The 55th Midwinter Conference of Immunologists at Asilomar

January 23 -26, 2016

Asilomar Conference Grounds, Pacific Grove, California

Christel Uittenbogaart, Executive Director
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Chairpersons

The Dan H. Campbell Memorial Lecture

Sponsored by The American Association of Immunologists

Saturday, January 23, 8:00 PM

The Chapel Auditorium

Michel C. Nussenzweig

The Rockefeller University

"The challenges of an HIV vaccine"

Council Members

Gregory Barton
Daniel Campbell
Michael Cancro
Rachel Caspi
Hilde Cheroutre
Nicholas Crispe
Shane Crotty
Jason Cyster
Laurie Dempsey
Pamela Fink
Ananda Goldrath
Wendy Havran
Stephen M. Hedrick
Kristin Hogquist
Christopher Hunter
Mitchell Kronenberg
Terri Laufer
David Lewis
Ann Marshak-Rothstein
Diane Mathis
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Shannon Turley
Christel Uittenbogaart
David Webb
Arthur Weiss
Steven Ziegler
Martha Zuniga

NAME BADGES are issued at the time of Registration and must be worn at all Conference meetings and Receptions. Guests are issued a badge at the time of their Registration for admittance to Receptions. All names remain on the **CONFERENCE e-LIST** for two years after last attendance. Update your e-mail address with the Registrar for assured delivery of special notices.

The Midwinter Conference of Immunologists

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The 2016 Midwinter Conference of Immunology at Asilomar
Pacific Grove, California (USA)

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CONFERENCE SCHEDULE

All Sessions: The Chapel Auditorium

Saturday, January 23rd		
3:00 pm	**Registration**	
8:00 pm	***The Dan H. Campbell Memorial Lecture***	
9:00–11:00 pm	***Reception in the Nautilus Room***	
Sunday, January 24th		
8:30–12:00 Noon	**Session I**	**New insights into adaptive immunity**
4:00– 6:00 pm	**Poster Session**	***Fred Farr Forum and Kiln Room***
7:30–10:00 pm	**Session II**	**Homeostasis and Inflammation at Barrier Surfaces**
10:00–11:00 pm	***Reception***	***Fred Farr Forum and Kiln Room***
Monday, January 25th		
8:30–12:00 Noon	**Session III**	**Cancer Immunology**
3:30-4:00 PM	**NIH/NIAID**	**Conrad Mallia, NIAID funding opportunities**
4:00–6:00 PM	**Oral Presentations**	***The Chapel Auditorium***
7:30–10:00 PM	**Session IV**	**Complexities of T and B cell differentiation**
10:00–11:00 PM	***Reception***	***Fred Farr Forum and Kiln Room***
Tuesday, January 26th		
8:30–12:00 Noon	**Session V**	**Humans: Immunology *in natura***
Sunday through Monday	**Posters on Display**	***Fred Farr Forum and Kiln Room***

CONFERENCE PROGRAM

SESSION I — *New insights into adaptive immunity*

Sunday Morning
8:30-12:00 Noon

Chairperson: Kenneth M. Murphy

Kenneth M. Murphy, Washington University
"Specification and commitment of CD8*a* DCs involve distinct transcriptional events"

Jason G. Cyster, University of California, San Francisco
"Dynamics of the early B cell response"

Stephen C. Jameson, University of Minnesota
"Immune system characteristics in mice with normal immunological experience"

K. Christopher Garcia, Stanford University
"Eighteen views of Interleukin-2"

Two short presentations chosen from abstracts

Sunday Afternoon
4:00 – 6:00 PM

POSTER SESSION and informal discussion groups.

SESSION II *Homeostasis and Inflammation at Barrier Surfaces*

Sunday Evening
7:30–10:00 PM

Chairperson: Yasmine Belkaid

June L. Round, University of Utah
"The microbiota promotes systemic T cell survival through a novel apoptotic factor"

Adam Lacy-Hulbert, Benaroya Research Institute
"Regulation of Immunity by Integrins"

Bana Jabri, University of Chicago
"Viruses and tolerance to dietary antigens"

Yasmine Belkaid, National Institutes of Health
"Long-term consequences of infection for tissue immunity and metabolism"

SESSION III *Cancer Immunology*

Monday Morning
8:30-12:00 Noon

Chairperson: Shannon J. Turley

Shannon J. Turley, Genentech
"Regulation of immune cell function and spatiality by stroma"

Troy J. Randall, University of Alabama, Birmingham
"Role of the omentum in immunity to peritoneal tumors"

Darrell J. Irvine, Massachusetts Institute of Technology
"Recruiting synergistic innate and adaptive immune responses against tumors"

Melody A. Swartz, University of Chicago
"Tumor-associated lymphatic vessels modulate the immune microenvironment to promote immune suppression and tolerance"

Two short presentations chosen from abstracts

SESSION IV *Complexities of T and B cell differentiation*

Monday Evening
7:30 -10:00 PM

Chairperson: Vijay K. Kuchroo

Vijay K. Kuchroo, Harvard University
"Single-cell genomics identifies novel regulators of pro-inflammatory function in Th17 cells"

Michael D. Rosenblum, University of California, San Francisco
"Tregs in skin facilitate epithelial stem cell differentiation"

Ryan M. O'Connell, University of Utah
"Context-dependent regulation of CD4+ T cell responses by miR-155"

Justin J. Taylor, Fred Hutchinson Cancer Research Center
"Investigating the potential of individual naïve B cells"

Awards Presentations to Graduate, Postdoctoral and Young Investigators

Poster Awards:

Ray Owen Poster Awards **(Sponsored by AAI)**
Council Poster Awards **(Sponsored by MCI Council)**
Ray Owen Young Investigator Poster Awards **(Sponsored by Cellular Immunology)**

Oral Presentation Awards:

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

SESSION V Humans: Immunology *in natura*

Tuesday Morning
8:30-12:00 Noon

Chairperson: Shane Crotty

Shane Crotty, La Jolla Institute for Allergy and Immunology
"Genetics of T follicular helper cell (Tfh) differentiation and function"

Ira Mellman, Genentech
"The mechanistic basis of cancer immunotherapy"

Vijayanand Pandurangan, La Jolla Institute for Allergy and Immunology
"17q21 asthma-risk variants switch CTCF binding and regulate IL-2 production by T cells"

Antonio Lanzavecchia, Institute for Research in Biomedicine, Bellinzona, Switzerland
"From broadly neutralizing antibodies to a new mechanism of diversification"

Specification and commitment of CD8α+ DCs involve distinct transcriptional events

Kenneth M. Murphy

Kenneth M. Murphy[1*], Gary E. Grajales-Reyes[1], Xiaodi Wu[1], Arifumi Iwata[1], Jorn Albring[2], Roxane Tussiwand[1,3], Nicole M. Kretzer[1], Carlos Briseno[1], Vivek Durai[1], Prachi Bagadia[1], Malay Haldar[1], Jörg Schönheit[4], Frank Rosenbauer[5], and Theresa L. Murphy[1]

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Abstract Dendritic cells (DCs) include several lineages with non-redundant roles in defense against pathogens[1-3]. pDCs produce type I IFNs that limit viral infection, but play a limited role in antigen presentation. Classical DCs (cDC) comprise several distinct branches that appear are somewhat specialized in promoting different effector modules in the T cells that they prime. One subset, the CD8α+ DC, appears specialized for promoting responses against intracellular pathogens. This cDC subset expresses high levels of IRF8 and its development is dependent on Batf3, although the basis for this requirement has remained unclear. By identifying and characterizing the clonogenic progenitor for subset, we determined that Batf3 functions only in the commitment, but not the specification of CD8α+ cDCs[4]. Specification of the CDP into the pre-CD8 DC is accompanied by the loss of Zeb2 and induction of Id2, Batf3, and CIITA. Upon specification, IRF8 autoactivation, which was established at the MDP stage and was previously dependent on PU.1, but not on Batf3, acquires a dependence for Batf3, which cooperates with IRF8 at a enhancer locate 32Kb downstream of the IRF8 TSS. This enhancer element exhibits selective activity in CD8α+ cDCs, but not CD4 cDCs or pDCs, and contains three AICE sites. In the absence of Batf3, CDPs specify normally to the pre-CD8 DC progenitor but fail maintain *Irf8* autoactivation and develop into normal CD4 (IRF4+) cDCs. Conditional deletion of Zeb2 in MDPs or CDPs induces uniform specification of progenitors into pre-CD8 DC progenitors, and a loss of nearly all pDCs and CD4 DCs. These results indicate that development of CD8α+ cDCs proceeds by at least two separate steps, one involving a Zeb2-sensitive commitment process, and the second being the maintenance of IRF8 by the recently induced Batf3 factor.

We thank the Alvin J. Siteman Cancer Center at Washington University School of Medicine for use of the Center for Biomedical Informatics and Multiplex Gene Analysis Genechip Core Facility. Supported by the Howard Hughes Medical Institute, the US National Institutes of Health (1F31CA189491-01to G.G), the American Heart Association (12PRE12050419 to W.K.), and the National Cancer Institute (P30 CA91842) for support of the Alvin J. Siteman Cancer Center).

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3. Tussiwand, R. et al., Klf4 expression in conventional dendritic cells is required for T helper 2 cell responses. *Immunity* **42**, 916-928 (2015).
4. Grajales-Reyes, G. E. et al., Batf3 maintains autoactivation of Irf8 for commitment of a CD8alpha(+) conventional DC clonogenic progenitor. *Nat Immunol* **16**, 708-717 (2015).

Dynamics of the early B cell response

Jason G. Cyster

Howard Hughes Medical Institute and
Department of Microbiology & Immunology
University of California, San Francisco, California

Lymphoid tissues provide crucial organizing grounds for cellular interactions needed to initiate and sustain humoral immune responses. CXCL13 guides cells into B cell follicles while the oxysterol receptor, EBI2, helps distribute activated cells along the B-T zone interface and segregates naïve and germinal center (GC) B cells within follicles. EBI2 also functions to promote dendritic cell positioning in regions of the spleen exposed to blood borne antigens and this facilitates responses against altered-self red blood cells. In recent work we established a role for sphingosine-1-phosphate receptor-2 (S1PR2) signaling via Ga13 and Arhgef1 in promoting confinement and growth regulation of GC B cells. Loss of this signaling pathway led to GC B cell overgrowth and mutations in these genes are associated with development of GC B cell-derived diffuse large B cell lymphoma (DLBCL) in humans. We found that a second G-protein coupled receptor that is mutated in GCB-DLBCL, P2RY8, also acts to promote B cell confinement to the follicle center. P2RY8 promotes B cell clustering at the follicle center in a follicular dendritic cell (FDC) dependent manner. We find that a further GPCR, S1PR3, contributes to the egress of Ga13-deficient GC B cells into circulation. In parallel work, we have characterized properties of newly identified CXCL12-expressing reticular cells (CRCs) of the GC dark zone, and we have studied molecular changes that GC B cells undergo as they cycle between the dark zone and light zone state. These changes may affect the ability of GC B cells to interact successfully with Tfh cells and may be crucial for efficient antibody affinity maturation. Finally, we have been studying requirements for mounting IgA responses in Peyer's patches. Our current work in these areas will be discussed.

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2. Yi T, Li J, Chen H, Wu J, Xu Y, Hu Y, Lowell CA, Cyster JG. Splenic dendritic cells survey red blood cells for missing self-CD47 to trigger adaptive immune responses. *Immunity* 2015; 43: 764-775

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Immune system characteristics in mice with normal immunological experience

Stephen C. Jameson

University of Minnesota, Center for Immunology

The immune response in mice is typically studied using animals maintained under Specific Pathogen Free (SPF) housing conditions – i.e. in barrier facilities that avoid exposure to common mouse pathogens. While a powerful reductionist approach, it has been proposed that this lack of normal immunological experience may limit the utility of SPF animals as models for the human immune system.

To explore this, we studied immune system homeostasis and response to pathogens in animals that were not housed under SPF conditions, with special focus on CD8+ T cells. In feral mice and animals obtained from commercial pet stores (PS) we found marked differences in the subset distribution and anatomical localization of CD8+ T cells, compared to SPF animals. In particular, there was a substantial increase in the frequency of effector-memory phenotype cells in lymphoid and non-lymphoid tissues (the latter with features of resident memory CD8+ T cells). Importantly, characteristics of the PS CD8+ T cell population could be induced in normal SPF animals after prolonged co-housing with PS mice, suggesting transmissible microbes as a key driver of these differences, rather than genetic disparities between SPF and PS animals.

To determine how changes in immune homeostasis affect the response to novel pathogens, we tested whether C57BL/6 (B6) SPF mice that were or were not co-housed with PS animals differed in their capacity to resist a primary infection with *Listeria monocytogenes* (LM). Notably, co-housed B6 mice showed significantly greater control of LM, reaching levels of protection similar to those induced by active immunization of SPF B6 mice. In a different disease model – infection with P.berghei ANKA infected red blood cells – the rapid cerebral malaria that afflicts SPF B6 mice was significantly delayed in B6 mice co-housed with PS animals.

To study immune homeostasis at a more global level, we analyzed gene expression patterns in peripheral blood mononuclear cells from SPF, PS and co-housed mice, and compared these to similar data from human adult and cord blood cells. While gene expression characteristics of blood from SPF animals aligned with cord blood of humans, blood cells from PS and co-housed animals mimicked adult human blood gene expression patterns. Genes regulated by Type-I IFN were an important driver of these differences.

These data suggest that more physiological immunological experience, through natural exposure to transmissible microbes, has a radical effect on normal immune homeostasis and reactivity in inbred mice, and that such animals may more faithfully reflect certain characteristics of the human immune system.

Eighteen views of Interleukin-2

K. Christopher Garcia

Stanford University/HHMI

In my talk I will discuss my laboratories efforts in integrating structural biology, protein engineering and cell signaling to probe and manipulate the actions of the immune cytokine Interleukin-2. Beginning with gaining structural access to the IL-2/receptor complex in 2005, we have used this information as a blueprint to engineer variants of IL-2, as well as surrogate ligands, that have distinct biological activities and, in some cases, therapeutic applications. I will summarize ongoing studies in the lab.

Key citations:

J.B. Spangler, J. Tomala, V.C. Luca, K.M. Jude, S. Dong, A.M. Ring, P. Votavova, M. Pepper, M. Kovar, **K.C. Garcia**. Antibodies to Interleukin-2 Elicit Selective T Cell Subset Potentiation through Distinct Conformational Mechanisms. *Immunity.* 42(5):815-25 (2015). PMCID 4439582.

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The microbiota promotes systemic T cell survival through a novel apoptotic factor

June L. Round

Raymond Soto, Charisse Petersen, Camille L. Novis, Jason L. Kubinak, W. Zac Stephens, Rickesha Bell, Robert Fujinami, Alberto Bosque, Ryan M. O'Connell and June L. Round

Department of Pathology, Division of Microbiology and Immunology, University of Utah School of Medicine, Salt Lake City

Symbiotic microbes influence host immune system development, yet little is known regarding the mechanisms by which this is accomplished at extra-intestinal sites. Here we report that a novel T cell gene, Erdr1, is regulated by the microbiota to control cellular apoptosis. Erdr1 expression is elevated in systemic T cells from germfree and antibiotic treated mice, indicating that microbial cues suppress expression of this gene in T cells. Repression of Erdr1 depends on direct detection of circulating microbial products by TLRs on T cells and this pathway is conserved in human T cells. Functional studies reveal that Erdr1 is an autocrine factor that promotes T cell apoptosis through induction of Fas and is caspase-3 dependent. Consistent with elevated Erdr1, germfree mice have increased T cell death and forced expression of Erdr1 in colonized animals induces antigen specific apoptosis and alleviates inflammation during an immune response. Importantly, Erdr1 expression levels control survival of auto reactive T cells and clinical outcome of autoimmunity. Cellular survival is a fundamental feature regulating appropriate immune responses. We have identified a mechanism whereby the host integrates signals from the microbiota to control T cell survival, making Erdr1 a microbiota-controlled rheostat to govern immunity.

Kubinak JL, Petersen C, Stephens WZ, Soto R, Bake E, O'Connell RM, Round JL (2015). MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health. Cell Host Microbe, 17(2), 153-63.

Kubinak JL, Stephens WZ, Soto R, Petersen C, Chiaro T, Gogokhia L, Bell R, Ajami NJ, Petrosino JF, Morrison L, Potts WK, Jensen PE, O'Connell RM, Round JL (2015). MHC variation sculpts individualized microbial communities that control susceptibility to enteric infection. Nature Communications, 6, 8642.

Regulation of Immunity by Integrins

Adam Lacy-Hulbert

Benaroya Research Institute, Seattle, WA

The objectives of our laboratory are to understand the mechanisms by which the innate system initiates and maintains tolerance. We have a longstanding interest in how the innate immune system recognizes and removes dying cells. Defects in this process are associated with autoimmunity in animal models and in patients with systemic autoimmune diseases such as Systemic Lupus Erythematosus. We have previously shown that phagocytosis of apoptotic cells drives an immune-regulatory phenotype in macrophages and dendritic cells(1,2), and ongoing work is focused on understanding the mechanisms linking apoptotic cell recognition with tolerance.

Alpha-v integrins, a family of adhesion molecules, are key components of the apoptotic cell clearance machinery. Deletion of alpha-v, or its partners beta 3 and beta 5, inhibits phagocytosis of apoptotic cells(3), and loss of serum molecules such as MFGE8, which bridge alpha-v beta 3 to exposed phosphatidylserine on apoptotic cells., cause autoimmunity in mice. We have previously used conditional knockout mice to identify critical immune roles for alpha v integrins including activation of TGF-beta(3,4) and cell migration. More recently we have identified a new role for alpha v in regulation of signaling through toll-like receptors (TLRs).
We have found that deletion of alpha-v or beta 3 integrins from B cells causes increased responses to TLR stimulation and leads to increased responses to antigens associated with TLR ligands, including self nucleic acids. Alpha-v regulates TLR signaling by promoting the association of the autophagy component LC3 with TLR-containing endosomes, which is essential for progression from NF-κB to IRF signaling, and ultimately for traffic to lysosomes and signal termination. Disruption of LC3 recruitment leads to prolonged NF-κB signaling and increased B cell proliferation and antibody production. Mice lacking alpha-v in B cells are predisposed to development of autoantibodies associated with SLE, such as DNA and phosphatidylserine, suggesting that recognition of apoptotic cells by B cells through integrins may be an important component of B cell tolerance. In addition, this work identifies a previously unrecognized role for alpha-v and the autophagy components LC3 and atg5 in regulating TLR signaling and B cell immunity.

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Viruses and tolerance to dietary antigens

Bana Jabri

University of Chicago

The gut is continuously exposed to pathogens and dietary antigens, posing the question of how our intestinal immune systems promotes protection against pathogens while ensuring tolerance to dietary antigens, and whether protective immunity can be dissociated from immunopathology. This question applies to viral infections that have been implicated in the pathogenesis of celiac disease (1,2), an autoimmune-related disorder induced by dietary gluten in genetically susceptible individuals (3,4). We will provide evidence that an avirulent viral pathogen successfully cleared from the infected host can nonetheless induce loss of tolerance to dietary antigen by disrupting immune homeostasis. We will discuss a role for the compartmentalization of protective immunity and oral tolerance, and the definition of a viral pathotype for oral tolerance that is dependent on a signaling pathway dispensable for protective immunity. Finally, we will show that two viruses belonging to the same family and inducing a similar protective intestinal immune response can have distinct immunopathological properties.

References:

1. Troncone R, Auricchio S. 2007. Rotavirus and celiac disease: clues to the pathogenesis and perspectives on prevention. *J Pediatr Gastroenterol Nutr* 44: 527-8

2. Di Sabatino A, Pickard KM, Gordon JN, Salvati V, Mazzarella G, Beattie RM, Vossenkaemper A, Rovedatti L, Leakey NA, Croft NM, Troncone R, Corazza GR, Stagg AJ, Monteleone G, MacDonald TT. 2007. Evidence for the role of interferon-alfa production by dendritic cells in the Th1 response in celiac disease. *Gastroenterology* 133: 1175-87

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Long-term consequences of infection for tissue immunity and metabolism

Yasmine Belkaid

Denise Morais da Foncesca[1], Tim Hand[1,2] and Yasmine Belkaid[1]

[1]NIAID, NIH, Bethesda
[2]University of Pittsburg

Infections have been proposed as initiating factors for inflammatory disorders, however, identifying associations between defined infectious agents and the initiation of chronic disease has remained elusive. Here, we report that a single acute infection can have dramatic and long-term consequences for tissue-specific immunity. Following clearance of Yersinia psuedotuberculosis, sustained inflammation and associated lymphatic leakage in the mesenteric adipose tissue deviates migratory dendritic cells to the adipose compartment thereby preventing their accumulation in the mesenteric lymph node. Consequently, canonical mucosal immune functions, including tolerance and protective immunity are persistently compromised. Post-resolution of infection, signals derived from the microbiota maintain inflammatory mesentery remodeling and consequently transient ablation of the microbiota restores mucosal immunity. Mesentery damages is also associated with profound alteration in tissue metabolism including aberrant fat depot and dyslipidemia. Our results indicate that persisting disruption of communication between tissues and the immune system following clearance of an acute infection represents an inflection point beyond which, without intervention, restoration of tissue homeostasis, metabolism and immunity is not possible.

References:

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Askenase M, Byrd AL, Morais da Fonseca D, Bouladoux N, Wilhelm C, Konkel JE, Hand T, Lacerda-Queiroz N, Su X, Trinchieri G, Grainger JR and **Y Belkaid.** Regulatory priming of monocytes by bone marrow resident NK cells during infection. 2015. *Immunity.* 16: 1130-42.

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Regulation of immune cell function and spatiality by stroma

Shannon J. Turley

Viviana Cremasco[1], Flavian Brown[1], Angelo L. Grauel[1], Michael Wu[1], Jillian Astarita[1,8], Shilpa Keerthivasan[8], Stephen Santoro[8], Frank A. Schildberg[1], Matthew C. Woodruff[2,3], Lotte Spel[1], Zohreh Amoozgar[1], Martin LeFleur[1], Michael C. Carroll[2,6], Kai W. Wucherpfennig[1], Shannon J. Turley[1,7,8]
and the IMMGEN Consortium

[1]Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, Boston, Massachusetts, 02115; [2] Program in Cellular and Molecular Medicine, Children's Hospital, Boston, Massachusetts, 02115; [3]Division of Medical Sciences, Harvard Medical School, Boston, MA 02115, USA; [6]Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA; [7]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, USA; [8]Department of Cancer Immunology, Genentech, 1 DNA Way, South San Francisco, California, 94080.

Stromal cells have long been viewed as key structural determinants that form the infrastructure of tissues including lymphoid organs. However, these cells are now considered essential regulators of immune cell trafficking, fluid flow, and lymphoid tissue homeostasis and expansion. Major advances in the isolation and in vivo targeting of specific stromal populations such as fibroblastic reticular cells have yielded crucial new insights into their immunological functions. Recent discoveries from my laboratory highlighting pivotal roles played by $PDPN^{+}PDGFR\alpha^{+}$ fibroblastic reticular cells in orchestrating lymphoid organ homeostasis, stromal microarchitecture and adaptive immunity will be presented. New work from the lab elucidating the immunomodulatory potential of distinct stromal cell subsets in the tumor microenvironment will also be discussed.

References:

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Role of the omentum in immunity to peritoneal tumors

Troy D. Randall

University of Alabama, Birmingham

The omentum is a fatty tissue connecting the stomach, spleen and pancreas that contains clusters of lymphocytes and APCs known as milky spots. Fluid from the peritoneal cavity drains through the milky spots, making them an ideal site for immune recognition and responsiveness. The milky spots also collect tumor cells from the peritoneal cavity and are important sites for the metastasis for ovarian and other cancers. Despite the immune activities of the milky spots, the accumulation of tumor cells in the omentum leads to profound immunological tolerance to tumor-associated antigens. This tolerance is mediated by specialized omentum-associated Tregs, which impair local anti-tumor immunity and contribute to poor clinical outcomes in mice and patients with peritoneal tumors.

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Recruiting synergistic innate and adaptive immune responses against tumors

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Abstract:
Immunotherapies are often developed with a focus on either innate immunity (e.g., tumor-directed antibodies) or adaptive immunity (e.g., adoptive T-cell therapy or cancer vaccines). We recently reported on an impressive synergy of combining anti-tumor antibodies with an extended-PK interleukin-2 molecule (exPK IL-2; IL-2 fused with an Fc protein or albumin) in the B16F10 mouse model of melanoma, which was strikingly dependent on components of both adaptive (CD8 T-cell) and innate (NK cell, neutrophils, eosinophils) immunity.[1] In parallel, we developed a very potent therapeutic vaccine strategy based on the conjugation of peptide antigens to albumin-binding lipid tails, where upon injection the conjugated peptides are efficiently targeted to lymph nodes through non-covalent hitchhiking on endogenous albumin.[2] Recognizing the complementary nature of these approaches, we examined immune responses induced by a four-component immunotherapy seeking to optimally merge “adaptive-centric” (a lymph node-targeted therapeutic vaccine and anti-PD-1 checkpoint blockade) with “innate-centric” (anti-tumor antibody and ex-PK IL-2) that enabled the endogenous immune response to destroy large established B16F10 tumors (>50 mm^2) that to our knowledge have previously only been treated by combination treatments involving transfer of large numbers of exogenous tumor-specific T-cells.[3] Similarly, this combination therapy applied to treatment of large established tumors in models of cervical cancer, breast cancer, and an inducible genetically-engineered model of melanoma all led to cures in a majority of animals. This treatment depended on both innate and adaptive immune cells, and was superior to subcombinations of each of the four individual components. Importantly, the combination treatment was critically dependent on cross presenting dendritic cells, and promoted antigen acquisition by CD8a+ dendritic cells, leading to priming of T-cell responses against new antigens not included in the vaccine. These findings illustrate the capacity of the endogenous immune response to eliminate very large established tumors and provide insights into the features of successful anti-tumor immunity.

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Tumor-associated lymphatic vessels modulate the immune microenvironment to promote immune suppression and tolerance

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Lymphatic vessels and cancer metastasis have long been correlated: the presence of the lymphatic growth factors VEGF-C/D in the tumor microenvironment associates with increased metastasis and poor prognosis, and function-blocking antibodies against their main receptor VEGFR-3, which prevent new lymphatic growth, are being explored in clinical trials to prevent metastasis (1). On the other hand, VEGF-C likely plays complex roles in the tumor microenvironment; in addition to driving lymphatic expansion locally and in the draining lymph node (LN), it has also been shown to increase lymphatic drainage and alter immune cell transport to the LN (2). Additionally, we have shown that VEGFR-3 activation on lymphatic endothelial cells increases their production of the lymphoid homing chemokine CCL21 (3), which attracts certain leukocyte subsets, naïve T cells, and regulatory T cells, and which can also drive lymphoid-like stromal remodeling through the recruitment of lymphoid tissue inducer cells (4). VEGF-C can be secreted by tumor-associated macrophages, stromal cells, or even tumor cells themselves. Here, we asked how VEGF-C-driven lymphatic activation alters the tumor stroma, both biomechanically as well as immunologically, particularly in its support of immune cell infiltrates and suppressive cytokine environment. In addition to causing lymphatic expansion, we found that VEGF-C stimulates multiple and complex alterations in the tumor stroma that promote immune suppression, tolerance, and invasion. These include lymphatic secretion of TGF-beta, which drives stromal myofibroblast activation and pro-invasive stromal remodeling, as well as secretion of immune suppressive factors like IDO. Furthermore, we found that VEGF-C enhances lymphatic endothelial cell scavenging and presentation of tumor antigens on MHCI and MHCII molecules as well as PD-L1, in turn dampening anti-tumor T cell responses. Together, these data suggest that VEGF-C/VEGFR-3 targeting could potentially be important in strategies that aim to alter the tumor microenvironment in order to improve the efficacy of immunotherapies more.

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Single-cell genomics identifies novel regulators of pro-inflammatory function in Th17 cells

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Recently a subset of interleukin (IL)-17-producing T cells (T_H17) distinct from T_H1 or T_H2 cells was described and shown to have a crucial role in the induction of autoimmune tissue injury. In contrast to the effector T cells, $CD4^+CD25^+$, $Fox\text{-}P3^+$ regulatory T cells (T-regs) inhibit autoimmunity and protect against tissue inflammation. TGF-β is a critical differentiation factor for the generation of T-regs and using Foxp3-GFP "knock-in" mice we have shown that IL-6, an acute phase protein induced during infection, inflammation and injury inhibits the generation of $Foxp3^+$ T-reg cells and induces proinflammatory Th17 cells (Bettelli et al., 2006). Our data therefore suggests a reciprocal relationship in the generation of pathogenic (Th17) T cells that induce autoimmunity and regulatory ($Foxp3^+$) T cells that inhibit autoimmune tissue injury. Accumulating data suggests that there are three distinct steps in Th17 differentiation: Induction, Amplification and Stabilization mediated by distinct cytokines and loss of any of the cytokines (TGF-b, IL-6, IL-21 or IL-23) in the pathway results in a defect in generation of Th17 (Korn et al., 2009). However not all Th17 cells are pathogenic and induce autoimmunity, IL-23 is a key cytokine that induces pathogenicity in Th17 cells (Lee et al., 2012). Using expression profiling at very high temporal resolution, novel computational algorithms and innovative nano-wire based "knock-down" approaches, we have developed a regulatory network that governs the development of Th17 cells. The Th17 transcriptional network consists of two self-enforcing but mutually antagonistic modules, which are essential for maintaining a balance between Th17 and other CD4 T cell subsets including Tregs (Yosef et al. 2013, Wu et al., 2013).

In addition to high-density temporal microarray analysis, we have performed single-cell RNA-seq of Th17 cells in order to characterize cellular heterogeneity, identify subpopulations, functional states and learn how gene expression variation affects Th17 effector functions. We have identified novel regulators of Th17 cells both *in vivo* and *in vitro* that do not affect Th17 differentiation but affect pathogenic vs. non-pathogenic functional states of Th17 cells.

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Tregs in Skin Facilitate Epithelial Stem Cell Differentiation

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Abstract:
Regulatory T cells (Tregs) play an indispensable role in suppressing inflammation. However, emerging data suggest that these cells have specialized functions in tissues that are independent of immune regulation. A relatively large proportion of Tregs reside in skin where they preferentially localize to hair follicles (HFs). We hypothesized that cutaneous Tregs play a role in HF biology. To test this hypothesis, we comprehensively phenotyped and functionally characterized HF-associated Tregs. Tregs localize to the bulge region of the resting HF in the steady-state and become activated upon induction of HF cycling. Transient depletion of Tregs in a depilation-induced model of hair follicle cycling resulted in pronounced attenuation of hair regeneration, suggesting that Tregs play a major role in facilitating HF cycling. Examination of the CD34+ HF stem cell (HFSC) compartment in Treg depleted mice showed a significant reduction in depilation-induced proliferation and differentiation. Whole transcriptome RNA sequencing of Tregs and HFSCs revealed that skin Tregs express the Notch ligand, Jagged-1, and that the Notch pathway is activated in HFSCs in the presence of Tregs. In functional experiments, recombinant Jagged-1 was able to rescue HF cycling in the absence of Tregs. Taken together, our data suggest that Tregs in skin directly influence HFSC function independent of their ability to suppress inflammation and further contribute to our expanding knowledge of the alternative functions of Tregs in tissues.

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Context-dependent regulation of CD4+ T cell responses by miR-155

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MicroRNAs play indispensable roles in regulating cellular development and function in the immune system. In particular, miR-155 has emerged as a critical regulator of both innate and adaptive immune responses following its upregulation in activated immune cells. Through the use of miR-155 conditional knockout mice, we have extensively investigated miR-155's role in the CD4+ T cell compartment. This work has identified critical, cell-intrinsic functions for miR-155 during Th1, Th17 and Tfh cell-mediated responses using mouse models of solid tumor growth, neuroinflammation and chronic low-grade inflammation, respectively. These data point to a pro-inflammatory role for this evolutionarily conserved microRNA. However, our recent work has also determined that T regulatory (Treg) cell-specific deletion of miR-155 impairs Treg functions resulting in heightened inflammatory responses *in vivo*. Together, these findings further refine our understanding of miR-155's role in the immune system: In addition to functioning in effector T cells to promote immune responses that mediate inflammation, miR-155 also acts in Tregs to ensure that immune responses have the proper magnitude and resolution. Thus, miR-155 has evolved to be a multi-faceted regulator of CD4+ T cell plasticity and context dependent functions, and its future use as a therapeutic target to treat inflammatory diseases should be carefully considered to achieve optimal clinical outcomes.

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Investigating the potential of individual naive B cells

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Most successful vaccines provide protection by inducing the production of antibodies that can bind to a pathogen and block infection. Unfortunately, there are many dangerous viruses in which the development of a vaccine has been elusive despite decades of intense research. These failures highlight gaps in knowledge about the type of cell that produces antibodies: the B cell. Under optimal conditions, a vaccine would induce “naive” B cells to produce large numbers of long-lived antibody-secreting and memory B cells that express high affinity protective antibodies. Unfortunately, the mechanisms controlling naive B cell activation and differentiation are complex and not fully understood. This gap in knowledge prevents the development of vaccines that can ensure the generation of a protective response. Our work is focused upon understanding the intrinsic and extrinsic mechanisms limiting the generation of a protective B cell response. To do this, we study B cell responses in humans and murine models beginning with the rare antigen-specific naive B cells present prior to the vaccination using enrichment methods we have developed. In this presentation, we will describe the results of experiments assessing the capability of individual naive B cells using an in vivo limiting dilution approach. In addition, we will describe experiments probing the mechanisms that explain the surprising finding that most antigen-binding naive B cells fail to respond to antigen immunization.

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Genetics of T follicular helper cell (Tfh) differentiation and function

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T cell help to B cells was one of the earliest discovered functions of T cells, 47 years ago, resulting in the coining of the term 'helper cell' [1]. The vast majority of currently licensed human vaccines work on the basis of long-term protective antibody responses. Generation of long term humoral immunity is a complex process predominantly dependent on germinal centers and CD4 T cell help to B cells. Follicular helper T cells (Tfh) are the specialized CD4 T cells for B cell help. We and others have now resolved many of the stages of Tfh differentiation, and molecules involved. We have also explored human Tfh cell biology and Tfh cell memory. In mechanistic studies of Tfh cells, we have identified novel genetic regulators of Tfh differentiation and function [2-6].

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The mechanistic basis of cancer immunotherapy

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The rapid advance of cancer immunotherapy is changing fundamentally the way we think about cancer care and cancer biology. To a significant degree, recent progress in the clinic was made possible by the understanding that the immunosuppressive mechanisms deployed by tumors to subvert T cell attack represented a key rate-limiting step in many patients. Interestingly, these mechanisms are not tumor-specific specializations but rather manifestations of the normal regulatory mechanisms used by all cells and tissues to maintain immune homeostasis. The best validated of these is the interaction between the negative regulator of T cells PD-1 with its ligand PD-L1 expressed by tumor cells or tumor infiltrating immune cells in response to IFN release. Antibodies that block this interaction, and the interaction of PD-L1 with a second T cell negative regulator CD80, have proved to be highly effective in the clinic over a wide array of solid and hematologic tumor indications. Yet, in important tumor types such as non-small cell lung cancer, only a fraction of patients respond clinically: ~20% overall, with responses enriched 2-3 fold in patients whose tumors express PD-L1 at baseline. To improve and extend the benefit of this therapy, it has now become critical to understand the use of biomarkers to aid patient selection and also to devise combinations of anti-PDL1/PD-1 with other immunological, oncogene-targeted, or standard of care chemotherapies.

Progress in the clinic has been so rapid, however, that our understanding of how the PD-L1/PD-1 axis works at the mechanistic level has lagged considerably. Mechanistic understanding is not only a scientifically compelling problem but also will be invaluable to therapeutic efforts. We have, therefore, engaged a series of investigations guided by results of our clinical studies, particularly by the detailed biomarker analysis to discover the immune correlates of “response” and “lack of response” in large patient cohorts. Our analysis to date has revealed a number of fundamental insights into the mechanism of action of PD-1, which in turn has informed strategies for undertaking combination therapies in the clinic.

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17q21 asthma-risk variants switch CTCF binding and regulate IL-2 production by T cells

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ABSTRACT

Asthma and autoimmune disease susceptibility has been strongly linked to genetic variants in the 17q21 haploblock that alter the expression of *ORMDL3*; however, the molecular mechanisms by which these variants perturb gene expression and the cell-types in which this effect is most prominent are unclear. We found several 17q21 variants overlapped enhancers present mainly in primary immune cell types, implying that these cell types are likely to be most affected by the 17q21 variants. Primary T cells showed the greatest increase (3-fold) in *ORMDL3* expression in individuals carrying the asthma-risk alleles, and ORMDL3 negatively regulated activation-induced interleukin (IL)-2 transcription and protein production. We identified potentially functional SNPs in the 17q21 haploblock that affect the activity of an enhancer in the intron of *ORMDL3*. The asthma-risk variant rs4065275 introduces a CTCF binding site in this enhancer region, and simultaneously, another linked risk variant rs12936231 disrupts CTCF binding at a downstream region, resulting in switching of CTCF binding sites by 17q21 variants in the locus. Overall, our results suggest that T cells are one of the most prominent cell types affected by 17q21 asthma-risk variants, a finding with important implications for understanding the genetic basis of asthma.

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From broadly neutralizing antibodies to a new mechanism of diversification

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We have developed cell culture-based high-throughput methods to interrogate with high efficiency human memory B cell and plasma cell repertoires and to isolate antibodies selected on the basis of their neutralizing potency and breadth. Relevant examples are antibodies that neutralize all influenza A viruses[1] or even four paramyxoviruses[2]. By targeting conserved structures, these broadly neutralizing antibodies are less prone to select escape mutants and are therefore promising candidates for prophylaxis and therapy of infections, as well as tools for vaccine design[3]. The value of such a target-agnostic approach to vaccine design is illustrated by our discovery of extremely potent antibodies that neutralize human cytomegalovirus, which led to the identification of their viral ligand[4], a pentameric complex that was finally produced and tested as an effective vaccine[5]. By reconstructing the genealogy trees of specific B cell clones, we investigate the role of somatic mutations in affinity maturation and in generation of antibody variants with broader or different specificity. We found that affinity maturation is achieved rapidly, often through a single mutation, but numerous redundant mutations accumulate, leading to extensive intraclonal diversification that may broaden reactivity against homologous viruses[6]. In some cases however, somatic mutations appear to be able to generate autoantibodies, as found in patients with pemphigus and autoimmune pulmonary alveolar proteinosis[7.8]. Recently, while searching for antibodies that broadly react with malaria variant antigens, we discovered a new mechanism of antibody diversification, which relies on the interchromosomal transposition of genomic DNA sequences into rearranging immunoglobulin genes, followed by somatic mutations[9]. In my presentation I will briefly review our work on the role of somatic mutations in affinity maturation and intraclonal diversification and discuss, in more details, the recent findings on the antibodies against malaria variant antigens generated by interchromosomal DNA transposition.

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