The 58th Midwinter Conference of Immunologists at Asilomar

January 26 -29, 2019

Asilomar Conference Grounds, Pacific Grove, California

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The Dan H. Campbell Memorial Lecture

Saturday, January 26, 8:00 PM
The Chapel Auditorium

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University of Minnesota

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The 2019 Midwinter Conference of Immunologists

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Contributions by Members of the Midwinter Conference of Immunologists

Contribution in Memory of Pierre Cairs

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The 2019 Midwinter Conference of Immunology at Asilomar
Pacific Grove, California (USA) www.midwconfimmunol.org

CONFERENCE SCHEDULE

All Sessions: The Chapel Auditorium

Saturday, January 26th		
4:00 pm	**Registration**	
8:00 pm	***The Dan H. Campbell Memorial Lecture***	
9:00–11:00 pm	***Reception in the Nautilus Room***	
Sunday, January 27th		
8:30–12:00 Noon	**Session I**	**The Great White North**
4:00– 6:00 pm	**Poster Session**	***Fred Farr Forum and Kiln Room***
7:30–10:00 pm	**Session II**	**South by SouthWest**
10:00–11:00 pm	***Reception***	***Fred Farr Forum and Kiln Room***
Monday, January 28th		
8:30 – 12:00 Noon	**Session III**	**Nor' Easter**
3:30 – 4:00 PM	**Conrad Mallia**	**NIAID**
4:00 – 6:00 PM	**Oral Presentations**	***The Chapel Auditorium***
7:30 – 10:00 PM	**Session IV**	**The Great West/NorthWest**
10:00–11:00 PM	***Reception***	***Fred Farr Forum and Kiln Room***
Tuesday, January 29th		
8:30–12:00 Noon	**Session V**	**Coast to Coast**
Saturday through Monday	**Posters on Display**	***Fred Farr Forum and Kiln Room***

CONFERENCE PROGRAM

SESSION I **The Great White North**

Sunday Morning ***Chairperson: Brian Rudd***

Speakers:

Peter A. Savage, University of Chicago
"Regulatory T cell choreography directed by endogenous self ligands"

Jennifer Gommerman, University of Toronto
"Recirculating intestinal IgA-producing cells regulate neuroinflammation via IL10"

~~Tatyana V. Golovkina, University of Chicago~~ *replaced by Dan Stetson.* **"Innate immune detection of DNA"**

Brian Rudd, Cornell University
"The developmental layers in the CD8+ T cell response to infection"

Two short presentations chosen from abstracts

Anna Beaudin, University of California, Merced
"The lymphoid-associated interleukin 7 receptor (IL-7R) regulates fetal tissue resident macrophage development"

Dominik Schenten, University of Arizona
"Control of Humoral Immunity by the Innate Signaling Adaptor MAVS"

Sunday Afternoon
4:00 – 6:00 PM

POSTER SESSION and informal discussion groups.

SESSION II
Sunday Evening
7:30–10:00 PM

South by South West
Chairperson: Michael S. Kuhns

Speakers:

Maximiliano D' Angelo, Sanford Burnham Institute
"**Nuclear pore complexes in T cell homeostasis"**

Wendy Havran, Scripps Research Institute
"γδ **T cells and the regulation of immune responses at epithelial surfaces"**

Deepta Bhattacharya, University of Arizona
"**B cell recall responses to flaviviruses"**

Gianna Hammer, Duke University
"**A roadmap to inflammation paved by dendritic cells"**

SESSION III
Monday Morning
8:30-12:00 Noon

Nor' Easter
Chairperson: Leslie J. Berg

Speakers:

Eric Huseby, University of Massachusetts
"**Dwelling on neonatal T cell development"**

Thorsten R. Mempel, Mass General Hospital
"**Disrupting the CBM complex selectively converts intratumoral Treg into IFNγ-secreting anti-tumor effector cells"**

Thomas Serwold, Joslin Diabetes Center
"**Immunotherapy and Autoimmunity"**

Leslie J. Berg, University of Massachusetts
"**Linking T cell receptor signal strength to variations in gene expression"**

<u>Two short presentations chosen from abstracts</u>

Amanda Jamieson, Brown University
"Immune Triage: Prioritization of the innate immune response when faced with multiple simultaneous insults."
Nikolaos Sgourakis, University of California, Santa Cruz
"Getting in the groove: The selection of MHC-I antigen repertoires by molecular chaperones is governed by a network of protein dynamics"

Monday Afternoon
3.30 – 4.00 PM
4:00 – 6:00 PM

"NIAID funding opportunities" Conrad Mallia
ORAL POSTER PRESENTATIONS

SESSION IV **The Great West/NorthWest**

Monday Evening ***Chairperson: Marion Pepper***
7:30 -10:00 PM

Speakers:

Mark Ansel, University of California, San Francisco
"**Post transcriptional regulation of immunity"**

Evan W. Newell, Fred Hutchinson Cancer Research Center
"**High dimensional cellular profiling to ask T cells about what they see in health and disease"**

Kevin B. Urdahl, Seattle Children's Research Institute
"**TGFβ restricts T cell-mediated immunity within tuberculous granulomas**

Marion Pepper, University of Washington
"**Understanding the differentiation of memory B cells"**

Awards Presentations to Graduate, Postdoctoral and Young Investigators

Poster Awards:

Ray Owen Poster Awards **(Sponsored by AAI)**
Young Investigator Travel Awards **(Sponsored by BioLegend)**
Ray Owen Young Investigator Poster Awards **(Sponsored by Cellular Immunology)**
Science Signaling Poster Awards **(Sponsored by AAAS)**

Oral Presentation Awards:

Ray Owen Young Investigator Awards **(Sponsored by AAI)**
Young Investigator Presentation Awards **(Sponsored by BioLegend)**

Excellence in Research Awards in memory of P. Cairs

SESSION V **Coast to Coast**

Tuesday Morning ***Chairperson: Sunny Shin***
8:30-12:00 Noon

Speakers:

Sunny Shin, University of Pennsylvania
"Bypassing bacterial blockade of innate immune signaling to ensure antimicrobial defense"

Susan R. Schwab, New York University
"**S1P and T cell migration"**

Jackson G. Egen, Amgen
"**T cell-mediated tumor killing: Key mechanisms regulating therapeutic efficacy"**

Sue Kaech, Salk Institute
"**Making Memories the EZ(H2)-way"**

Regulatory T cell choreography directed by endogenous self ligands

Peter A. Savage
University of Chicago

Abstract:
Foxp3-expressing regulatory T (Treg) cells are critical for the prevention of autoimmunity, and are thought to be a major barrier to the induction of robust anti-tumor immune responses. Whereas thymus-derived Treg cells are thought to be reactive to endogenous self-peptides, the lack of knowledge regarding the identity of natural self-ligands has restricted progress in the field. In recent work, we have identified natural self-peptide ligands that are recognized by naturally occurring populations of Treg cells in mice. Using unique tools to study monoclonal and polyclonal T cell populations reactive to these self-peptides, we aim to address fundamental questions about the development, specificity, and function of Treg cells. Specifically: 1) What determines whether overtly self-reactive thymocytes will undergo clonal deletion vs. Treg cell differentiation? 2) Are single specificities of Treg cells required to prevent autoimmune reactions? 3) What are the mechanisms of Treg-mediated suppression? The latest developments in our work in these areas will be discussed.

References:
1. Leonard, J.D., Gilmore, D.C, Dileepan, T., Nawrocka, W.I., Chao, J.L., Schoenbach, M.H., Jenkins, M.K., Adams, E.J.*, and **Savage, P.A.*** (2017). Identification of natural regulatory T cell epitopes reveals convergence on a dominant autoantigen. *Immunity* 47, 107-117. * Denotes co-corresponding authors.
2. Malchow, S., Leventhal, D.S., Lee, V., Nishi, S., Socci, N.D., and **Savage, P.A.** (2016). Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity* 44, 1102-1113.
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4. Malchow, S., Leventhal, D.S., Nishi, S., Fischer, B.I., Shen, L., Paner, G.P., Amit, A.S., Kang, C., Geddes, J.E., Allison, J.P., Socci, N.D., and **Savage, P.A.** (2013). Aire-dependent thymic development of tumor-associated regulatory T cells. *Science* 339, 1219-1224.

Recirculating Intestinal IgA-producing cells Regulate Neuroinflammation via IL10

Jen Gommerman
University of Toronto

Plasma cells (PC) are found in the central nervous system (CNS) of multiple sclerosis (MS) patients, yet their source and role in MS remains unclear. We find that some PC in the CNS of mice with Experimental Autoimmune Encephalomyelitis (EAE) originate in the gut and produce immunoglobulin A (IgA). Moreover, we show that IgA^+ PC are dramatically reduced in the gut during EAE, and likewise, a reduction in IgA-bound fecal bacteria is seen in MS patients during disease relapse. Removal of PB/PC resulted in exacerbated EAE that was normalized by the introduction of gut-derived IgA^+ PC. Furthermore, mice with an over-abundance of IgA^+ PB/PC were specifically resistant to the effector stage of EAE, and expression of IL10 by PB/PC was necessary and sufficient to confer resistance. Our data show that IgA^+ PB/PC mobilized from the gut play an unexpected role in suppressing neuroinflammation.

Please refer to:

Rojas et. al. Recirculating Intestinal IgA-producing cells Regulate Neuroinflammation via IL10; *Cell* In press.

Retroviruses, leukemia and the gut microbes

Tatyana Golovkina
University of Chicago

Retroviruses induce a broad range of tumors in vertebrates. Murine Leukemia Virus (MuLV), which can spread as an oral and as a blood-borne pathogen, is highly proficient in causing leukemia. Intriguingly, tumor incidence within the same strain of infected mice varies between different facilities. Among environmental factors that may differ between the research labs, the variation in gut microbiota stands out. Thus, we sought to determine whether the microbiota contributes to virally-induced leukemogenesis. Accordingly, we monitored virally-induced leukemia in germ-free (GF, sterile) and specific pathogen free (SPF) conventionally raised BALB/cJ mice. Even though MuLV replication and spread were not affected in the absence of the microbiota, GF mice were significantly more resistant to the leukemia than SPF mice. Colonizing GF mice with a defined group of commensal bacteria (Altered Schaedler's Flora), or with a single bacterium such as *Lactobacillus murinus* but not *Parabacteroides distasonis* or *similar to E. coli and shigella* (SECS) did not change virus replication but abolished the tumor-resistant phenotype of the colonized mice, indicating that some gut microbes have tumor-promoting properties. At the same time, GF mice lacking adaptive immune system (T and B lymphocytes) developed leukemia at a high rate suggesting that the gut microbes facilitate leukemia promotion by counteracting the adaptive immune response.

The gut microbiota has been implicated in both progression of cancers of colon and liver (*1-3*) ('local' influence), as well as in systemic anti-cancer effect by enhancing the effect of anti-cancer immunotherapy (*4, 5*). We report the first example suggesting for the role of the gut microbes in the development of tumors outside the gut or organs connected to the gut.

1. B. S. Reddy *et al.*, Colon carcinogenesis with azoxymethane and dimethylhydrazine in germ-free rats. *Cancer research* **35**, 287-290 (1975).
2. S. Yoshimoto *et al.*, Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* **499**, 97-101 (2013).
3. J. C. Arthur *et al.*, Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* **338**, 120-123 (2012).
4. A. Sivan *et al.*, Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **350**, 1084-1089 (2015).
5. M. Vetizou *et al.*, Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **350**, 1079-1084 (2015).

The developmental layers in the CD8+ T cell response to infection

Brian Rudd
Cornell University

Norah L. Smith[1], Ravi Patel[2], Arnold Reynaldi[3], Jennifer K. Grenier[4], Jocelyn Wang[1], Neva B. Watson[1], Kito Nzingha[1], Kristel Yee Mon[1], Seth A. Peng[1], Andrew Grimson[2], Miles P. Davenport[3], Brian D. Rudd[1]

[1]Department of Microbiology and Immunology, Cornell University; [2]Department of Molecular Biology and Genetics, Cornell University; [3]Kirby Institute for Infection and Immunity, UNSW Australia; [4]RNA Sequencing Core, Department of Biomedical Sciences, Cornell University.

Vertebrate development comprises three germ layers that give rise to all tissues and organs. Similarly, the immune system is formed from distinct waves of hematopoietic stem cells that give rise to unique populations of immune cells at different stages of development. Although recent studies have indicated that conventional T cells produced in early life are functionally different from those made later in life, it remains unclear whether these cells persist into adulthood and maintain their cell-intrinsic differences during infection. To address this question, we employed a novel fate mapping system to 'timestamp' CD8+ T cells produced from the thymus at various stages of life. We found that fetal-derived CD8+ T cells persist into adulthood as a distinct developmental layer, maintaining a phenotype of early response to infection and inflammatory signals. These data indicate that there are developmental layers in the adult CD8+ T cell response to infection, and that the heterogeneity in the effector pool is linked to variation in the developmental origins of responding cells. More recently, we have employed a mathematical modeling approach to obtain a comprehensive picture of how the developmental layers in the CD8+ T cell compartment change over the life of the animal. Collectively, our data provides a framework for understanding the ontogeny of the CD8+ T cell compartment and contextualizes age-related changes in the CD8+ T cell response to infection.

References:

1. Smith NL, Patel RK, Reynaldi A, Grenier JK, Wang J, Watson NB, Nzingha K, Yee Mon K, Peng SA, Grimson A, Davenport MP, Rudd BD. "Developmental origin governs CD8+ T cell fate decisions during infection." *Cell*. 2018. Jun 28; 174:117-130.
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Nuclear pore complexes in T cell homeostasis

Maximiliano A. D'Angelo

Sanford Burnham Prebys Medical Discovery Institute

Joana Borlido[1], Stephen Sakuma[1], Marcela Raices[1], Florent Carrette[2], Roberto Tinoco[2], Linda M. Bradley[2] and Maximiliano A. D'Angelo[1, 2]

[1]Development, Aging and Regeneration Program and NCI-Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA.

[2]Infectious and Inflammatory Disease Center and NCI-Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA.

Nuclear pore complexes (NPCs) are multiprotein channels that connect the nucleus with the cytoplasm. These structures are built by the repetition of 32 different proteins known as nucleoporins or nups. In recent years, it has become evident that the expression of many NPC components varies among different cell types and tissues and that mutations in several nups result in tissue-specific phenotypes. These findings indicate that NPCs can be specialized to play specific roles. What the physiological functions of these cell type-specific structures are remain largely unknown. We recently discovered that the loss of the tissue-specific NPC component Nup210 causes a severe deficit of naïve $CD4^+$ T cells. Nup210-deficient $CD4^+$ T lymphocytes develop normally but fail to survive in the periphery due to their inability to transmit tonic T cell receptor (TCR) signals, and to increased sensitivity to Fas-mediated cell. We have now identified that depletion of another NPC component from a different pore domain also significantly reduces the number of naïve $CD4^+$ T cells. Notably, our evidence suggests that these nucleoporins play distinct roles in maintaining T cell homeostasis. Our results establish NPCs as important cell-intrinsic regulators of T cell physiology and expose these structures as novel players in the adaptive immune system.

Borlido, J., Sakuma, S., Carrette, F., Trinoco, R., Bradley, L., and **D'Angelo, M.A.** Nuclear pore complex-mediated modulation of TCR signaling is required for naïve $CD4^+$ T cell homeostasis. ***Nature Immunology.*** 2018 Jun; 19, 594-605. PMCID: PMC5976539

Raices M, Bukata L, Sakuma S, Borlido J, Hernandez L.S., Hart D.O., and **D'Angelo, M.A**. Nuclear pores regulate muscle development and maintenance by assembling a localized Mef2C complex. ***Developmental Cell*** 2017,41(5):540-554. PMCID: PMC5515297

Raices, M and **D'Angelo, M.A**. Nuclear pore complex composition: a new regulator of tissue-specific and developmental functions. ***Nature Reviews. Molecular Cell Biology*** 2012, 13(11):687-699***.***

γδ T Cells and the Regulation of Immune Responses at Epithelial Surfaces

Wendy L. Havran
The Scripps Research Institute

Margarete Johnson[1], Mike McGraw[1], Srinivas Tekkam[2], Stephen Crooke[2], M.G. Finn[2], Deborah Witherden[1], and Wendy L. Havran[1]
[1] Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA 92037 USA
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Intraepithelial γδ T cells play unique roles in homeostasis, tissue repair, inflammation and protection from malignancy in mice and man. Human epidermal T cells contribute to wound healing and are defective in patients with chronic wounds. Increasing numbers of elderly and diabetic patients have defects in tissue repair leading to chronic, non-healing wounds. We have identified key regulators of intraepithelial γδ T cell recognition of damaged epithelial cells leading to activation and participation in local immune responses, including the JAML and CAR costimulatory molecules. Expression of the CAR costimulatory ligand is upregulated on epithelial cells around human healing wounds but not in chronic wounds. Surgical debridement to remove non-healing tissue can create a new acute wound, lead to upregulated CAR expression, and result in improved healing. Since expression of CAR correlates with effective wound healing, we hypothesize that defective costimulation due to lack of CAR expression is responsible for the T cell dysfunction in chronic wounds. We are utilizing biodegradable hydrogels to deliver costimulatory ligands directly into non-healing wounds as a new therapeutic strategy targeting the dysfunctional T cells. Further characterization of the molecules and mechanisms that regulate interactions between tissue-resident T lymphocytes and neighboring epithelial cells may allow for the development of this and other new therapeutic strategies to treat chronic wounds and other epithelial disorders.

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2. Verdino, P., Witherden, D.A., Havran, W.L. and Wilson, I.A. The molecular interaction of CAR and JAML recruits the central cell signal transducer PI3K. Science, 329:1210-1214, 2010.
3. Nielsen, M.M, Witherden, D.A. and Havran, W.L. γδ T cells in homeostasis and host defense of epithelial barrier tissues. Nature Rev. Immunology 17:733-745, 2017.
4. Witherden, D.A., Johnson, M.D., and Havran, W.L. Coreceptors and their ligands in epithelial γδ T cell biology. Frontiers in Immunology 9:731, 2018.

B cell recall responses to flaviviruses

Deepta Bhattacharya
University of Arizona

Mosquito-borne flavivirus infections, such as Yellow Fever (YFV), West Nile (WNV), Japanese Encephalitis (JEV), Dengue (DENV), and Zika (ZIKV) viruses, have a long history of causing human disease and epidemics. This family of viruses poses a unique conundrum for generating immunity. Though primary infections by flaviviruses (e.g. DENV) readily generate neutralizing antibodies and lifelong protection, they also leave behind many memory B cells and antibodies with non-neutralizing specificities. Upon subsequent infection with a different flavivirus (e.g. ZIKV), these memory B cells and antibodies cross-react with non-neutralizing epitopes and enhance infection through Fc receptor-mediated uptake. Recent live-attenuated DENV vaccine trials have indeed led to enhanced disease severity in a subset of children. By focusing on potent neutralizing epitopes as candidates for subunit vaccines, we sought to define how germinal center diversification shapes the memory B cell vs. long-lived plasma cell decision in primary responses, and how protective antibodies to secondary heterologous flavivirus infections are generated by memory B cells.

Using antigen-specific tetramers for the neutralizing lateral ridge epitope, we found that memory B cells were more tolerant to WNV escape mutations than were long-lived plasma cells, indicating a more antigenically diverse repertoire. To determine if this diversity is generated from the failure of the B cell receptor (BCR) to reach a certain affinity threshold to promote a long lived plasma cell fate, we generated a novel mouse (AID-TamCre) where deletion of activation induced cytidine deaminase (AID) is driven by hCD20-CreERT2. Deletion of AID during an ongoing germinal center reaction to WNV did not affect the antigen specificity or quantity of memory B cells or bone marrow plasma cells. Quantification of the affinities of a panel of memory B cell and long-lived plasma cell-derived monoclonal antibodies revealed similar binding kinetics between these two cellular compartments. These data indicate that an absolute final BCR affinity threshold is not responsible for segregating memory B cells from long-lived plasma cells. However, reversion of these monoclonal antibodies to their germline sequences demonstrated that the naïve B cell precursors of long-lived plasma cells have substantially higher BCR avidities than do precursors of memory B cells. We propose that the initial avidity for antigen imprints the memory B cell vs. plasma cell fate at an early timepoint, irrespective of the final avidity reached during the germinal center reaction. Memory B cell diversity is thus likely promoted by recruitment of a broad range of naïve B cells and corresponding avidities in the initial response.

We next defined how this diversity in the memory B cell pool is utilized in a recall response. Deletion of AID from WNV-immune mice did not alter the subsequent response to JEV. Thus, clonal selection of pre-existing cross-reactive B cells dominates the response to heterologous flavivirus infections with no requirement for additional affinity maturation.

References (* denotes equal contribution):

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2) Jash A, Wang Y, Weisel FJ, Scharer CD, Boss JM, Shlomchik MJ, Bhattacharya D, ZBTB32 restricts the duration of memory B cell recall responses. Journal of Immunology 2016: 197:1159-1168

3) Lam WY*, Becker AM*, Kennerly KM*, Wong R, Curtis JD, Payne EM, McCommis KS, Fahrmann J, Pizzato, HA, Nunley RM, Lee J, Wolfgang MJ, Patti GJ, Finck BN, Pearce EL, Bhattacharya D, Mitochondrial pyruvate import promotes the long-term survival of antibody-secreting plasma cells. Immunity 2016: 45:60-73

4) Wong R, Bhattacharya D, Basics of Memory B cell Responses: Lessons from and for the Real World. Immunology 2018: epub ahead of print

A roadmap to inflammation paved by dendritic cells

Gianna E. Hammer
Duke University

Abstract not available

Dwelling on neonatal T cell development

Eric Huseby
University of Massachusetts

Neonatal thymus-derived Foxp3$^+$ regulatory T cells (tT_{regs}) are critical in preventing multi-organ autoimmunity. Despite their central role in controlling immune homeostasis, the influence of self-antigen specificity on their development and their relationships with Tconv cells and negative selection is poorly understood. We have characterized TCRs expressed on neonatal tT_{regs}, revealing specificity for self-antigens that are presented by antigen presenting cells in an age-dependent and inflammation-dependent manner. To characterize the specificity of TCRs expressed on neonatal Treg cells, we have developed a self-peptidome identification platform. This approach has lead to the pairing of many TCRs with their self-antigen ligands, including both tissue-specific and inflammation-sensitive specificities. Detailed studies of a set of Peptidyl arginine deiminase, type IV, (Padi4)-specific tT_{regs} reveal their restricted development during the neonatal window, while thymocytes expressing these same TCRs undergo negative selection in adult mice. TCRs expressed on Padi4-specific tT_{regs} engage self-pMHC with moderate dwell times using a conventional docking orientation. In contrast, Padi4-specific TCRs that have short dwell times with I-A^b-Padi4 are heavily biased towards the conventional CD4$^+$ T cell repertoire, whereas TCRs with long dwell times are greatly limited from entering the mature T cell repertoire. These results argue for a kinetic selection model of neonatal tT_{reg} development.

Disrupting the CBM complex selectively converts intratumoral Treg into IFNγ-secreting anti-tumor effector cells

Thorsten R. Mempel and Mauro Di Pilato
Massachusetts General Hospital and Harvard Medical School

Solid tumors are infiltrated by effector T cells (Teff) with the potential to control or reject them, as well as by regulatory T cells (Treg) that restrict the function of Teff and thereby promote tumor growth.[1] The anti-tumor activity of Teff can be therapeutically unleashed and is now being exploited for the treatment of some select forms of human cancer. However, weak tumor-associated inflammatory responses and the immune-suppressive function of Treg remain major hurdles to broader effectiveness of tumor immunotherapy.[2] Here we show that upon disruption of the CARMA1-BCL10-MALT1 (CBM) signalosome, the majority of tumor-infiltrating Treg produce IFN-γ, followed by stunted tumor growth. Remarkably, genetic deletion of both or even just one allele of CARMA1 in only a fraction of Treg, which avoided systemic autoimmunity, was sufficient to produce this anti-tumor effect, showing that not mere loss of suppressive function, but gain of effector activity by Treg initiates tumor control. Treg production of IFN-γ was accompanied by macrophage activation and up-regulation of MHC-I on tumor cells. However, tumor cells also up-regulated expression of PD-L1, indicating activation of adaptive immune resistance.[3] Consequently, PD-1 blockade concomitant with CARMA1-deletion caused rejection of tumors that otherwise do not respond to anti-PD-1 monotherapy. This effect was reproduced by pharmacological inhibition of the CBM protein MALT1. Our results demonstrate that partial disruption of the CBM complex and induction of IFN-γ-secretion in the preferentially self-reactive Treg pool does not cause systemic autoimmunity but is sufficient to prime the tumor environment for successful immune checkpoint therapy.

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Immunotherapy and Autoimmunity

Thomas Serwold
Joslin Diabetes Center, Harvard Medical School

Shio Kobayashi[1], Martin Thelin[1], Neha Deshpande[2], Heather Parrish[2], Mark Lee[2], Michael Kuhns[2] and Thomas Serwold[1]
[1]Joslin Diabetes Center, Harvard Medical School, Boston, MA
[2]University of Arizona, Tucson, AZ

Central and peripheral tolerance effectively prevent most T cell responses toward self-antigens, but T cells that escape these mechanisms can cause autoimmunity. There is an unmet demand for interventions that can eliminate autoimmune T cells and halt progression of autoimmune diseases. We have developed a cell-based approach to eliminate T cells of known specificities. We engineered 5 module chimeric antigen receptors (^{5M}CARs) that combine the extracellular domains of the peptide MHC complex, and the transmembrane and intracellular domains of the T cell receptor. These chimeric receptors fold and assemble with endogenous CD3 modules in T cells, and when they are co-expressed with an additional chimeric CD80-LCK co-receptor, transduce a TCR signal upon binding to the T cells bearing a cognate TCR. In a model of type 1 diabetes, ^{5M}CAR-expressing CD8 T cells effectively target and kill autoimmune T cells in an antigen specific fashion, preventing diabetes development. ^{5M}CAR T cells have promise for targeting unwanted T cells that cause autoimmunity as well as other T mediated diseases.

Linking T cell receptor signal strength to variations in gene expression

Leslie J. Berg, James Conley III, Michael Gallagher, Pranitha Vangala, and Manuel Garber
University of Massachusetts Medical School

Stimulation of the T cell receptor (TCR) induces signals that promote T cell development, differentiation, and activation. Numerous studies have documented that variations in the strength of TCR stimulation, via modulation of TCR binding affinity for pMHC or by varying antigen dose, can produce differential outcomes in responding cells. Our studies have aimed to dissect the molecular and biochemical mechanisms by which variations in TCR signal strength generate differential programs of gene expression. We have addressed this question by a targeted approach focused on the regulation of *Irf4* gene transcription coupled with modulation of the Tec family tyrosine kinase, ITK, a signaling protein activated by TCR stimulation. These studies have been complemented by more general studies of transcription factor activation in primary T cells stimulated with peptide-APCs. An unbiased study using RNA-seq analysis of T cells stimulated with strong versus weak TCR stimulation has provided further insight into the mechanisms linking TCR signal strength to differential programs of gene expression.

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Post-transcriptional regulation of immunity

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Compared to coding sequences, untranslated regions of the transcriptome are not well conserved, and functional annotation of these sequences is challenging. Global relationships between nucleotide composition of 3′ UTR sequences and their sequence conservation have been appreciated since mammalian genomes were first sequenced, but the functional relevance of these patterns remain unknown. To identify RNA cis-regulatory elements, we developed GCLiPP, a biochemical technique for detecting RNA binding protein (RBP) occupancy transcriptome-wide. GCLiPP sequence tags corresponded with known RBP binding sites, specifically correlating to abundant cytosolic RBPs. We used these RBP occupancy profiles to guide functional dissection of 3' UTRs with CRISPR/Cas9 genome editing. For example, two RBP occupied sites in the CD69 3' UTR destabilized the transcript of this key regulator of lymphocyte tissue egress. In addition, we systematically measured the effect on gene expression of the sequences of >25,000 RBP occupied sites in primary mouse T cells using a massively parallel reporter assay. In this system, GC-rich sequences were destabilizing of reporter mRNAs and came from more rapidly evolving regions of the genome. These sequences were more likely to be folded in vivo, and contained a number of structural motifs that reduced accumulation of a heterologous reporter protein. Comparison of full-length 3′ UTR sequences across vertebrate phylogeny revealed that strictly conserved 3′ UTRs were GC-poor and enriched in genes associated with organismal development. In contrast, rapidly evolving 3′ UTRs tended to be GC-rich and derived from genes involved in metabolism and immune responses. By reducing gene expression, GC-rich RBP occupied sequences act as a rapidly evolving substrate for gene regulatory interactions that govern immunity and other biological processes.

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High dimensional cellular profiling to ask T cells about what they see in health and disease

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Blood and tissue samples taken as part of clinical studies and trials can provide critical information on the roles of the immune response in patient outcome. However, the cellular compositions of these samples are often highly diverse, and important information can be lost if rare cells are overlooked. For instance, antigen specific T cells are critical initiators and orchestrators of the adaptive immune response, but cells specific for any given pathogen or cancer can be exceedingly rare, especially in blood. Here, the utility of high dimensional mass cytometry analysis together with rapidly evolving computational analysis tools for the study of T cell responses in human cancer and infectious disease will be discussed. In infectious disease, we are analyzing the large degree variation seen within broad lymphocyte activation during acute stages of infection to gain insights about factors that can influence the profiles of dengue-specific T cells seen after the clearance of the infection. In cancer, we are using information about the specificity of infiltrating T cells to better understand the basis for the large degree of heterogeneity seen within and between patient tumors. Overall, these examples will hopefully highlight the utility of combining broad cellular immune profiling together with the analysis of antigen-specific T cells to gain insight about immunological mechanisms in humans.

TGFβ restricts T cell-mediated immunity within tuberculous granulomas

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The biggest barrier to developing new vaccines and immunotherapeutics to combat tuberculosis (TB) is our poor understanding of protective immunity in the *Mycobacterium tuberculosis* (Mtb)-infected lung as well as the forces that restrict it. To address this problem, we have developed a new mouse tuberculosis (TB) model in which mice are infected with a physiologic, ultra-low dose (ULD) of aerosolized Mtb (1-3 CFU). We have shown that ULD Mtb-infected mice share several key features with human Mtb infection, including well-circumscribed granulomatous structures and heterogeneous outcomes. In order to monitor the degree of Mtb control in individual live mice, we identified a blood transcriptional signature that correlates with the pulmonary bacterial burden. Remarkably, this mouse-derived signature predicts human TB risk as well as a previously identified human-derived transcriptional signature, confirming the model's relevance to human Mtb infection.

Leveraging the well-organized granulomatous structures that develop in ULD Mtb-infected mice, we have begun to investigate the barriers to protective immunity in these structures. Because optimal control of Mtb infection requires direct interactions between CD4 T cells and Mtb-infected cells, we used immunohistochemistry and quantitative imaging to perform a spatial analysis of T cell receptor (TCR) signaling (using phospho-S6 or IRF4 expression) and IFNγ production relative to Mtb-infected cells within granulomas. Although many T cells localize near Mtb-infected cells, and a subset of these cells clearly undergo TCR signaling, few T cells produce IFNγ within the granuloma. Surprisingly, a high proportion of CD4 T cells within the granuloma express phospho-SMAD3, suggesting a role for TGFβ signaling in local immunosuppression. In support of this idea, we found that T cells that lack the ability to respond to TGFβ (TGFβRII-deficient) exhibit enhanced IFNγ production within TB granulomas and improve Mtb control. These findings help explain why IFNγ-producing T cells have a limited capacity to control pulmonary Mtb infection and could guide new strategies for vaccine and immunotherapeutic development.

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Understanding the differentiation of memory B cells

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Humoral immunity depends upon the development of long-lived, antibody-secreting plasma cells and rapidly responsive memory B cells (MBCs). The differentiation of high affinity, class-switched MBCs after immunization is critically dependent upon BCL6 expression in germinal center (GC) B cells and CD4+ T follicular helper (Tfh) cells. It is less well understood how more recently described MBC subsets are generated, like the CD73+CD80+ IgM+ MBCs that initially form antibody-secreting effector cells in response to a secondary *Plasmodium* infection. We therefore interrogated how BCL6 expression in both B and CD4+ T cells influenced the formation of heterogeneous *Plasmodium*-specific MBC populations. All *Plasmodium*-specific CD73+CD80+ MBCs required BCL6 expression for their formation and secondary responsiveness, suggesting germinal center dependence. Yet further dissection of the CD4+ T and B cell interactions revealed that CD73+ IgM+ MBCs form in a distinct manner that requires unique T-B interactions that differ from those required to form IgG+ MBCs.

Bypassing bacterial blockade of innate immune signaling to ensure antimicrobial defense

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Intracellular pathogens pose a unique set of challenges for the immune system. While eukaryotic organisms possess evolutionarily conserved innate immune sensors and signaling pathways to sense and respond to infection, intracellular pathogens likewise possess evolutionarily conserved mechanisms to disrupt, evade, or modulate cellular signaling networks. Thus, while the mechanisms of PRR activation by isolated PAMPs are reasonably well-understood, how the immune system successfully generates responses against pathogens that utilize virulence factors to shut off cellular responses is less clear. Many bacterial pathogens deploy virulence factors that disrupt key immune processes, raising the question of how a robust immune response can be generated during bacterial infection. I will discuss our recent findings elucidating how the immune system overcomes bacterial virulence activities in order to generate a robust antimicrobial response.

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S1P and T cell migration

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The signaling lipid sphingosine 1-phosphate (S1P) guides immune cell egress from tissues into circulation. Although S1P signaling regulates the migration of multiple types of leukocyte, its role has been best characterized for T cells. Upon maturation, T cells follow S1P gradients out of the thymus into blood. Naïve T cells then circulate among secondary lymphoid organs, following S1P gradients from the spleen into blood and from lymph nodes and Peyer's patches into lymph. Like naïve T cells, activated T cells follow S1P gradients out of secondary lymphoid organs, but once they reach blood they may enter infected tissues. In some cases, activated T cells at a site of infection again follow S1P gradients back into lymph. Much progress has been made in understanding how S1P distribution is regulated at steady-state, but little is known about how these gradients are altered during the course of an immune response, and how in turn this affects the immune response. We will discuss how S1P levels in the draining lymph node change upon challenge.

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T cell-mediated tumor killing: Key mechanisms regulating therapeutic efficacy

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The clinical success of T cell-targeted immunotherapies, such as immune checkpoint blockade, in solid tumors has demonstrated that robust cytolytic T cell responses are critical for productive anti-tumor immunity. However, the responses of individual cancer patients and cancer patient populations to immunotherapy are extremely heterogenous, dictated by diverse and interrelated factors such as the inherent tumor immunogenicity, pre-treatment tumor-associated T cell infiltration, and the presence of immunosuppressive cell types and pathways. Identifying the specific mechanisms that limit the activity of clinically-validated, efficacious immunotherapies is critical for expanding patient populations who may benefit from these therapies, enabling development of optimal combination strategies and next generation therapeutic approaches. The ability of Blincyto®, a Bi-Specific T cell Engager (BiTE®) molecule, to successfully treat acute lymphoblastic leukemia has validated this class of anti-cancer immunotherapy agents in hematological malignancies and led to the exploration of this approach in solid tumors. BiTE® molecules consist of an Fc domain with tandem single chain variable fragments (scFv) recognizing the CD3 receptor on T lymphocytes and a tumor-associated antigen and induce redirected T cell cytotoxicity and tumor cell lysis. Despite the clinical efficacy of BiTE® molecules, there has been little elucidation of the parameters governing their in vivo activity. We are using a novel genetically engineered mouse model to study the mechanisms regulating BiTE® molecule activity in solid tumors, focusing on elucidating pharmacokinetic, pharmacodynamic, and efficacy relationships. These studies have identified novel correlates between the phenotype of the pre-treatment tumor-associated T cell compartment and the ability of BiTE® molecules to successfully eradicate solid tumors and are beginning to define key components of the anti-tumor immune response that could be targeted in order to maximize the potential of BiTE® molecules in the clinic.

Making Memories the EZ(H2)-way

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Memory CD8 T cells arise following infection from a heterogenous population of effector T cells that contains cells of various differentiation states. Many of these effector CD8 T cells develop into end-stage terminal effector cells that die following infection and a smaller portion develops into cells with greater memory cell potential and longevity. Understanding how effector CD8 T cell differentiation is regulated to generate cells of diverse cell fates is important and much progress has been made in identifying several transcriptional factors that regulate effector and memory cell fates, function and phenotypes. In this talk we will discuss how the epigenetic landscape of different subsets of effector T cells varies and impacts their long-term fates and multipotency.