ iTreg cells in the gut.

Relevant citations of our work in this area:

Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, Santacruz N, Peterson DA, Stappenbeck TS, Hsieh CS. Peripheral education of the immune system by colonic commensal microbiota. *Nature*, 2011: 478:250-4.

Supported by NIH/NIAID, Crohn's Colitis Foundation, and the Burroughs Wellcome Fund.

Retroviruses and commensal microbiota

Tatyana Golovkina
Department of Microbiology
University of Chicago

Most viral pathogens launch acute infections, whereby the virus replicates rapidly and disseminates to another organism prior to immune clearance or death of the host. In contrast, some viruses are able to establish persistent infections through adoption of complex relationships with their hosts and manipulation of a wide array of cellular mechanisms for their own advantage. Even though persistent viruses have evolved distinct mechanisms to enable long-term survival in the host, they all share a common trait—the ability to evade the immune system. These viruses are often transmitted most efficiently through mucosal surfaces rich in microbiota, as in the case of Mouse Mammary Tumor Virus. We found that MMTV, when ingested by newborn mice, stimulates unresponsiveness towards viral antigens (1). This unresponsiveness alleviates TLR7-mediated anti-retrovirus immunity (2) and allows for virus indefinite persistence. This process requires intestinal microbiota, as antibiotic-treated mice or germ-free mice do not transmit infectious virus to their offspring (1). The MMTV-induced tolerance pathway involves activation of Toll-like receptor 4 by lipopolysaccharide and subsequent IL-6-dependent production of the inhibitory cytokine IL-10 (1). Thus, MMTV has evolved to rely on the interaction with omnipresent microbiota to induce the neonatal oral tolerance pathway.

We have expanded our studies on the role of commensal microbiota in transmission of another retrovirus, which can be spread as both blood borne and an oral pathogen.

References

1. **Kane, M., L. K. Case, K. Kopaskie, A. Kozlova, C. MacDearmid, A. V. Chervonsky, and T. V. Golovkina.** 2011. Successful transmission of a retrovirus depends on the commensal microbiota. Science **334:**245-249.
2. **Kane, M., L. K. Case, C. Wang, L. Yurkovetskiy, S. Dikiy, and T. V. Golovkina.** 2011. Innate Immune Sensing of Retroviral Infection via Toll-like Receptor 7 Occurs upon Viral Entry. Immunity **35:**135-145.

Innate Lymphoid Cells in Mucosal Immunity

MARCO COLONNA
Washington University School of Medicine, St. Louis, Missouri

Mucosal innate lymphoid cells (ILCs) promote immune responses to pathogens by producing distinct signature cytokines in response to changes in the cytokine microenvironment. We previously identified human NKp44+CCR6+ROR[?]t+AHR+ ILCs distinguished by IL-22 secretion. Here we characterized a human NK cell-related ILC subset, which we defined tissue-resident NK cells (NKTR). NKTR produced IFN-[g] in response to IL-12 and IL-15 and were unique for intraepithelial location, integrin profile, hallmarks of TGF-[ß] imprinting and memory/activated phenotype. Because this profile is shared by tissue-resident memory CD8 T cells, NKTR may represents their innate counterpart. In mice, NKTR were distinguished by CD160 and required the NK cell transcription factor NFIL3 for development, but not ROR[?]t, AHR or intestinal microbiota. Notably, NKTR were increased in Crohn's disease patients, while in mice contributed to intestinal pathology in the anti-CD40-induced model of colitis. Thus, NKTR may initiate IFN-[?] response against pathogens, but contribute to pathology when deregulated.

References

A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity.
Cella M, Fuchs A, Vermi W, Facchetti F, **Otero** K, Lennerz JK, Doherty JM, Mills JC, **Colonna** M.
Nature. 2009 Feb 5;457(7230):722-5.

AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch.
Lee JS, **Cella** M, McDonald KG, Garlanda C, Kennedy GD, Nukaya M, Mantovani A, Kopan R, Bradfield CA, Newberry RD, **Colonna** M.
Nat Immunol. 2011 Nov 20;13(2):144-51.

AHR and the Transcriptional Regulation of Type-17/22 ILC.
Lee JS, **Cella** M, **Colonna** M.
Front Immunol. 2012;3:10.

Regulatory T cells control NK cells by limiting IL-2-mediated T cell help

Georg Gasteiger[1,2], Saskia Hemmers[1,2], Matthew A. Firth[2], Joseph C. Sun[2], Alexander Y. Rudensky[1,2]

[1]Howard Hughes Medical Institute and [2]Immunology Program, Memorial Sloan-Kettering Cancer Center, Immunology Program, New York City, NY 10065

The emergence of the adaptive immune system took a toll in the form of pathologies mediated by self-reactive cells. Suppression by regulatory T (Treg) cells is essential for immune homeostasis and for "self" tolerance of T and B lymphocytes. We asked whether Treg cells are required to restrain NK cells, the third lymphocyte lineage, whose features combine innate and adaptive immune cell properties. We found that NK cell responses to "missing-self", but not "non-self" were contained by Treg cells through limiting IL-2 availability. Furthermore, we found that Treg cells restrained the expansion of immature $CD127^+$ NK cells, which also expressed IL2Rα (CD25). These results suggest that by limiting IL-2 availability Treg cells restrain the crosstalk between adaptive $CD4^+$ T cells and innate lymphocytes and, thereby, control their homeostasis and function.

Control of immune tolerance by Aire-expressing cells

Mark S. Anderson
University of California, San Francisco
San Francisco, California

Thymic epithelial cells play a central role in properly educating developing T cells to be simultaneously responsive to foreign antigens and tolerant to self-antigens. The latter process is completed in the medulla, where medullary thymic epithelial cells (mTECs) expressing the autoimmune regulator (Aire) synthesize a wide array of tissue-specific antigens (TSAs) to drive negative selection of autoreactive T cells. Aire is present in a distinct, non-dividing MHC II^{hi} subset that has been described as terminally differentiated. Here, we show that a novel *Aire-DTR* transgenic mouse strain allows specific ablation of Aire+ mTECs, but that repeated Aire+ mTEC ablation affects the entire mTEC compartment, leading to a breakdown in central tolerance. Using an inducible Aire-Cre fate mapping system, we find that ablation outside of Aire+ mTECs results in part from the existence of a post-Aire state. These post-Aire mTECs retain TSA expression while losing MHC II expression, suggesting that post-Aire mTECs may have a unique role in driving tolerance, and highlighting the existence of distinct developmental mTEC stages among mTEC subsets.

Therapeutic targeting of tumor antigens that are self-proteins with T cells

***Philip Greenberg*[1]*, Aude Chapuis*[1]*, Gunnar Ragnarsson*[1]*, Merav Bar*[1]*, Sebastian Ochsenreither*[1]*, Ravi Majeti*[3]*, Irv Weissman*[3]*, Tom Schmitt*[1]*, David Aggen*[2]*, David Kranz*[2]*, Ingunn Stromnes*[1]*, Cassie Chou*[1]*, and Andrea Schietinger*[1]

[1]Fred Hutchinson Cancer Research Center and University of Washington, Seattle, Washington; [2]University of Illinois, Urbana, Illinois; and [3]Stanford University, Palo Alto, California

Modulating T cell immunity to treat human malignancies is showing increasing promise, but substantive obstacles remain. Reproducibly effective therapy requires many factors to be in place, including having an appropriate antigenic target, generating a high avidity and high magnitude T cell response, and targeting the tumor with T cells that have the ability to infiltrate and retain function in the tumor microenvironment. We have been systematically investigating strategies to address these issues. Targetable tumor antigens need to be preferentially expressed by tumor compared to normal cells. To identify such targetable human leukemia antigens, we compared gene expression profiles in purified leukemic stem cells with profiles in normal hematopoietic stem cells and other somatic cells, and identified two promising targets that contribute to the leukemic phenotype, WT1 and Cyclin A1, and demonstrated that CD8 T cells can be generated that lyse leukemic cells. Generating sufficient numbers of such T cells with high avidity for the target in each patient is a substantive problem. We will discuss a just completed trial targeting WT1 in leukemia patients, which highlights the potential benefit of providing potent T cell responses to this pro-oncogenic protein. For this trial, we generated panels of WT1-specific CD8 T cells clones for each patient and then selected and expanded for adoptive therapy the highest avidity clone isolated. However, efficacy with this approach is limited by the quality of responses that can elicited for each patient, who often have compromised repertoires, and could be overcome by isolating T cell receptor (TCR) genes from a defined high avidity leukemia-reactive T cell clone that can be introduced into large numbers of patient T cells to create a standardized reagent for treatment. Even with this approach, the avidity of transduced T cells used for therapy is limited by the affinity of the introduced TCR, and high affinity TCRs for tumor antigens that are also normal self-antigens may not be readily identified in normal repertoires. Our lab has developed in collaboration with David Kranz' lab methods to mutate/alter the CDR3 regions of the isolated antigen-specific TCR chains prior to introduction into recipient T cells to improve the affinity for the target antigen, as well as methods to interrogate the full repertoire of β-chain rearrangements capable of pairing with a defined TCR α-chain and forming a high affinity TCR before negative selection. Strategies to evaluate the *in vivo* activity and safety of such TCRs in relevant mouse models will be described.

Unfortunately, providing a high avidity T cell response does not necessarily result in tumor eradication. Major obstacles include the development of tolerance/anergy and/or exhaustion/dysfunction in tumor-reactive T cells, particularly within the tumor microenvironment. We have explored these issues in T cell therapy models in mice with leukemia or that "spontaneously" develop solid tumors as a consequence of regulated tissue-specific expression of an oncogene. These studies highlight the difficulties inherent in sustaining responses to tumor antigens that are self-proteins as well as the inhibitory pathways commonly operative within the tumor microenvironment, and have provided insights into how to potentially sustain activity by selecting or genetically modifying T cells to be resistant to obstacles that prevent tumor eradication. However, different tumor types can engage distinct pathways and have unique characteristics, and thus understanding the immunobiology of the tumor to be treated will likely be essential for designing effective therapies.

1) Schietinger A, Delrow JJ, Basom RS, Blattman JN, Greenberg PD. Rescued tolerant CD8 T cells are preprogrammed to reestablish the tolerant state. *Scienc*e. 335:723-7 (2012).
2) Provasi E, Genovese P, Lombardo A, Magnani Z, Liu PQ, Reik A, Chu V, Paschon DE, Zhang L, Kuball J, Camisa B, Bondanza A, Casorati G, Ponzoni M, Ciceri F, Bordignon C, Greenberg PD, Holmes MC, Gregory PD, Naldini L, Bonini C. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. *Nat Med*. 18:807-15 (2012).
3) Ochsenreither S, Majeti R, Schmitt T, Stirewalt D, Keilholz U, Loeb KR, Wood B, Choi YE, Bleakley M, Warren EH, Hudecek M, Akatsuka Y, Weissman IL, Greenberg PD. Cyclin-A1 represents a new munogenic targetable antigen expressed in acute myeloid leukemia stem cells with characteristics of a cancer-testis antigen. *Blood*. 119:5492-501 (2012)
4) Chou CK, Schietinger A, Liggitt HD, Tan X, Funk S, Freeman GJ, Ratliff TL, Greenberg NM, Greenberg PD. Cell-intrinsic abrogation of TGF-β signaling delays but does not prevent dysfunction of self/tumor-specific CD8 T cells in a murine model of prostate cancer. *J Immunol*. 189:3936-46 (2012).

TLR recognition of DNA and RNA in autoimmunity and inflammation

Robert L. Coffman, Dynavax Technologies, Berkeley, California

The recognition of self molecules by cells of the innate immune system can be an important contributor to autoimmune disease. The clearest example of this comes from studies of the autoimmune disease, systemic lupus erythematosis. Lupus is characterized by circulating immune complexes containing self DNA or RNA that stimulate plasmacytoid dendritic cells (PDC) through 2 important pattern recognition receptors, TLR9 and TLR7, respectively. This stimulation of PDC leads to chronic elevation of type I Interferons in many patients and is thought to be important in disease pathogenesis. A second consequence of TLR7 and TLR9 signaling in PDC is to render them resistant to killing by glucocorticoids, possibly accounting for the need for very high doses of these agents to treat acute lupus flares. Signaling by self DNA and RNA through TLR9 and TLR7 makes a major contribution to other forms of non-microbial inflammation as well, such as skin injury mediated by repeated tape stripping. The innate response to self nucleic acids can contribute to wound healing, but can lead to chronic autoimmune inflammation in the presence of certain genetic or environmental cofactors. In contrast toTLR7 and TLR9, the contribution of TLR8, a second single-stranded RNA receptor, to inflammation and autoimmunity is poorly understood. This is partly due to the substantial differences in specificity and function of human TLR8 and the TLR8 ortholog in mice and rats. To develop a model for studying the functions of human TLR8, we have constructed a series of transgenic mouse lines expressing different copy numbers of the human TLR8 gene under control of its own promoter. Chimeras with high levels of humanTLR8 expression spontaneously develop a multiorgan inflammatory disease with indications of arthritis and pronounced involvement of the pancreas, leading to death within 2-4 months. This complex disease state can be transferred to wild-type mice by transfer of bone marrow from TLR8 transgenic chimeras, demonstrating that human TLR8 expression by hematopoietic cells is sufficient for inflammation. A human TLR8 transgenic line with relatively low copy number was fertile, generating a mouse strain that was healthy but significantly more susceptible to an induced model of rheumatoid arthritis. Thus, recognition of self ligands, presumably RNA, by human TLR8 can lead to a pattern of autoimmune inflammation that is distinct from that mediated by TLR7 or TLR9, reflecting the different cellular expression patterns of these receptors.

References:

1. Development of TLR inhibitors for the treatment of autoimmune diseases. F.J. Barrat & R.L. Coffman (2008) Immunol Rev. 223:271-8
2. Autoimmune skin inflammation is dependent on plasmacytoid dendritic cell activation by nucleic acids via TLR7 and TLR9. Guiducci C, Tripodo C, Gong M, Sangaletti S, Colombo MP, Coffman RL, Barrat FJ. (2010) J Exp Med. 207:2931-42
3. TLR recognition of self nucleic acids hampers glucocorticoid activity in lupus. Guiducci C, et al. (2010) Nature 465:937-41.