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The 2018 La Jolla Immunology Conference will be held at The Salk Institute, overlooking the Pacific Ocean just west of the University of California, San Diego.

Driving Directions (From North)

1. Use I-5 South
2. Exit Genesee Ave and turn right (heading West)
3. Turn left onto North Torrey Pines Road
4. Drive 1/8 mi, and turn right onto Torrey Pines Scenic Drive

Driving Directions (From South)

1. Use I-5 North
2. Exit Genesee Ave and turn left (heading West)
3. Turn left onto North Torrey Pines Road
4. Drive 1/8 mi, and turn right onto Torrey Pines Scenic Drive

***Alternative means of transportation is recommend***
Tuesday, October 16th 2018

11:00am - 1:00pm:  REGISTRATION & LUNCH

1:00pm - 2:00pm:  Keynote Lecture
       Jenny Ting (UNC)
   Intracellular Innate Immune Receptors: Regulators of Inflammation, Cancer and Immunity

Session I: IMMUNE AND NERVOUS SYSTEM  Chair: Axel Nimmerjahn

2:15pm - 2:35pm:  Thomas Riffelmacher (LJI)
   Lymphotoxin receptor beta expression by neutrophils prevents metabolic activation and colitis pathogenesis

2:35pm – 3:05pm:  Monica Carson (UCR)
   TREM2 a molecular link between infection, injury and dementia

3:05pm - 3:35pm:  Axel Nimmerjahn (Salk)
   How do microglia recognize and respond to viral infection?

3:35pm - 3:55pm:  Shivashankar Othy (UCI)
   Visualizing the Dynamics of Regulatory T cells During Autoimmune Neuroinflammation

4:00pm - 4:30pm:  COFFEE BREAK

4:30pm - 5:00pm:  Christopher Glass (UCSD)
   Nature and nurture of human microglia identity and function

5:00pm - 5:20pm:  Li-En Hsieh (UCSD)
   Human natural regulatory T cells recognize immunodominant peptides of the heavy constant region of immunoglobulins endogenously processed and presented by IgG+ B cells
5:20pm - 5:40pm: Julie Burel (LJI)
No cell is an island: circulating T cell: monocyte complexes are markers of immune perturbations

5:45pm - 7:30pm: POSTER SESSION I (sponsored by Gilead Science)

Wednesday, October 17th 2018

**Session II: IMMUNE REGULATION OF THE GENOME** Chair: Sonia Sharma

9:00am - 9:30am: Sonia Sharma (LJI)
Metabolic-epigenetic priming of cell-intrinsic innate immunity in a systemic inflammatory disease

9:30am - 9:50am: Ty D. Troutman (UCSD)
Exploiting altered enhancer landscapes to decode pathogenic changes in Kupffer cell gene expression

9:50am - 10:20am: Ferhat Ay (LJI)
Distal enhancer regulatory landscapes of primary human immune cells

10:20am - 10:50am: COFFEE BREAK

10:50am - 11:10am: Joana Borlido (SBPMDI)
Nucleoporins and T cell functions

11:10am - 11:40pm: Kristian Andersen (TSRI)
Outbreakiomics

11:40am - 12:00pm: Kyla Omilusik (UCSD)
Sustained Id2 regulation of E-proteins is required for terminal differentiation of effector CD8+ T cell

12:00pm - 1:30pm: Meet the Expert Lunch: INDUSTRY / BIOTECH / EDITORS (sponsored by Pfizer)

**Session III: PATHOGENS AND IMMUNITY** Chair: Elina Zuniga

1:30pm - 2:00pm: Elina Zuniga (UCSD)
Immune Adaptations During Chronic Viral Infections

2:00pm - 2:30pm: Elise Landais (TSRI)
Broadly Neutralizing Antibodies to HIV-1: Lessons from Protocol C Studies

2:30pm - 2:50pm: Lindsey E. Padgett (LJI)
Exploring a Novel CD8+ T cell that Intensifies Cardiovascular Disease and Atherosclerosis
2:50pm - 3:10pm: Christophe Pedros (LJI)  
Control of regulatory T cell function by PKC-eta (PKCη), a novel target for cancer immunotherapy

3:10pm - 3:40pm: COFFEE BREAK

Session IV: MOLECULAR IMMUNOLOGY Chair: Timothy O'Sullivan

3:40pm - 4:10pm: Timothy O'Sullivan (UCLA)  
Tissue-Resident Responses at Sites of Initial Viral Infection

4:10pm - 4:30pm: Chad R. Dufaud (TSRI)  
PD-1 blockade releases commensal-specific B cell immunity and disrupts gut microbiome composition

4:30pm - 5:00pm: Lili Yang (UCLA)  
Stem Cell-Engineered Invariant Natural Killer T cells for Cancer Immunotherapy

5:00pm - 5:10pm: MELVIN COHN AWARD  
Intro by Suzanne Bourgeois-Cohn

5:10pm - 5:40pm: MEL COHN TALK: Alexandra Kuhlmann (Salk)  
Metabolic adaptation of tissue-resident macrophages in cancer

5:45pm - 7:30pm: POSTER SESSION II (sponsored by Celgene)

Thursday, October 18th 2018

Session V: CANCER IMMUNOLOGY Chair: Enfu Hui

9:00am - 9:30am: Aaron Miller (UCSD)  
Letting Biology Drive Neoantigen Discovery

9:30am - 9:50am: Joyce Chen (LJI)  
Nr4a transcription factors limit CAR T cell function in solid tumors

9:50am - 10:20am: Enfu Hui (UCSD)  
Mechanistic dissection of PD-1 signaling using reconstitution approaches

10:20am - 10:50am: COFFEE BREAK

10:50am - 11:10am: James Clarke (LJI)  
Single-cell analysis of tissue-resident memory T cells in human cancer

11:10am - 11:40am: Susan Kaech (Salk)  
Making Effector T cells the EZ(H2)-way
11:40am - 12:00pm: Joseph S. Dolina (LJI)
Functional identification of neoantigen-specific T cell responses targeting murine squamous cell carcinoma (SCC) VII

12:00pm - 1:30pm: Meet the Expert Lunch: ACADEMIA/FACULTY (sponsored by Janssen)

Session VI: REGULATION OF GENE EXPRESSION Chair: Cornelis Murre

1:45pm - 2:15pm: Hilde Cheroutre (LJI)
A long noncoding RNA in the Cd8 locus controls functional differentiation of CD4 T cells

2:15pm - 2:35pm: Cheng-Jang Wu (UCSD)
MiR-23~27~24-mediated control of humoral immunity reveals a TOX-driven regulatory circuit in follicular helper T cell differentiation

2:35pm - 3:05pm: Cornelis Murre (UCSD)
Non-Coding Transcription Establishes T Cell Identity

3:05pm - 3:25pm: Nazia Abbasi (UCSD)
DDX5 regulates RNA editing in Th17 cell differentiation

3:30pm - 4:00pm: COFFEE BREAK and FINAL VOTING

4:00pm - 5:00pm: Charles Surh Memorial Lecture
Jonathan Sprent (Garvan Institute, Australia)
Self tolerance: new thoughts on an old issue (sponsored by BD Life Sciences)

5:00pm - 5:15pm: Poster and Oral Presentation Awards (sponsored by American Association of Immunologists)

5:15pm: RECEPTION @ SALK INSTITUTE (sponsored by BioLegend)
Thank you to our Sponsors!
Experts Lunches

Industry Experts
Lunch Wednesday, Oct.17th noon – 1:30pm

Editor Table 1:
Ifor Williams, Science Immunology
Brian Kelsall, Nature Immunology

Editor Table 2:
Sara Hamilton, Cell Reports
Alejo Chorny, Journal Experimental Medicine

Editor Table 3:
João Lucas Duarte, Nature Biomedical Engineering

Industry Table 4:
Marcos Steinbert, Celgene
Margo Roberts, Kite Pharma

Industry Table 5:
Kelly Lundsten, BioLegend
Mohd Mushtag Husain, Fate Therapeutics

Industry Table 6:
Flavia Pernasetti, Pfizer
Art Levin, Avidity Bio

Industry Table 7:
Lawrence Fourgeaud, Jansen
Kurt Wong, United Therapeutics Corp.

Academia Experts
Lunch Thursday, Oct.18th noon – 1:30pm

Table 1:
Sonia Sharma (LJI)
Aaron Miller (UCSD)

Table 2:
Timothy O'Sullivan (UCLA)
Susan Kaech (Salk)

Table 3:
Lili Yang (UCLA)
Ferhat Ay (LJI)

Table 4:
Cornelis "Kees" Murre (UCSD)
Gislaine Martins (Cedars-Sinai)

Table 5:
Monica Carson (UCR)
Michael McHeyzer Williams (TSRI)

Table 6:
Lynn Hedrick (LJI)
Li Fan (UCSD)
Invited Keynote Speaker

Jenny Ting, Ph.D.

William Rand Kenan Professor of Genetics, Microbiology and Immunology
Department of Genetics
Immunology Program Leader, Lineberger Comprehensive Cancer Center
Director, Center for Translational Immunology
Co-Director, Inflammatory Disease Institute
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599-7295

“Intracellular Innate Immune Receptors: Regulators of Inflammation, Cancer and Immunity”

The innate immune system is unique that much of the pathogen detection occurs intracellularly, involving intracellular receptors. The NLR (NBD-LRR or NOD-like receptor) family is a conserved family of innate immune receptors and sensors defined by a conserved nucleotide-binding domain and leucine rich repeats. Members of this family play important regulatory roles in inflammatory diseases, cancer and multiple types of infection. Mutations or polymorphisms within this family have been associated with a number of monogenic or complex genetic diseases. The inflammasome NLRs form macromolecular complexes with a variety of adaptors and enzymes to regulate anti-microbial and inflammatory responses. The inflammasome complex leads to caspase-1, caspase-11, IL-1beta and IL-18 cleavage and cytokine secretion. We have recently humanized a mouse line model by syntenic replacement of NLRP3 and will discuss the outcome of this model. Although many NLRs are positive regulators of inflammation, others mediate negative regulatory functions in a number of disease models and appear to be important in maintaining a homeostatic state of health. Several are regulators of NFκB, STAT and MAPK, and a have profound impact on inflammation, infection and cancer. In addition, recent findings show that NLRs have a profound impact on the microbiome, resulting in resistance to metabolic and inflammatory diseases.
Oral Presenter for Voting

Thomas Riffelmacher (LJI)

Thomas Riffelmacher (1), Daniel Giles (1), Sonja Zahner (1), Esmé van der Gracht (1), Venetia Morris (1), Alexei Tumanov (2), Zbigniew Mikulski (3), Mitchell Kronenberg (1)

(1) Division of Developmental Immunology and (3) Microscopy Core, La Jolla Institute for Allergy & Immunology, 9420 Athena Circle, La Jolla, CA 92037. (2) Department of Microbiology, Immunology and Molecular Genetics, University of Texas Health Science Center San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229.

Lymphotoxin receptor beta expression by neutrophils prevents metabolic activation and colitis pathogenesis

Inflammatory bowel disease represents a group of intestinal disorders characterized by exacerbated intestinal immune activation. However, the mechanisms that lead to immune activation remain to be fully defined. We have previously reported that a member of the TNF superfamily, TNFSF14 (LIGHT), is required for preventing severe disease in a mouse model of dextran sulfate sodium (DSS) induced colitis. Additionally, antibody mediated blocking of lymphotoxin beta receptor (LTβR), which recognizes LIGHT, also led to exacerbated colitis pathogenesis. Thus, we aimed to determine the cell type(s) and mechanism critical to LTβR mediated exacerbation of DSS-colitis. Employing the use of LTβRflox/flox mice and tissue-specific Cre-mediated deletion, we evaluated the impact of LTβR signaling on disease severity in a variety of immune and intestinal cell types. We found that neutrophils were a critical LTβR expressing cell type and that specific deletion of neutrophil LTβR expression, via Mrp8-Cre, resulted in exacerbated DSS-induced colitis and three-fold increased intestinal neutrophil accumulation. Mechanistically, RNASeq analysis of neutrophils revealed alterations in cellular metabolism, and mitochondrial function in particular. Neutrophils from LTβRflox/flox Mrp8-Cre mice had increased mitochondrial mass and number, which was accompanied by excessive mtROS production and activated glycolysis at steady state and in response to LPS. In sum, our results demonstrate that neutrophil LTβR signaling and metabolic activation, through LIGHT ligand, play a critical role in the immune activation associated with DSS-induced colitis.
Invited Speaker

Monica Carson (UCR)

TREM2 a molecular link between infection, injury and dementia

Microglia are highly plastic CNS-resident tissue macrophages. In response to both homeostatic and pathologic changes within the CNS, microglia are able to assume a broad spectrum of phenotypes. Thus, we find that microglia phenotype and function is altered not only by direct insults to the CNS but by systemic inflammation, environmental exposures, age and obesity as well as by sex and estrus cycle. Furthermore, microglia are long-lived cells and thus have the potential to provide a form of “innate immune memory” of life-long insults and environmental exposures. TREM2 is a molecule expressed on microglia and other cells of the myeloid lineage. Humans lacking a functional TREM2 pathway develop a form of early onset dementia in their 20’s. GWAS have further revealed 3-6 fold increased risk of late onset Alzheimer’s disease in individuals carrying mutations in TREM2. Using TREM2 as an example, we demonstrate that the functional consequences of microglial responses to insults and environmental cues are highly context dependent. These data also demonstrate that conclusions concerning the consequences of microglia phenotypes for CNS function can be highly dependent on the model system used.
Invited Speaker

Axel Nimmerjahn (Salk)

Associate Professor
Waitt Advanced Biophotonics Center, The Salk Institute for Biological Studies,
10010 North Torrey Pines Road, La Jolla, CA 92037

How do microglia recognize and respond to viral infection?

Microglia are the intrinsic immune sentinels of the central nervous system. Their activation restricts tissue injury and pathogen spread, but in some settings, including viral infection, this response can contribute to cell death and disease. Identifying mechanisms that control microglial responses is therefore an important objective. Using replication-incompetent adenovirus 5 (Ad5)-based vectors as a model, we recently investigated the mechanisms through which microglia recognize and respond to viral uptake. Transgenic, immunohistochemical, molecular-genetic, and fluorescence imaging approaches revealed that phosphatidylserine (PtdSer) exposure on the outer leaflet of transduced cells triggers their engulfment by microglia through TAM receptor-dependent mechanisms. We showed that inhibition of phospholipid scramblase 1 (PLSCR1) activity reduces intracellular calcium dysregulation, prevents PtdSer externalization, and enables months-long protection of vector-transduced, transgene-expressing cells from microglial phagocytosis. Our study therefore identified PLSCR1 as a potent target through which the innate immune response to viral vectors, and potentially other stimuli, may be controlled. In this talk, I will present some of our latest findings on this topic.
Oral Presenter for Voting

Shivashnakar Othy

Cornelia Ringer, Chijioke Akunwafo, Angel Zavala, Andriy V. Yeromin, Ian Parker, and Michael D. Cahalan.

Department of Physiology and Biophysics, University of California, Irvine, California, USA-92697.

Visualizing the Dynamics of Regulatory T cells During Autoimmune Neuroinflammation

Regulatory T lymphocytes (Tregs) play a crucial role in disease remission in several organ-specific autoimmune disorders. Our knowledge of the localization, real-time dynamics, and interactions within the target organs is limited. Using two-photon microscopy and explanted spinal cord preparation, we characterize the in situ behavior of endogenous Tregs during experimental autoimmune encephalomyelitis (EAE), a murine model of MS. During the onset of EAE, spinal cord homing Tregs infiltrate the subarachnoid space, and are primarily found in the leptomeninges, eventually localizing to the tertiary lymphoid-like structures (TLS) as the disease progresses. Spinal cord homing Tregs actively explore the meninges with an average velocity of $12 \pm 4 \, \mu m/min$ but their motility coefficients and meandering indices are significantly lower than effector T cells (both endogenous and adoptively transferred MOG-specific Th17 cells). Directionality and MSD analysis confirm that Tregs display localized high scanning motility behavior. Further, Tregs repeatedly visit the certain areas on their paths that are occupied by antigen-presenting dendritic cells (DCs). The unique mode of repetitive scanning behavior of Tregs on dendritic cells provides insight into the physical basis of downregulation of costimulatory molecules on the DCs and competition for pMHC complexes, thus preventing T cells from establishing immunological synapses in the CNS. Our results demonstrate a choreography based evidence for Treg-control of local re-activation of encephalitogenic T cells to limit neuroinflammation during EAE.
Invited Speaker

Chistopher K. Glass (UCSD)

*Nature and nurture of human microglia identity and function*

Microglia play essential roles in central nervous system (CNS) homeostasis and influence diverse aspects of neuronal function. Although dysregulation of microglia activity is genetically linked to neurodegenerative and psychiatric diseases, the transcriptional mechanisms that specify human microglia phenotypes are largely unknown. We characterized the transcriptomes and epigenetic landscapes of human microglia isolated from surgically resected brain tissue, revealing that genes associated with risk alleles or exhibiting altered expression in neurodegenerative diseases are preferentially or highly expressed in human microglia in comparison to intact brain tissue. The transition of human and mouse microglia from the brain to a tissue culture environment results in rapid and extensive downregulation of genes, identifying sets of environmentally sensitive transcripts. These findings revealed an environment-dependent transcriptional network specifying the microglial gene expression program and will facilitate efforts to better understand the roles of microglia in human disease. Moving forward, we are investigating the transcriptional networks that are responsive to alterations in brain environment in neurodegenerative diseases and the influence of natural genetic variation as a means of obtaining further insights into pathogenic mechanisms.
Human natural regulatory T cells recognize immunodominant peptides of the heavy constant region of immunoglobulins endogenously processed and presented by IgG+ B cells

Here we define a human population of natural regulatory T cells (nTreg) that controls naive T cell differentiation toward a pro-inflammatory phenotype and recognizes universal peptides derived from the heavy constant region of immunoglobulins (Fc). The fine specificity of nTreg and the immunodominance of the peptides has been determined in healthy donors and in rheumatoid arthritis (RA) patients as a model of systemic autoimmune disease. 8 Fc peptides were identified as highly immunogenic in healthy donors and in RA with great similarities within the ranking but with differences in the epitope spreading. 4 of 8 immunodominant Fc peptides bind multiple HLA alleles including DR, DP and DQ. This nTreg population is activated by IgG+ B cells in the germinal centers and expands by further stimulations with the exogenous Fc processed by classical antigen presenting cells via Fcg receptors internalization and pinocytosis. In RA, the nTreg response to the exogenous Fc has been found greatly defective resulting in the lack of Fc-specific nTreg in circulation.
Oral Presenter for Voting

Julie Burel

Julie G. Burel (1), Mikhail Pomaznoy (1), Cecilia S. Lindestam Arlehamn (1), Daniela Weiskopf (1), Ricardo da Silva Antunes (1), Veronique Schulten (1), Mariana Babor (1), Grégory Seumois (1), Jason A. Greenbaum (1), Sunil Premawansa (2), Gayani Premawansa (3), Ananda Wijewickrama (4), Rashmi Tippalagama (5), Aruna D. deSilva (1) (5), Robert H. Gilman (6) (7), Mayuko Saito (8), Randy Taplitz (9), Pandurangan Vijayanand (1) (10), Alessandro Sette (1) (10), Bjoern Peters (1) (10)

(1) Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA. (2) Department of Zoology and Environment Science, Science Faculty, University of Colombo, Sri Lanka. (3) North Colombo Teaching Hospital, Ragama, Sri Lanka. (4) National Institute of Infectious Diseases, Gothathuwa, Angoda, Sri Lanka. (5) Genetech Research Institute, Colombo, Sri Lanka. #present address: Dept of Paraclinical Sciences, Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka. (6) Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA. (7) Universidad Peruana Caytano Heredia, Lima, Peru. (8) Department of Virology, Tohoku University Graduate School of Medicine, Sendai, Japan. (9) Division of infectious diseases, University of California San Diego, La Jolla, CA, USA. (10) Department of Medicine, University of California San Diego, La Jolla, CA, USA.

No cell is an island: circulating T cell:monocyte complexes are markers of immune perturbations

We set out to detect a transcriptional signature in CD4 T cells of individuals at risk of progression to active tuberculosis. We indeed found such a signature which surprisingly could be tracked to a cell population expressing both T cell (CD3) and monocyte (CD14) markers. Imaging experiments revealed that the CD3+CD14+ population consisted of either T cells bound to monocyte fragments or intact Tcell:monocyte complexes that were so tightly bound that cytometric sorting did not disrupt them. T cells in these complexes showed dramatically increased TCR signaling activity, suggesting a functional interaction. Broadening our investigation beyond tuberculosis, we also found CD3+CD14+-complexes as a function of time post tetanus, diphtheria and pertussis (Tdap) vaccination and as a function of disease severity in dengue infection. Our data further suggest that circulating Tcell:monocyte complexes form in vivo as a result of T cells recognizing antigen presented on the monocytes, and can provide with valuable diagnostic and mechanistic information on a given immune perturbation. Thus, our results highlight for the first time that a significant proportion of ‘double positive’ cells in flow cytometry are the result of true biological interactions between immune cells and that the conventional wisdom in the field to avoid studying cell:cell complexes should be re-visited.
Invited Speaker

Sonia Sharma (LJI)

Authors: Rekha Dhanwani, Mariko Takahashi, Ian T. Mathews, Jeramie D. Watrous, Bartijn Pieters, Satarupa Banerjee, Catherine C. Hedrick, Chris A. Benedict, Joel Linden, Roland Nilsson, Mohit Jain and Sonia Sharma

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5. Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital, SE-17176, Stockholm, Sweden.

Title: Extracellular nucleoside metabolites trigger the innate interferon response by inhibiting DNA methylation

We screened human genes associated with multi-organ inflammatory disease and identified Adenosine Deaminase 2 (ADA2) as a new inhibitor of type I IFNβ signaling. We find that loss of ADA2, which catabolizes the extracellular purine nucleosides adenosine and deoxy-adenosine, drives IFNβ production independently of purinergic receptor signaling via cellular nucleoside transport. Inside the cell, deoxy-adenosine yields deoxy-inosine, an innate immuno-metabolite that acutely inhibits S-adenosyl-methionine (SAM) cycle metabolism and SAM-dependent cellular DNA methylation, driving transcription of germline-encoded endogenous retrovirus elements (ERV) that trigger innate cytosolic nucleic acid sensing pathways and IFNβ. Our data show that ADA2 activity guides a unique receptor-independent sensing strategy for the metabolite by-products of extracellular DNA, which is released in inflammatory microenvironments during infection, ischemia, and tumor growth. Thus, we uncovered a novel metabolite-coupled epigenetic rheostat linking external nucleoside danger signals to endogenous inflammatory triggers.
Oral Presenter for Voting

Ty D. Troutman

Ty D. Troutman (1), Jason S. Seidman (1), Mashito Sakai (1), Anita Gola (2), Zhengyu Ouyang (1), Nathanael J. Spann (1), Cassi M. Bruni (1), Hunter Bennett (1), BaoChau T. Vu (1), Xiaoli Sun (3), Martina Pasillas (1), Verena M. Link (1), Mojgan Hosseini (4), Lorane Texari (3), Sven Heinz (3), Ronald N. Germain (2), Joseph L. Witztum (3), and Christopher K. Glass (1)

(1) University of California, San Diego, Department of Cellular and Molecular Medicine, La Jolla, California
(2) National Institutes of Health, National Institute of Allergy and Infectious Disease, Bethesda, Maryland
(3) University of California, San Diego, Department of Medicine, La Jolla, California
(4) University of California, San Diego, Department of Pathology, La Jolla, California

Exploiting altered enhancer landscapes to decode pathogenic changes in Kupffer cell gene expression

Kupffer cells sense and respond to microbial associated molecular patterns from the portal system and clear senescent erythrocytes from circulation. During nonalcoholic fatty liver disease (NAFLD), Kupffer cells are exposed to an increased lipid burden and participate in inflammatory events promoting development of steatohepatitis and fibrosis. The mechanisms underlying development of NAFLD and progression to nonalcoholic steatohepatitis (NASH) are linked to increased inflammation, however, the transcriptional regulatory mechanism governing macrophage involvement in NAFLD and NASH are poorly characterized. We profiled hepatic CD45+ cells from mice subjected to a dietary NASH model using single cell RNA-seq and found both NASH induced transcriptional diversification of Kupffer cells, as well as expansion of additional myeloid cell types residing within the liver. Using candidate surface markers predicted by single cell RNA-seq, we confirmed Kupffer cell heterogeneity using surface expression of Tim4. During progression of NAFLD to NASH, we found expansion of Tim4+ Kupffer cells and traced their ontogeny to hematopoietic precursors. Additionally, we profiled chromatin accessibility and activity of the major NASH associated myeloid populations using a combination of ATAC-seq and ChIP-seq. Analysis of these data enabled predictions of increased NF-kB, AP1, and NRF transcription factor activity during NAFLD/NASH. We further predicted and confirmed a requirement for sustained LXR activity to maintain the population of Tim4+ Kupffer cells during NASH. Our studies establish for the first time gene regulatory events controlling diverse hepatic macrophages during homeostasis and NASH.

Support provided by NIDDK-P30DK063491, NIDDK-T32DK007044, NCI-T32CA009523, and by the Intramural Research Program of NIAID, NIH.
Invited Speaker

Ferhat Ay (UCSD)

*Distal Enhancer regulatory landscapes of primary human immune cells*
Nucleoporins and T cell functions

Nuclear pore complexes (NPCs) are multiprotein channels connecting the nucleus with the cytoplasm. NPCs were historically considered static structures whose sole function was to regulate nucleocytoplasmic transport. However, accumulating evidence from our lab and others has shown that NPCs are dynamic and perform several transport-independent functions, including gene expression regulation and chromatin remodeling.

We have recently identified that loss of the tissue-specific NPC component Nup210 causes a severe deficit of naïve CD4+ T cells (Borlido et al., Nat. Imm., 2018). 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 death. We have now identified that depletion of another NPC component, Nup37, also significantly reduces the number of naïve CD4+ T cells. Surprisingly, our evidence suggests that Nup210 and Nup37 play distinct roles in maintaining T cell homeostasis. Here we will discuss the causes for the observed phenotypes and their implications for immunity. Our results firmly establish NPCs as central regulators of T cell homeostasis and expose these structures as key players in the adaptive immune system.
Invited Speaker

Kristian G. Anderson

Outbreakomics

Our group is using viral genomics, computational biology, and traditional molecular biology, to gain insights into how viruses emerge and spread in human populations. By generating large-scale genomic datasets of Ebola virus and Lassa virus sequences from hundreds of infected patients, we dissected the trajectory of how these viruses evolved and spread across West Africa.

More recently, our group led efforts to sequence and analyze Zika virus dataset from local human transmissions and mosquitoes in across the Americas. Based on this data, we have been able to demonstrate that the Florida outbreak is much more complex than previously accepted. We show that multiple introductions happened into Florida in the spring of 2016 leading to sustained transmission chains. We show that these Zika virus lineages originated in the Caribbean and were likely brought to the United States via frequent cruise ship traffic. By modeling genomic data and mosquito abundance, we also show that Miami and Southern Florida is at particular risk for future Zika outbreaks.
Kyla Omilusik

Kyla D. Omilusik (1), Marija S. Nadjsombati (1), Laura A. Shaw (1), Bingfei Yu (1), J. Justin Milner (1) and Ananda W. Goldrath (1)

(1) Department of Biological Sciences, University of California San Diego, La Jolla, California, USA;

Sustained Id2 regulation of E-proteins is required for terminal differentiation of effector CD8+ T cells

CD8+ T cells responding to infection differentiate into a heterogeneous population composed of progeny that are short-lived and participate in the immediate, acute response and those that provide long-lasting host protection. While it is appreciated that distinct functional and phenotypic CD8+ T cell subsets persist, it is unclear whether there is plasticity among subsets and what mechanisms maintain subset-specific differences. Here, we show that continual Id2 regulation of E protein activity is required to maintain the KLRG1hi CD8+ T cell population following LCMV infection. Induced deletion of Id2 phenotypically and transcriptionally transformed the KLRG1hi “terminal” effector/effector-memory CD8+ T cell population into a KLRG1lo memory-like population, promoting a gene-expression program that resembled that of central memory T cells. Our results question the idea that KLRG1hi CD8+ T cells are necessarily terminally programmed and suggest that continual regulation is required to maintain distinct CD8+ T cell states.
Invited Speaker

Elina Zuniga (UCSD)

Immune Adaptations During Chronic Viral Infections

Viral persistence requires a long-term relationship between the host and the microbe that involves multiple layers of interactions from molecular to cellular to whole organisms. Our laboratory studies cellular and molecular aspects of virus-host interactions during acute versus chronic viral infections to determine general principles of viral immune-evasion, persistence and pathogenesis. The ultimate goal is to generate fundamental knowledge on immune-regulation that could help modulate immune responses to prevent or treat infectious diseases and may also have implications for other immune-related disorders such as cancer immune-evasion. I will be presenting data on how the innate immune system adapt during a chronic viral infection and the mechanisms involved.
Elicitation of broadly neutralizing antibodies (bnAbs - those capable of neutralizing a large fraction of global HIV-1 isolates) is thought to be essential to the development of an effective HIV-1 vaccine. However, such antibody responses develop naturally after years of chronic infection in rare HIV-1-infected individuals, but the bnAbs isolated from these individuals harbor unusual features suggesting complex maturation pathways. Recent publications offered hope that rational vaccine strategies, based on germline targeting followed by sequential immunizations with guiding immunogens, may succeed (Jardine 2015, 2016, Briney 2016). This reductionist approach could therefore greatly benefit from a deeper understanding of the Env variants and features that allowed the initiation and maturation of bnAb lineages during natural HIV infection.

The IAVI-Protocol C, an African longitudinal HIV-1 primary infection cohort, identified several broad neutralizers targeting the five main bnAb epitope regions of HIV-1 Env (Landais 2016). The recent advances in next-generation sequencing (NGS) technologies, first allowing unprecedented analysis of the memory-B-cell repertoire and now also providing exceptional resolution of the viral population diversity at the individual level, enabled comprehensive and multidimensional studies deciphering the molecular interplay between the virus and B-cell response over the course of infection. We have now completed co-evolution studies of bnAbs lineages with autologous Env escape in four Protocol C donors targeting the V2-apex, the high-mannose patch and the CD4 binding site (CD4bs). Comparisons of B-cell germlines, bnAb-eliciting Envs and maturation-selecting variants between individuals who developed bnAbs targeting the same epitopes provide important insight for immunogen design.
Exploring a Novel CD8+ T cell that Intensifies Cardiovascular Disease and Atherosclerosis

Cardiovascular disease (CVD) kills one in four people annually in the United States. Within atherosclerosis, the main underlying trigger of CVD, activated T cells infiltrate atherosclerotic plaques, leading to destabilization, rupture, and heart attack. As diagnosis requires invasive methods, there is an urgent need to easily and non-invasively identify at-risk individuals. CyTOF mass cytometry of 28-matched subject PBMCs revealed a profound loss of circulating naïve CD8+ T cells with severe compared to low CVD risk. Excitingly, severe CVD individuals showed a strong, 2-fold increase in naïve CD8+ T cells that expressed the memory antigen CD95. A CD8+ stem cell memory T cell, denoted by naïve T cell markers and expressing CD95, was identified in humans and mice, but has not been studied in atherosclerosis. We hypothesized that this CD8+ stem cell memory T cell was pro-atherogenic and a biomarker of CVD risk. CD8+ stem cell memory T cells positively correlated with CVD risk, while naïve CD8+ T cells inversely correlated. CD8+ stem cell memory T cells from severe CVD individuals secreted 2-fold increased synthesis of the pro-atherogenic cytokine IFN-gamma compared to low CVD. Atherosclerotic Apolipoprotein E (ApoE) knockout mice displayed significantly increased CD8+ stem cell memory T cells by 1.8-fold within aorta-draining para-aortic lymph nodes compared to C57BL/6; importantly, CD8+ stem cell memory T cells were detected in ApoE knockout aorta, indicating that this novel population is pro-atherogenic. Ultimately, CD95 expression by naïve CD8+ T cells may constitute a predictive biomarker of CVD, potentially improving diagnosis of at-risk individuals.
Oral Presenter for Voting

Christophe Pedros

Christophe Pedros, Hsin-Yu Liu, Ann J Canonigo-Balancio, Kok-Fai Kong, Amnon Altman

La Jolla Institute for Allergy and Immunology

Control of regulatory T cell function by PKC-eta (PKCη), a novel target for cancer immunotherapy

Tregs play a critical role in maintaining immune system homeostasis, but also suppress anti-tumor immunity. We found that a Treg-intrinsic CTLA4-PKCη pathway mediates contact-dependent Treg suppression of tumor immunity via a trimolecular complex consisting of the GIT-PAK-PIX proteins, which, in turn, promotes dissociation of Treg-DC contacts and indirectly controls transendoctyosis of DC-expressed costimulatory CD86 by Tregs. Here we show that PKCeta is required for Treg-mediated suppression of tumor immunity in vivo. The presence of PKCη-deficient (Prkch−/−) Tregs in the tumor microenvironment was associated with increased CD86 expression on intratumoral CD103+ DCs, enhanced priming of antigen-specific CD8+ T cells, and greater levels of effector cytokines. Similar to Prkch−/− Tregs, Git2−/− Tregs also failed to suppress anti-tumor responses, highlighting the key role of the GIT-PAK-PIX complex in Treg suppression. PRKCH and GIT2 knockdown in human Tregs reduced their in vitro suppressive activity, demonstrating that The CTLA-4-PKCη signaling pathway also operates in human Tregs. Tumor growth was similarly reduced in Prkch−/− mice and in Prkchfl/fl Foxp3Cre recipients, implying that PKCη deficiency in T effector cells does not adversely affect their anti-tumor response.

We are exploring the therapeutic impact of PKCη deletion in tumor-bearing mice using tamoxifen treatment of Prkchfl/fl Foxp3ERT2Cre and Prkchfl/fl UBCERT2Cre as a therapeutic model and a surrogate for treatment with a highly selective PKCη inhibitor. Collectively, our data indicate that targeting the CTLA4-PKCη-GIT-PAK-PIX signaling pathway may represent a novel immunotherapeutic strategy to alleviate the negative impact of Tregs on anti-tumor immunity.
Invited Speaker
Timothy O'Sullivan (UCLA)

Tissue-Resident Responses at Sites of Initial Viral Infection

Infection is restrained by the concerted activation of tissue-resident and circulating immune cells. Whether tissue-resident lymphocytes confer early antiviral immunity at local sites of primary infection prior to the initiation of circulating responses is not well understood. Furthermore, the kinetics of initial antiviral responses at sites of infection remain unclear. Here, we show that tissue-resident type 1 innate lymphoid cells (ILC1) serve an essential early role in host immunity through rapid production of interferon (IFN)-γ following viral infection. Ablation of Zfp683-dependent liver ILC1 lead to increased viral load in the presence of intact adaptive and innate immune cells critical for mouse cytomegalovirus (MCMV) clearance. Swift production of IL-12 by tissue-resident XCR1+ conventional dendritic cells (cDC1) promoted ILC1 production of IFN-γ in a STAT4-dependent manner to limit early viral burden. Thus, ILC1 contribute an essential role in viral immunosurveillance at sites of initial infection in response to local cDC1-derived proinflammatory cytokines.
Oral Presenter for Voting

Chad R Dufaud

McHeyzer-Williams, LJ (1), McHeyzer-Williams, MG (1)

(1) Department of Immunology & Microbiology, The Scripps Research Institute, La Jolla, CA, USA

**PD-1 blockade releases commensal-specific B cell immunity and disrupts gut microbiome composition**

Programmed Death 1 (PD-1) blockade relieves CD8 T cell exhaustion in persistent infection and cancer. However, its role in B cell immunity remains unclear. Blocking PD-1 in vivo resulted in substantially expanded IgG1+ and IgA+ germinal center B cells in the spleen and Peyer’s patch of healthy, unimmunized mice. Concurrently, PD-1 blockade triggered rapid differentiation of IgG1-producing plasma cells, increased serum IgG1 and IgA specific for a subset of gut microbes, and altered gut microbiome composition. These data reveal a new germinal center B cell checkpoint that suppresses the evolution of microbe-specific B cell immunity, enables commensal formation, and provides an opportunity to modify the composition of the gut microbiome.
Invited Speaker

LiLi Yang (UCLA)

“Stem Cell-Engineered Invariant Natural Killer T cells for Cancer Immunotherapy”

Invariant natural killer T (iNKT) cells comprise a small population of αβ T lymphocytes. iNKT cells are attractive cancer therapy agents because of their capacity to target multiple types of cancers independent of tumor antigen- and MHC-restrictions. However, the therapeutic applications of iNKT cells are greatly limited by their small numbers in humans (~0.001-1% in blood). Here, we report a new method to generate therapeutic levels of iNKT cells in vivo through T cell receptor (TCR) gene engineering of hematopoietic stem cells (HSCs). When tested in a mouse melanoma lung metastasis model, the HSC-engineered iNKT cells effectively protected mice from tumor metastasis. Using a humanized BLT (human bone marrow-liver-thymus engrafted) mouse model, we further proved that engineering human CD34+ HSCs with human iNKT TCR gene can effectively program these cells to develop into human iNKT cells. This method takes advantage of the self-renewal and longevity of HSCs and has the potential to provide to cancer patients a long-term supply of cancer-fighting iNKT cells, thus opening up a new avenue for iNKT cell-based cancer immunotherapy.
Tissue resident immune cells play a critical role in maintaining homeostasis and orchestrating host defense. Resident macrophages represent cell populations that are uniquely adapted to their specific niche, integrating multiple complex environmental cues to generate a maximally beneficial response for their client epithelia. We propose a model in which the deregulated proliferation of malignant epithelia co-opts resident macrophage homeostatic networks to favor tumorigenesis. Using a genetically inducible mouse model of lung adenocarcinoma we observed a striking expansion in the resident cells of the alveolus, the alveolar macrophage (AM). By engaging AM lipid metabolism—a metabolic program known to skew macrophages towards an M2 like state—AM homeostatic clearance of the lipid-rich alveolar surfactant aids in the maintenance of airway tolerance, critically important in the lungs where there is a high cost to inflammation. As tumors progressed, AMs up-regulated surface expression of markers associated with alternatively activated macrophages, decreased their production of inflammatory cytokines, while increasing their lipid uptake and storage. AMs had functionally altered metabolic states with tumor-associated AMs having significantly increased rates of basal respiration as well as mitochondrial uncoupling. Induction of the transcription factor PPARγ is necessary for AM maturation, integrating AM surfactant clearance and consequently lipid catabolism with their tolerant immune state. Pharmacological inhibition of PPARγ delayed tumor progression, reduced AM recruitment, while restoring their inflammatory cytokine production. Our data suggests that rewiring of AM metabolism, specifically via antagonism of PPARγ, has the potential to be a novel therapeutic target in the treatment of lung cancer.
Invited Speaker

Aaron M. Miller (UCSD)

Letting Biology Drive Neoantigen Discovery

Through a collaborative effort between UCSD and LJI we have developed a set of novel bioinformatic and cellular tools which allows for the functional validation of NeoAgs recognized by both CD4+ and CD8+ T cells at a higher rate than previously reported. We have also applied a novel cellular reprogramming technology which allows for the routine generation of patient-specific xenograft cell lines that preserve expression of identified neoantigens and are recognized by a patient’s autologous T cells in vitro and in vivo as tumors growing in immunodeficient NSG mice. This has allowed effective identification and targeting NeoAgs in solid tumors with low to moderate mutational burden through precision immunotherapy.
Joyce Chen
Chen J (1,2), Scott-Browne JP (1,7), Lopez-Moyado IF (1,3), Seo H (1), Lio C-W (1), Hempleman, LJ (1), Sekiya T (4), Yoshimura A (5), & Rao A (1,6)

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Nr4a transcription factors limit CAR T cell function in solid tumors

CD19-targeted CAR T cells are clinically efficacious against B cell leukemias and lymphomas, but are less effective against solid tumors. This is in part because CAR T cells enter a hypo-responsive (“exhausted” or “dysfunctional”) state that is triggered by chronic antigen stimulation and characterized by upregulation of several inhibitory receptors and loss of effector function. To identify transcriptional regulators and other candidates contributing to the diminished CAR T cells function in solid tumors, we developed a CAR T cell model in which recipient mice bearing murine melanoma tumors expressing human CD19 were adoptively transferred with CD19-targeted CAR T cells. We demonstrate that in both CAR T tumor-infiltrating lymphocytes (TILs) and endogenous CD8+ TILs expressing high levels of PD-1 and TIM3, the Nr4a proteins Nr4a1 (Nur77), Nr4a2 (Nurr1), and Nr4a3 (Nor1) are prominent effectors of the transcriptional program downstream of NFAT: they promote the expression of inhibitory receptors and genes associated with early hypo-responsive state, and limit effector function. Most importantly, treatment of tumor-bearing mice with CAR T cells lacking all three Nr4a factors (Nr4aTKO) resulted in tumor regression and prolonged survival. Nr4aTKO CAR T TILs displayed a transcriptomic profile characteristic of effector function, including upregulation of granzymes and cytokines, and many of these gene expression changes were associated with altered regulatory element accessibility near effector genes. Our data identify Nr4a transcription factors as major players in the cell-intrinsic program of T cell hypo-responsiveness and point to Nr4a inhibition as a promising strategy for cancer immunotherapy.
Invited Speaker

Enfu Hui (UCSD)

Mechanistic dissection of PD-1 signaling using reconstitution approaches

The PD-1 pathway, consisting of the coinhibitory receptor PD-1 on T cells, and its ligand PD-L1 on antigen-presenting-cells (APCs), is a major mechanism of tumor immune evasion. PD-1 and PD-L1 blockade antibodies have produced remarkable clinical activities against a subset of cancers. However, the mechanistic understanding of the PD-1 pathway has been rudimentary. In this talk, I will discuss a novel regulatory mechanism of the PD-L1/PD-1 pathway based on cellular and in vitro reconstitution approaches.
Single-cell analysis of tissue-resident memory T cells in human cancer.

High numbers of tissue-resident memory T (TRM) cells are associated with better clinical outcomes in cancer patients. However, the molecular characteristics that drive their efficient immune response to tumors are poorly understood. Here, single-cell and bulk transcriptomic analysis of TRM and non-TRM cells present in tumor and normal lung tissue from patients with lung cancer, revealed that TRM cells in lung and tumors expressed high levels of the immune-checkpoint molecule PD-1. However, present exclusively in the tumors was a distinct population of TIM-3+IL-7R- TRM cells that were clonally expanded and enriched for transcripts linked to cell proliferation and cytotoxicity. This PD-1+TIM-3+IL-7R- TRM subset likely contributes to successful anti-tumor immune responses induced by PD-1 inhibitors. PD-1 expression by TRM cells in normal lung tissue may explain the “off-target” adverse reactions caused by PD-1 inhibitors. Our findings have implications for the design of therapies that preferentially target TRM cells in tumors.
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.
Functional identification of neoantigen-specific T cell responses targeting murine squamous cell carcinoma (SCC) VII

The comparative resistance of some cancers including head and neck squamous cell carcinoma (HNSCC) to immune checkpoint blockade immunotherapy has been speculated to derive from the low frequency of expressed somatic mutations targeted by T cells as neoantigens. SCC VII, a spontaneously arising C3H murine squamous carcinoma resembling human HNSCC, is similarly poorly immunogenic as irradiated tumor cells alone fail to induce protective immunity within syngeneic hosts. Using SCC VII to describe T cell responses to neoantigens, we show that both CD4+ and CD8+ T cells are detectable and essential for vaccine efficacy of SCC VII and polyI:C co-administration. Whole-exome sequencing tumor versus normal genome identified 39 nonsynonymous missense mutations that were synthesized into 81 representative 20-mers. Neoantigen-specific CD4+ IFN-γ responses were found against mutations of Pik3ca, Ctnnd1, and Otud5 while both CD4+ and CD8+ IFN-γ responses were stimulated by a single Cltc mutation after in vitro recall T cell assays. Prophylactic immunization with a mix of all IFN-γ-stimulatory peptides protected hosts from subsequent tumor challenge. However, these same peptides were not therapeutically beneficial in vivo unless the Cltc neoantigen, eliciting both CD4+ and CD8+ T cell responses, was used as an immunotherapy alone. Anti-PD-1 blockade resulted in synergistic tumor rejection via boosting Cltc-specific T cell responses while simultaneously increasing epitope spreading. These data show neoantigen-specific T cell responses to tumors are viable when combined with checkpoint blockade and that a filtered selection of neoepitopes that co-prime both CD4+ and CD8+ T cell responses is superior for immunotherapeutic intervention.
Invited Speaker

Hilde Cheroutre (LJI)

A long Noncoding RNA Transcribed from the Cd8 Locus Controls Functional Differentiation of CD4 T Cells

Master transcription factors drive CD4 T cells to develop into functional subsets, characterized by gene transcription signatures. This process is critical for normal immune function. The mechanisms that control the actions of these key transcription factors are poorly understood. Here, we identify a long noncoding RNA, encoded by the Cd8 locus (Cd8LncRNA), as a transcriptome regulator for CD4 T cells. Through RNA-protein interactions, Cd8LncRNA directs expression of T-BET and counteracts RUNX1 function. Absence of Cd8LncRNA results in differentiation of pathogenic CD4 effector lymphocytes in vivo. These findings define Cd8LncRNA as a key regulator of CD4 T cell function and classify interactions of LncRNA with master transcription factors as a higher order regulation mechanism that determines the functional outcome for activated T cells.
MiR-23~27~24-mediated control of humoral immunity reveals a TOX-driven regulatory circuit in follicular helper T cell differentiation

Follicular helper T (Tfh) cells are essential for generating protective humoral immunity. To date, microRNAs (miRNAs) have emerged as important players in regulating Tfh cell biology. Here, we show that loss of miR-23~27~24 clusters in T cells resulted in elevated Tfh cell frequencies upon different immune challenges whereas overexpression of this miRNA family led to reduced Tfh cell responses. Mechanistically, miR-23~27~24 clusters coordinately control Tfh cells through targeting a network of genes that are crucial for Tfh cell biology. Among them, thymocyte selection-associated HMG-box protein (TOX) was identified as a central transcription regulator in Tfh cell development. TOX is highly up-regulated in both mouse and human Tfh cells in a BCL6-dependent manner. In turn, TOX promotes the expression of multiple molecules that play critical roles in Tfh cell differentiation and function. Collectively, our results establish a key miRNA regulon that maintains optimal Tfh cell responses for resultant humoral immunity.
The role of non-coding transcription in establishing T cell identity

We have recently identified an ensemble of non-coding transcripts that are associated with the developmental progression of lymphoid cells. The presentation will be focused on one such non-coding RNA, named ThymoD (thymocyte differentiation factor). ThymoD is located 20 kbp upstream of the Bcl11b enhancer. ThymoD-deficient mice displayed a defect at the onset of T cell development and developed lymphoid malignancies. We found ThymoD acts to reposition the Bcl11b enhancer from the lamina into the nuclear interior. The repositioning brings the Bcl11b enhancer and promoter into a single loop domain to permit efficient enhancer-promoter communication and activation of Bcl11b expression. This process involves multiple steps: (i) CTCF occupancy across the ThymoD locus and the Bcl11b promoter region, (ii) activation of cohesin-dependent looping to juxtapose the enhancer and promoter into a single loop domain that is anchored by CTCF sites in the ThymoD locus and the Bcl11b promoter region, (iii) repositioning the enhancer from a heterochromatic to an euchromatic environment and (iv) activation of Bcl11b expression and specification of T cell fate. How non-coding transcription acts at a global scale to modulate developmental progression and immunity will be discussed.
Oral Presenter for Voting

**Nazia Abbasi**

Nazia Abbasi, Brian A.Yee, Benjamin Cho, Evelyn Ma, Gene W. Yeo, Wendy Huang

Department of Cellular and Molecular Medicine, University of California San Diego School of Medicine
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**DDX5 regulates RNA editing in Th17 cell differentiation**

DDX5, a nuclear RNA binding protein, and member of the DEAD box family of RNA helicases is abundantly expressed in T cells at different stages of development. Interestingly, we have recently found that it confers effector functions in a type of helper T cells called T helper 17 (Th17). These specialized cells secrete specific cytokines that contribute to chronic inflammation in human diseases, including inflammatory bowel disease, multiple sclerosis, and rheumatoid arthritis. We employed the Enhanced Cross-Linked Immunoprecipitation (eCLIP) coupled to high-throughput sequencing to identify DDX5-bound RNAs in both mature and immature T cells from mice. For DDX5-bound messenger RNAs, DDX5 shows preferential binding to 5` untranslated region (UTR) and introns with notable cell-type-specific motif preferences. Gene ontology analysis of DDX5 bound mRNA transcripts revealed tissue-specific pathways downstream of DDX5 in immature thymocytes and mature Th17 cells. Surprisingly, we found that DDX5 binds to mRNAs encoding members of the RNA editing enzymes in mature Th17 cells, but not in immature thymocytes, and controls their RNA abundance. Our RNAseq and RNA-editing analyses with SAILOR revealed that A-to-I RNA editing in Th17 cells from DDX5 deficient animals were significantly reduced. Furthermore, RNA interference to knockdown adarb1 suggests a key role of RNA editing in Th17 differentiation and function.
Invited Keynote Speaker

Jonathan Sprent (Garvan Institute, Sydney, Australia)

*Self tolerance: new thoughts on an old issue*

Thymic selection is known to generate a repertoire of mature T cells with low but significant reactivity to self MHC/peptide ligands, recognition of these ligands being important for keeping naïve T cells alive; overt T cell recognition of self ligands is avoided by a combination of negative selection in the thymus and suppression by Foxp3+ T regulatory cells (Tregs). Studies with Foxp3.DTR mice have shown that acute removal of Tregs leads to prominent lymphadenopathy and autoimmune disease, though whether this disease is directed to self antigens or foreign antigens is unclear. Charlie Surh began addressing this issue by raising a colony of germ-free and antigen-free mice at his research institute in Pohang, Korea. Based on studies on both Foxp3.DTR and Rag-deficient mice raised in an antigen-free environment, I will discuss how removal of Tregs allows a subset of high-affinity T cells to become overtly reactive to self ligands, both *in vivo* and *in vitro*. 
Poster Abstract #1

Christina Chang

Christina Chang(1), Silin Sa(1), Chip Lomas(1), Devon Jensen(1), Jing Hu(1), Suraj Saksena(1), Eleen Shum(1)

1. BD Biosciences

Single-cell protein and gene expression profiling of stem memory T cells by AbSeq

High-throughput single-cell RNA sequencing is a powerful tool for profiling heterogeneous cell populations. However, the lack of protein expression information can make identifying cell types that have conventionally been defined by cell-surface markers challenging. T cells in particular contain relatively low abundance of transcripts, and different T-cell subsets often exhibit highly similar transcriptional profiles.

Here we utilized BD(TM) AbSeq, a novel protein sequencing technology enabled by oligo-conjugated antibodies, to study stem memory T cells (Tscm), a rare long-lasting memory T-cell population. A high parameter oligo-conjugated antibody panel was used for protein profiling alongside gene expression profiling in single cells. From human PBMCs, we enriched for Tscms, and naïve and memory subsets by FACS. The sorted samples were labeled with antibodies barcoded with unique sample IDs, which allowed us to pool multiple samples for single cell capture. The antibody-specific and sample ID-specific oligos were captured, amplified and sequenced alongside mRNAs with BD Rhapsody(TM), a massively-parallel single-cell analysis system. The resulting sequencing data provided a combined output of gene expression, protein expression and sample identity.

We observed consistent protein expression patterns when comparing BD AbSeq data to flow cytometry data of the same samples. The addition of protein marker expression provided more robust clustering of single-cell data compared to clustering by mRNA expression alone, which allowed us to better characterize the different subsets. Our study demonstrates the power of combining BD AbSeq with scRNA-seq to gain a more comprehensive understanding of cell lineage and function at the single-cell level.
Bi-Huei Yang

Bi-Huei Yang (1), Xiaomei Yuan (1), Yan Liang (2), Yi Dong (1,6), Sunglim Cho (3), Wenjia Huang (1), Gen-Sheng Feng (2,3,4), Hai-Hui Xue (5), Li-Fan Lu (3,4) and Wenxian Fu (1,4)

(1) Pediatric Diabetes Research Center, Department of Pediatrics
(2) Department of Pathology
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(5) Department of Microbiology, University of Iowa, Iowa City, Iowa, USA.
(6) Present address: The Johns Hopkins University School of Medicine.

TCF1 and LEF1 control Treg homeostatic differentiation and Tfr generation to maintain immune tolerance

CD4+ Foxp3+ T regulatory (Treg) cells are key players in preventing lethal autoimmunity and deleterious tissue inflammation. To fulfill these roles, Treg cells undertake activation and differentiation processes to acquire diverse functional properties. However, how Treg’s functional specifications are regulated during their differentiation process remains poorly understood. Here we show that two TCF/LEF family TFs, TCF1 and LEF1 act redundantly as checkpoint regulators in peripheral Treg homeostatic differentiation and effector subset generation. We find that the gradient expression of TCF1 and LEF1 distinguishes Treg cells into three distinct subsets. Sustained expression of TCF1/LEF1 retains Treg at their resting stage. Conversely, conditional knockout of TCF1 and LEF1 promotes effector-like phenotype in bulk Treg cells, but surprisingly renders the mice susceptible to early onset of systemic autoimmunity. This uncoupling between Treg effector-like phenotype and their function in suppressing autoimmunity is attributed by two TCF1/LEF1-centered mechanisms. First, the ablation of TCF1 and LEF1 in Treg cells abolishes the generation of T follicular regulatory (Tfr) cells, leading to unrestrained T follicular helper (TFh) and germinal center B cell responses. Second, TCF1 and LEF1 are required for Treg survival under competitive conditions. Thus, TCF1 and LEF1 play critical roles in Treg homeostatic maintenance and Tfr generation.
Poster Abstract #3

Peter Pioli

David Casero (1), Encarnacion Montecino-Rodriguez (1), Sherie L. Morrison (2), and Kenneth Dorshkind (1)

(1) Department of Pathology and Laboratory Medicine, (2) Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095

Plasma Cells are Obligate Enforcers of Myeloid Skewing in Aging Bone Marrow

Aging results in reduced lymphopoiesis and increased myelopoiesis, but how changes in the bone marrow microenvironment influence these events is not understood. We show that plasma cells increase in number in the bone marrow over time and demonstrate through in vivo antibody depletion that they play an obligate role in age-related myeloid skewing. We further demonstrate that plasma cells constitutively and upon Toll-like receptor ligation produce known myelopoietic cytokines and also stimulate the production of these factors from bone marrow stromal cells. Disruption of this circuitry with IL-1 and TNF-α inhibitors results in attenuated myelopoiesis in the aging bone marrow. Finally, we show that plasma cells contribute to declines in lymphopoiesis but that redundant mechanisms exist to inhibit that process during aging. These observations provide a basis for understanding the age-related increase in myelopoiesis and define a regulatory role for plasma cells in this process.
Human Natural Killer Cells Mediate Adaptive Immunity to Viral Antigens

While Natural Killer (NK) cells have traditionally been considered cells of the innate immune system, mounting evidence in mice and non-human primates (NHP) warrants reconsideration of the existing paradigm that only B and T cells mediate adaptive immunity. Adaptive immune responses are defined as antigen-sensitization-dependent, antigen-specific, long-lived immunological memory. While adaptive immune responses have been demonstrated in murine and NHP-derived NK cells, it is currently unknown whether human NK cells mediate adaptive immune responses. We therefore tested whether human NK cells mediate adaptive immunity to virally encoded antigens using humanized mice and human volunteers. We found that human NK cells mediate vaccination-dependent, antigen-specific killing in vitro, when isolated from livers of humanized mice previously vaccinated with human immunodeficiency virus (HIV) encoded Envelope (Env) protein. Further, we discovered that large numbers of actively degranulating NK cells with a hepatic phenotype are recruited to sites of varicella zoster virus (VZV) skin test antigen-challenge in VZV-experienced human volunteers. These NK cell-mediated recall responses in humans occur years to decades after initial VZV exposure, thus NK memory in humans is long-lived. Our data demonstrates that human NK cells mediate adaptive immune responses, similarly to subsets of NK cells in mice and NHP. These data are important, as the existence of human memory NK cells may allow for the development of novel clinical
approaches that direct potent NK cell-mediated memory functions towards host protection via vaccination.
High modality assessment of aortic leukocytes uncovered for the first time extrathymic presence of CD4CD8 T cell progenitors.

Atherosclerosis is a chronic inflammatory disease that is characterized by the build-up of leukocyte-rich plaques in the intimal layer of arteries. Here, we applied a novel multi-modal approach including mass cytometry (CyTOF) and single cell RNA-sequencing (scRNA-seq.) to define leukocyte heterogeneity in murine aortae. Unsupervised clustering resulted in 27 distinct leukocyte clusters covering known and several novel leukocyte subpopulations. To gain insights into the transcriptional regulation of aortic leukocyte subsets, we applied scRNA-seq. Clustering of single cell transcriptomes confirmed enhanced leukocyte heterogeneity with functionally segregated subpopulations. Among the newly identified aortic leukocytes by CyTOF and scRNA-seq., which are CD4CD8 cells which were present at different stages at disease. Out of 37 marker, CD4CD8 cells were only positive for CD4 and CD8. Transcriptomically, they resemble thymic CD4CD8 cells, progenitors of conventional T cells which are believed to not leave the thymus. Flow cytometric screening and imaging analysis confirmed presence of CD4CD8 cells in the mediastinal fat and aortic arch also in wildtype mice, pointing towards a general phenomenon. Fat and aortic arch-resident CD4CD8 cells depend on the presence of RAG and the thymus, latter for continuous replenishment. Of note, the expression of RAG gradually declines between thymus, fat, and aortic arch located CD4CD8 cells. Functionally, fat-isolated CD4CD8 cells can develop into conventional T cells. High modality assessment of aortic leukocytes identified the presence of new immune cells, which are thymic-derived CD4CD8 cells. This indicates a yet undescribed and novel escape mechanism of thymic CD4CD8 cells before successful development into T cells.
Poster Abstract #6

Shelley Das

Damon B Cook, Brian C McLucas, Leticia A Montoya, Chris M Brotski, Shelley Das, Markus Miholits, Thao H Sebata

Thermo Fisher Scientific, Carlsbad, CA 92008

Multiplexing Protein and Gene Level Measurements on a Single Luminex Platform

Background: The ability to accurately measure both proteins and genes is expected for comprehensive analysis from a single sample. Bottlenecks to multiplexing assays using a single starting sample include limited sample volume, time consuming experimental procedures, and complicated data analysis. Here, we utilize Luminex® xMAP® technology to measure multiple proteins or genes in a single well. Purpose: Our study examines ProcartaPlex and QuantiGene Plex assays to provide both protein and gene expression data from the same starting sample. Experimental procedures: We demonstrate two high throughput assays measuring genes and proteins and run on a Luminex platform. Human peripheral blood mononuclear cells (hPBMCs) were treated with lipopolysaccharide (LPS) and harvested at 24 and 72 hours. The treated cells were centrifuged and secreted cytokines were measured using the ProcartaPlex Human 65-plex Cytokine Panel, and the cell pellets were lysed and intracellular mRNA was measured with the QuantiGene Plex Human Cytokine Panel. Summary of data: Upon further examination, a subset of gene expression and analyte levels corresponds, namely IL-1β, IL-6, TNF-α, and MIP-1b. Conclusion: These results show that sample can be conserved and produce targeted results.
Poster Abstract #7

Zhiheng He

Zhiheng He (1). Jing Zhang (1,2), Zhaofeng Huang (3), Qian Du (1), Yuan Chen (4) and Zuoming Sun (1)

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Sumoylation of RORgt regulates TH17 differentiation and thymocyte development

RORgt controls the differentiation of TH17 cells, which are involved in autoimmunity such as experimental autoimmune encephalomyelitis (EAE). RORgt also regulates thymocyte development and lymph node genesis. Here we show that RORgt function is regulated by its sumoylation. Loss of Sumo3, but not Sumo1, dampens TH17 differentiation and delays the progression of thymic immature CD8 single-positive cells (ISP). RORgt is SUMO3-modified at lysine 31 (K31), and mutation of K31 to arginine (RORgt-K31R) in mice prevents RORgt sumoylation, leading to impaired TH17 differentiation, resistance to TH17-mediated EAE, accumulation of thymic ISP, and lack of Peyer’s patches. Mechanically, RORgt-K31 sumoylation by E3 ligase PIAS4 stabilizes the binding of SRC1 through recruiting histone acetyltransferase KAT2A to enhance RORgt transcription factor activity. This study thus demonstrates sumoylation as a critical mechanism for regulating RORgt function, and reveal new drug targets for preventing TH17-mediated autoimmunity.
Poster Abstract #8

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M cells in the intestine are broadly induced by TNF over-expression independent of organized lymphoid tissue

The intestinal epithelium provides a physical barrier against invading pathogens, but for epithelium overlying organized lymphoid aggregates, specialized epithelial cells called M cells provide active transcytosis of luminal particles across the epithelial barrier to aid immune surveillance. However, in the setting of intestinal inflammation, widespread recruitment of immune cells results in accumulation of disorganized lymphoid aggregates with more random induction of M cells. For example, in a transgenic TNFΔARE mouse model of chronic inflammation, we found extensive induction of Peyer’s Patch type Microfold (M) cells along the villous epithelium. These cells were induced without underlying organized lymphoid tissues yet remained fully functional in luminal particle uptake. The disorganization in recruited immune cells includes a random assortment of dendritic cells, specialized fibroblastic reticular cells, B cells and CD4+ T cells rather than a clear segregation of T- and B-dependent compartments. Functional transcytosis by inflammation-induced epithelial M cells in the absence of organized lymphoid tissues may promote microbial access to the lamina propria, exacerbating innate immune triggers of inflammation and amplifying inflammatory mechanisms in intestinal inflammatory disease.
Poster Abstract #9

Yanfang Peipei Zhu

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Identification of an Early Unipotent Neutrophil Progenitor with Pro-tumoral Activity in Mouse and Human Bone Marrow

Neutrophils are short-lived cells that play important roles in both health and disease. Neutrophils and monocytes originate from the granulocyte monocyte progenitor (GMP) in bone marrow; however, unipotent neutrophil progenitors are not well defined. Here, we use cytometry by time of flight (CyTOF) and single-cell RNA sequencing (scRNA-seq) methodologies to identify a committed unipotent early stage neutrophil progenitor (NeP) in adult mouse bone marrow. Importantly, we found a similar unipotent NeP (hNeP) in human bone marrow. Both NeP and hNeP generate only neutrophils. NeP and hNeP both significantly increase tumor growth when transferred into murine cancer models, including a humanized mouse model. hNeP are present in the blood of treatment-naive melanoma patients but not of healthy subjects. hNeP can be readily identified by flow cytometry and could be used as a biomarker for early cancer discovery. Understanding the biology of hNeP should allow the development of new therapeutic targets for neutrophil-related diseases, including cancer.
Poster Abstract #10

Karl-Johan Malmberg

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Modulation of Secretory Lysosomes During NK Cell Education Leads to Accumulation of Granzyme B and Enhanced Functional Potential

Inhibitory signaling during natural killer (NK) cell education translates into increased responsiveness to activation; however the intracellular mechanism for functional tuning by inhibitory receptors remains unclear. We found that educated NK cells expressing self-MHC specific inhibitory killer cell immunoglobulin-like receptors (KIR) show accumulation of granzyme B, localized in dense-core secretory lysosomes, converged close to the centrosome. This discrete morphological phenotype persists in self-KIR+ NK cells independently of transcriptional programs that regulate effector function, metabolism and lysosomal biogenesis. The granzyme-B dense, large secretory lysosomes in self-KIR+ NK cells were efficiently released.
upon target cell recognition, contributing to their enhanced cytotoxic capacity. Secretory lysosomes are part of the acidic lysosomal compartment, which has been shown to channel calcium and mediate intracellular signalling in several cell types. Interference of signaling from acidic Ca2+ stores in primary NK cells reduced both target-specific Ca2+-flux, degranulation and cytokine production. Furthermore, inhibition of PI(3,5)P2 synthesis or genetic silencing of the PI(3,5)P2-regulated lysosomal Ca2+-channel TRPML1 led to increased levels of granzyme B and enhanced functional potential. These results indicate an intrinsic role for lysosomal homeostasis in NK cell education.
Poster Abstract #11

Youtong Huang

Youtong Huang, Kaisa Happonen, Patrick Burrola and Greg Lemke

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Investigating Microglial TAM Receptors as Modulators for Alzheimer’s Pathology

TAM receptor tyrosine kinases – Tyro3, Axl, and Mer - are essential regulators of apoptotic cell (AC) clearance and immune homeostasis in macrophages, dendritic cells, and immune sentinels throughout the body. Our lab recently demonstrated that these receptors play equivalent roles in microglia, the specialized tissue macrophages of the central nervous system, where they are required for the clearance of the ACs generated during adult neurogenesis. Alzheimer’s disease (AD) features formation of neurotoxic Abeta oligomers and deposition of plaques, along with prominent neuroinflammation, which give rise to synapse and neuronal loss and cognitive decline in patients. Recent transcriptomic studies show that microglial Axl mRNA is dramatically up-regulated in both AD and its mouse models. In addition, nuclear receptor (e.g., PPAR-gamma) agonists, which elevate both Mer expression and phagocytosis in microglia and macrophages, have been shown to ameliorate mouse AD pathology in a Mer-dependent fashion. These findings notwithstanding, the biological role of microglial TAMs signaling in Alzheimer’s disease remains unclear. We hypothesize that microglial TAM receptor signaling restrains AD by (a) regulating the production of neurotoxic proinflammatory cytokines and (b) promoting microglial phagocytosis of neurotoxic Abeta peptides and plaques. Here we sho
Poster Abstract #12

Alba Grifoni

John Pham, Yuan Tian, Sandy L. Rosales1, Grégory Seumois, John Sidney, Bjoern Peters, Pandurangan Vijayanand, Daniela Weiskopf and Alessandro Sette

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Transcriptional immunoprofiling of ZIKV-specific CD8+ T cells reveals multifunctional and cytotoxic antiviral responses

ZIKV has been spreading rapidly throughout the Americas, constituting an increasing public health problem. While most ZIKV infections are mild or asymptomatic, rare neurological complications can occur including microcephaly during pregnancy or Guillain–Barré syndrome (GBS) and other autoimmune neurological diseases in adults. We have defined antigenic targets of the ZIKV-specific CD8+ T cell response in humans. Here we characterized the quality and phenotypes of ZIKV antigen-specific CD8+ T cell responses, by a combined use of cytometry and transcriptomic methods, using peripheral blood mononuclear cells (PBMCs) from donors from different geographical locations collected in the convalescent phase of ZIKV infection. We show that ZIKV antigen-specific CD8+ T cells are characterized by a polyfunctional IFNγ and cytotoxic signature not influenced by previous DENV exposure, geographical location or time of sample collection after infection. This work elucidates the first in-depth characterization of human CD8+T cells responding to ZIKV infection. These new insights are applicable for the design of vaccines that elicit robust cellular immune responses.
**Poster Abstract #13**

Hsin Yu Liu

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Leveraging a novel Treg-intrinsic CTLA4-PKCη signaling pathway for immunotherapy of hepatocellular carcinoma

Hepatocellular carcinoma (HCC) patients have limited treatment options, poor prognosis, low survival rate and frequent recurrences. Inhibition of regulatory T cells (Tregs) or blockade of the inhibitory receptor CTLA4 have been recognized as potential therapeutic strategies to enhance antitumor immunity in HCC. We previously reported that protein kinase C-eta (PKCη) plays an important role in the contact-dependent suppressive activity of Tregs via its association with CTLA4, and that PKCη-deficient (Prkch–/–) Tregs fail to suppress anti-melanoma tumor immunity. Here we extend this study to two genetically engineered mouse models of HCC driven by a MYC transposon or CRISPR-Cas9-driven deletion of Pten and p53. In the first model, immunodeficient mice reconstituted with T effector cells and Prkch–/– Tregs developed smaller and fewer tumors than recipients of wild-type (WT) Tregs. In addition, intratumoral CD103+ dendritic cells, the most efficient stimulators of tumor immunity, displayed higher levels of the costimulatory ligand CD86. Moreover, we observed enhanced production of granzyme B in tumor-infiltrating CD8+ T cells in HCC-bearing mice that received Prkch–/– Tregs. In the second Pten/p53 HCC model, germline Prkch–/– mice developed fewer tumors and showed significantly higher CD8+ T cell:Treg ratios than tumor-bearing WT mice. These results indicate that Treg-expressed PKCη is required for Treg-mediated suppression of anti-HCC tumor immunity and that its deletion is associates with a more immunogenic tumor microenvironment. Thus, inhibition of the CTLA4-PKCη signaling axis in Tregs may represent a novel strategy for treatment of HCC, particularly when combined with other therapeutic modalities.
Poster Abstract #14

Clarissa Paw U

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Differential effects of nicotine on flu response in various dendritic cell populations

Smoking is well known for its negative impact on health, such as an increased frequency and severity of flu infection. The effect of nicotine on dendritic cells (DCs) functions have not been studied in detail and most previous studies have used monocyte derived dendritic cells (moDC) due to the low quantities of mDCs and pDCs in peripheral blood. Here we examined whether nicotine affects the functions of human myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) which play a vital role in the immune system particularly in response to infections including influenza.

We hypothesize that nicotine compromises dendritic cell response to the flu; specifically, nicotine exposure alters cytokine profiles, reducing the efficacy of the immune system.

In our research, peripheral blood mononuclear cells (PBMC) from human blood samples were used for the isolation of mDC/pDC and to generate moDC. We analyzed them with flow cytometry and cytokine assays, to quantify DC populations, assess activation, and obtain the cytokine profiles of IFN\(\alpha\), IFN\(\gamma\), TNF\(\alpha\), IL-10, IL-6, and IL-1\(\beta\).

Nicotine had differential effects on different DC populations. PDC numbers were decreased after nicotine exposure. Secretion of IFN-\(\alpha\) in response to influenza was also reduced in pDCs. Nicotine had no effect on mDC numbers but the secretion of IFN-\(\alpha\) was decreased. The effect on cytokine secretion in moDCs was variable with some cytokines displaying an increase and some a decrease. Thus, the effect of nicotine on various DC populations seems variable and warrants further investigation.
T cell Antigen discovery using Signaling and Antigen-presenting Bifunctional Receptors

The antigen specificity of a T cell is derived from the interaction of its surface T cell receptor (TCR) with a short peptide epitope presented on Major Histocompatibility Complex (MHC) molecules on its target cell. Peptide epitopes recognized by T cells can be derived from self-peptides, commensal or pathogenic microbes, viruses, and cancer associated antigens. The specificity of the TCR-peptide-MHC interaction is critical in invoking the function of the T cell only when it recognizes the correct target. One of the biggest unmet needs in immunology is for techniques that allow identification of the cognate antigen of a T cell. Current technologies such as pMHC multimers, functional screens, and yeast display are non-robust, not scalable, and technically challenging. Here, we describe the use of chimeric receptors called Signaling and Antigen-presenting Bifunctional Receptors (SABRs) in a novel cell-based platform for T Cell antigen discovery. SABRs present an extracellular peptide-MHC complex and induce intracellular signaling upon binding with a cognate TCR. We devised a strategy for antigen discovery using SABR libraries to screen thousands of antigenic epitopes. We validated this platform by identifying the targets recognized by public TCRs of known specificities. Moreover, we extended this approach for personalized neoantigen discovery. The antigen discovery platform reported here will provide a scalable and versatile way to identify the cognate epitope for any given T cell, and will be immensely useful in understanding and engineering T cell immunity.
Poster Abstract #16

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Characterizing tissue-resident memory CD4 T cells in acute viral infections

In response to an acute infection, naïve T cells proliferate and differentiate into effector cells, which aid in clearing pathogens, or long-lived memory cells, which mediate protection from reinfection. CD4+ T lymphocytes play a key role in this response because they mediate the activity of innate cell, B cell, and CD8+ T cell immunity. Memory T lymphocytes have classically been categorized into central memory cells (TCM) or effector memory cells (TEM), both of which circulate between blood, secondary lymphoid organs, and non-lymphoid tissues. Recently, a third subset of memory cells, referred to as tissue-resident memory T cells (TRM), has been described to migrate to barrier surfaces, such as skin and intestinal mucosa, in response to initial infection. TRM serve as sentinels for potential reinfection, coordinating the initial response to pathogens and providing a significant boost to immunity. The ontogeny and regulation of CD8+ TRM differentiation have been characterized in recent years, but little is known about their CD4+ counterparts within the context of antiviral immunity. Using adoptive transfers of viral-specific CD4+ T cells, flow cytometry, bulk RNA sequencing, and chromatin profiling, we demonstrate infection-induced CD4+ TRM are distinct from circulating memory CD4+ T cells, both phenotypically and transcriptionally. Additional studies will focus on elucidating the developmental pathway of CD4+ TRM focusing on contributions from early TFH cells to resident populations, and on identifying novel transcriptional regulators of CD4+ TRM fate, specifically the transcription factor Runx3 in establishing the CD4+ TRM population in intestinal tissues.
Poster Abstract #17

Cecilia Lindestam Arlehamn
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Widespread tau-specific CD4 T cell reactivity in the general population

Tau protein is found to be aggregated and hyper-phosphorylated (p-tau) in many neurological disorders, including Parkinson’s disease (PD) and related parkinsonisms, Alzheimer’s disease, traumatic brain injury and even in normal aging. While not known to produce autoimmune responses, we hypothesized that the appearance of aggregated tau and p-tau with disease could activate the immune system. We thus compared T cell responses to tau and p-tau derived peptides between PD patients, age matched healthy controls, and young healthy controls (< 35 y.o.; who are less likely to have high levels of tau aggregates). All groups exhibited CD4+ T cell responses to tau-derived peptides that were associated with secretion of IFN-γ, IL-5 and/or IL-4. The PD and control participants, exhibited a similar magnitude and breadth of responses. Some tau-derived epitopes, consisting of both unmodified and p-tau residues, were more highly represented in PD participants. These results were verified in an independent set of PD and control donors (either age matched or young controls). Thus, T cell recognizing tau epitopes escape central and peripheral tolerance in relatively high numbers, and the magnitude and nature of these responses is not modulated by age or PD disease.
Poster Abstract #18

Joseph McGraw

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JAML-CAR – A Mechanism of T Cell Antitumor Immunity

Dendritic epidermal T cells (DETC) are a subset of γδ T cells that reside in the murine epidermis and have critical roles in wound healing and tumor surveillance. In addition to their invariant Vγ3Vδ1 TCR, DETC express the costimulatory molecule junctional adhesion molecule-like protein (JAML), which binds to the Coxsackie and adenovirus receptor (CAR) expressed on keratinocytes, and this costimulation is required for proper wound healing. Mice lacking these cells are more susceptible to tumorigenesis, but the role that DETC play in antitumor immunity is not well defined. We hypothesize that this JAML-CAR interaction is an additional mechanism of recognition and elimination of tumors by DETC. Additionally, other immune cell types, such as CD8 T cells, lymphoid γδ T cells, and neutrophils can express JAML. Published studies suggest that JAML-CAR interactions regulate transepithelial migration of these cell types, and therefore, JAML-CAR interactions may be required for tumor infiltration. Initial results utilizing the B16F10 melanoma model show that tumors in mice lacking JAML develop and grow faster than in wild-type mice. This increased susceptibility to tumor growth is due in part to a defect in DETC-mediated recruitment of other immune effector subsets and/or defects in migration of peripheral immune cells to tumor sites as seen by reduced tumor-infiltrating lymphocyte (TIL) populations in mice lacking JAML. Therefore, we are investigating the possibility that JAML-CAR may be a target for cancer immunotherapy.
Poster Abstract #19

Jerry Lio

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TET enzymes augment AID expression via 5hmC modifications at the Aicda superenhancer

TET enzymes are dioxygenases that promote DNA demethylation by oxidizing the methyl group of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Here we report a close correspondence between 5hmC-marked regions, chromatin accessibility and enhancer activity in B cells, and a strong enrichment for consensus binding motifs for basic region-leucine zipper (bZIP) transcription factors at TET-responsive genomic regions. Functionally, Tet2 and Tet3 regulate class switch recombination (CSR) in murine B cells by enhancing expression of Aicda, encoding the cytidine deaminase AID essential for CSR. TET enzymes deposit 5hmC, demethylate and maintain chromatin accessibility at two TET-responsive elements, TetE1 and TetE2, located within a superenhancer in the Aicda locus. Transcriptional profiling identified BATF as the bZIP transcription factor involved in TET-dependent Aicda expression. 5hmC is not deposited at TetE1 in activated Batf-deficient B cells, indicating that BATF recruits TET proteins to the Aicda enhancer. Our data emphasize the importance of TET enzymes for bolstering AID expression, and highlight 5hmC as an epigenetic mark that captures enhancer dynamics during cell activation.
Poster Abstract #20

Angeline Chen

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Dietary Antigens Drive the Generation of Protective CD8 alpha beta T Cells in the Small Intestine Epithelium Prior to Pathogen Exposure

Intestinal intraepithelial T cells (IETs) consist of tissue resident alpha beta and gamma delta T lymphocytes, which display immediate effector function. The alpha beta T cell population contains an innate self-specific CD8 alpha alpha+ subset and a major subpopulation of mainstream CD8 alpha beta T cells that migrate there before any pathogen-encounter. How CD8 alpha beta T cells are generated as pre-existing fully differentiated protective cells is poorly understood. In this report, we show that at steady state, CD8 alpha beta+TCR alpha beta+IETs depend on dietary antigens for their recruitment to the small intestine epithelium and functional maturation. Unlike pathogen-induced memory CD8 alpha beta+ T cells, dietary antigen-induced CD8 alpha beta+TCR alpha beta+IETs require continuous exposure to their cognate dietary antigens for their long-term persistence and protective capacity. Moreover, the generation of pre-existing functional CD8 alpha beta+TCR alpha beta+IETs depends on T-BET but not on IL-15. Instead, IL-12 family members, IL-27 and IL-12 play differential roles in the recruitment and functional differentiation of CD8 IETs at steady state. Notably, mice lacking diet antigen-induced T cells in the intestinal epithelium have significantly decreased immunity against an oral infection with Listeria monocytogenes. Collectively, the data show that dietary
antigens are critical for the generation of protective T cells that are pre-existing at the mucosal border prior to pathogen challenge and contribute to a rapid and efficient defense against foodborne pathogens.
Poster Abstract #21

Ankita Shukla

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CD11a expression distinguishes infiltrating myeloid cells from plaque-associated microglia in Alzheimer's Disease

Alzheimer's Disease (AD) is the leading cause of age-related neurodegeneration and is characterized neuropathologically by the accumulation of insoluble beta-amyloid (Aβ) peptides. In AD brains, plaque-associated myeloid (PAM) cells cluster around Aβ plaques but fail to effectively clear Aβ by phagocytosis. PAM cells were originally thought to be brain-resident microglia. Several studies have also suggested that Aβ-induced inflammation causes peripheral monocytes to enter the otherwise immune-privileged brain. The relationship between AD progression and inflammation in the brain remains ambiguous because microglia and monocyte-derived macrophages are extremely difficult to distinguish from one another in an inflamed brain. Whether PAM cells are microglia, peripheral macrophages, or a mixture of both remains unclear. CD11a is a component of the β2 integrin LFA1. We have determined that CD11a is highly expressed on peripheral immune cells, including macrophages, but is not expressed by mouse microglia. These expression patterns remain consistent in LPS-treated inflamed mice, as well as in two mouse models of AD. Thus, CD11a can be used as a marker to distinguish murine microglia from infiltrating peripheral immune cells. Using CD11a, we show that PAM cells in AD transgenic brains are comprised entirely of microglia. We also demonstrate a novel Fluorescence-Assisted Quantification Technique (FAQT), which reveals a mild but significant increase in peripheral immune cell populations in the brains of AD mice. Our findings support the notion that microglia are the lead immunological players in AD and that rejuvenating their phagocytic potential may be an important therapeutic strategy.
NFκB/RelA dynamics control developmental pacing in early B lymphopoiesis

B lymphopoiesis consists of a series of developmental stages ensuring the generation of functional B cells capable of mounting immune responses. Inflammatory disease and aging alter hematopoiesis and diminish the B cell repertoire. It remains unclear how NFκB, a key mediator of inflammation, is regulated during early B lymphopoiesis and whether NFκB dysregulation, as seen in inflammatory conditions, affects B lymphopoiesis. Using a new RelA-Venus knock-in reporter mouse, we found that NFκB RelA levels are dynamically regulated along early B lymphopoiesis, and that this dynamic regulation is partially impaired in old mice. We developed a genetic model for impaired dynamic NFκB regulation by combinatorial IkB deletion. These mice show a much reduced B cell output that originate from developmental defects in the bone marrow. Following bone marrow transplantation, the phenotype is preserved. Yet, we found no evidence for either a developmental block or survival defects. To quantitatively interpret population numbers, we used a mathematical model of hematopoietic development and found that accelerated pro-B cell to pre-B cell transition best explained the phenotype. This was confirmed by in vitro differentiation experiments. In vivo aberrant cell size dynamics and premature pre-BCR target gene signature suggest NFkB dysregulation results in the skipping of a proliferation phase, resulting in reduced pre-B cell output. Our results uncover the paradoxical role of NFkB in early B lymphopoiesis: while basal NFkB activity is essential for cell survival during development, elevated NFkB accelerates developmental pacing of B cells; because a highly proliferative phase is shortened, B lymphopenia ensues.
Poster Abstract #23

Marcelo Freire

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Immunoresolvents in Chronic Inflammatory Diseases

Host-microbial relationships provide dynamic cues to the establishment of health, and disruption of the finely tuned equilibrium leads to disease. Throughout life, microbiota-derived molecules signal host immune cells shaping their development, plasticity, and overall functions. At the interface of oral mucosal tissues, the microbiome and immune crosstalk is very dynamic. Chronic inflammation in the mucosa is influenced by environmental changes, genomic and microbiome-immune interactions leading to dysbiosis of oral tissues including, periodontal diseases, peri-implant conditions, caries, endodontic lesions, and oral cancers. Microbiome-induced inflammatory diseases with disruptions of tissues surrounding periodontal tissues present irreversible connective and bone tissue loss. This is aggravated when chronic systemic conditions are present as clusters of inflammatory diseases including type 2 diabetes. This process is tightly regulated by a new genus of molecules called immunoresolvents (e.g., resolvins, lipoxins, maresins and protectins). As pro-resolution molecules, immunoresolvents increase phagocytosis of bacteria, clearance of dead cells and turn on signals to return to homeostasis. Metagenomic sequencing of human and mice oral microbiota has revealed a pathobiont enrichment, P. gingivalis, as important to promote dysbiosis. The next frontier of microbiome-host integration requires mechanistic studies to validate and to understand the details of health and disease spectrum. We demonstrate that in a murine chronic inflammatory model for type 2 diabetes (db/db) and periodontal diseases, P. gingivalis induced inflammation and tissue loss which is controlled by the specific interaction of immunoresolvents and its cognate receptors present in innate immune cells. Using a workflow of human, murine transcriptomic innate immune evaluations,
Serine 203 mutation of RORγt enhances the pathogenic autoimmune Th17 responses

Th17 cells are associated with several chronic autoimmune diseases. Previous reports suggest the differentiation and function of Th17 cells depend on a nuclear receptor, RORγt, which is tightly controlled and/or modulated by post-translational modifications (PTMs). While several RORγt phosphorylation sites have been detected, including the phosphorylation at serine 203, none of them has been functionally characterized in vivo. Here we generated the phosphor-null knock-in mutant of RORγt by replacing serine at residue 203 to alanine using CRISRP technology. We found that this mutation of RORγt did not affect protein stability, mice expressing RORγtS203A maintained normal thymocyte development and lymph node genesis. Interestingly, RORγtS203A succumbed to more severe diseases pathology when challenged in a model of experimental autoimmune encephalomyelitis (EAE) and experienced delayed recovery when challenged with DSS-induced gut injury. We will discuss the contribution of the RORγt serine 203 residue to the generation and function of pathogenic Th17 and Tγδ17 cells, and their roles in inflammation and autoimmune conditions.
Cytomegalovirus evades ILC1 defenses by blocking TRAIL signaling

Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) regulates cellular apoptosis and inflammation, and growing evidence indicates a key role in antiviral defense. Cytomegalovirus (CMV, a herpesvirus) establishes a lifelong persistent/latent infection that depends upon viral subversion of host innate and adaptive defenses. Our lab has shown that both human and mouse CMV (HCMV and MCMV) inhibit TRAIL death receptors (DR) signaling through the use of specific viral proteins. MCMV viral protein m166 blocks TRAIL-DR expression and an MCMV mutant lacking m166 (MCMVm166Stop) displays severely attenuated early replication in the visceral organs and, persistent-phase replication in salivary glands (SG). Recent publications have revealed a critical role of group 1 Innate Lymphoid cells (ILCs) in viral control and clearance during MCMV infection. Accordingly, we investigated the role of the 2 main subpopulations of group 1 ILCs (conventional NK and ILC1) in the control of MCMV infection in the liver during early infection and in the SG during persistence. Depleting group 1 ILCs prior to infection restored MCMVm166Stop SG replication to normal levels, and similar results were seen in TRAIL-/− mice. In addition, Liver and SG ILC1 display a unique phenotype and express high levels of TRAIL during MCMV infection when compared to their conventional counterpart. Together our data reveal a critical role for m166 inhibition of TRAIL-DR expression in promoting viral infection by blocking TRAIL-mediated ILC1 effector functions, indicating that this cytokine plays a key role in innate antiviral defenses.
Poster Abstract #26

Nicolas Thiault

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A novel double edged sword in T cell development and function: Receptor-Interacting Protein Kinase 1 (RIPK1)

Ripk1 is a key regulator of survival and death. This dual function is controlled by post-translational modifications: ubiquitination of RIPK1 leads to activation and survival whereas its deubiquitination or phosphorylation triggers death. In addition to its involvement in downstream signaling by many death receptors RIPK1 also functions in TCR-induced signaling cascades that either lead to activation-induced death or survival and NFkb activation. The role of Ripk1 in T cells development and functions are still poorly understood mainly because of the perinatal lethality of Ripk1 genomic deletion. To circumvent this problem, we generated T cell specific conditional KO mice by crossed Ripk1fl/fl mice with CD4Cre. This deletion during development greatly affected the generation of TCRab thymocytes and in particular the agonist selected T cell subsets, including the NKT cells and the TCRab DN T cells. Similarly, TCRab T cells in the periphery were greatly reduced, in particular the NK T cell and DN subsets, whereas as expected TCRgd T cells were not affected. We also analyzed Ripk1d/d transgenic mice which possess a mutation in the kinase domain making it inactive. Whereas the thymic development seems not affected, most TCRab T cells displayed a CD44+ activated phenotype and differentiation to cytotoxic cells, including the CD4+ T cells. A similar effect was seen among the mucosal T cells in the gut, where most of the intraepithelial TCRab T cells were cytotoxic T cells with high levels of Granzyme expression.
Investigating Transcriptional Regulators of Memory T Follicular Helper Cells

Generation of T-cell memory is crucial in conferring vaccine-induced immunity, particularly against pathogens where neutralizing antibodies alone are insufficient at providing long-term protection. While great advances have been made in understanding the generation and maintenance of memory CD8+ T cells and B cells, mechanisms underlying the generation of CD4+ T cell memory have remained relatively elusive. This limitation is in part due to both the multipotency as well as lineage plasticity exhibited by CD4+ T helper (TH) cells. Using the LCMV viral infection model, we show that the T follicular helper (TFH) subset seems to be the predominant CD4+ memory cell type based on its relative abundance following pathogen elimination as well as its multi-potent potential upon antigen re-challenge. This multipotency of TFH memory cells during secondary challenge has also been observed in influenza infection and acute bacterial infection, suggesting that TFH memory subset is most capable of providing a comprehensive and robust secondary response, thus conferring proper immunity.

From advancements in CD8+ memory T cell studies, it has become abundantly clear that transcription factors (TF) serve as crucial arbiters for the cell-fate decisions between short-lived effector and memory. To investigate the transcriptional mechanisms underlying TFH formation, we employ a novel bioinformatics analysis utilizing the PageRank algorithm that combines both RNA-seq and ATAC-seq to decipher key TF responsible for memory TFH differentiation. Further understanding of T-FH memory formation will undoubtedly unveil new insights into CD4+ T cell memory in hopes of improving vaccine-based immunity.
Impact of genetic polymorphisms on human immune cell gene expression

While many genetic variants have been associated with risk for human diseases, how these variants affect gene expression in various cell types remains largely unknown. To address this gap, the DICE (Database of Immune Cell Expression, Expression quantitative trait loci (eQTLs) and Epigenomics) project was established. Considering all human immune cell types and conditions studied, we identified cis-eQTLs for a total of 12,254 unique genes, which represent 61% of all protein-coding genes expressed in these cell types. Strikingly, a large fraction (41%) of these genes showed a strong cis-association with genotype only in a single cell type. We also found that biological sex is associated with major differences in immune cell gene expression in a highly cell-specific manner. These datasets will help reveal the effects of disease risk-associated genetic polymorphisms on specific immune cell types, providing mechanistic insights into how they might influence pathogenesis (http://dice-database.org).
Poster Abstract #29

Greet Verstichel

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Thymic selection of innate self-specific T cells: Timing of TCR signaling matters

Naïve CD4 and CD8 T cells are selected in the thymus in response to low-avidity TCR interactions. Such weak TCR signals are crucial for installing a quiescent naïve program. In contrast, agonist-selected double negative (DN) T cells receive strong TCR signals already in the thymus and are instructed to guard immune homeostasis at the mucosal borders. Although the role for strength of TCR activation is clear in instructing these different outcomes, the generation of protective self-specific T cells remains enigmatic. Previous work suggests that the DN T cell lineage already diverges early during T cell development, before the expression of a fully rearranged TCR. We have explored the role of developmental timing by Cre-mediated deletion of components of the TCR pathway. As TCR targets we investigated the lack of Themis expression, essential for the generation of conventional naïve T cells, but dispensable for agonist selection. Vice versa, we analyzed the requirement for Stim1 and Stim2, driving agonist selection, but not conventional positive selection. Deletion of Themis lead to a drastic decrease in naïve T cells regardless of the timing. However, DN T cells appeared particularly dependent on early strong TCR signals as only pre- but not post-beta selection deletion of Stim1 and Stim2 eliminated this subset. Our results suggest that strong early TCR signals are crucial for differentiation of innate self-specific T cells.
A Single-step Chemoenzymatic Reaction for the Construction of Antibody-cell Conjugates

Employing live cells as therapeutics is a direction of future drug discovery. An easy and robust method to modify the surfaces of cells directly to incorporate novel functionalities is highly desirable. However, genetic methods for cell-surface engineering are laborious and limited by low efficiency for primary cell modification. Here we report a chemoenzymatic approach that exploits a fucosyltransferase to transfer biomacromolecules, such as an IgG antibody (MW=150 KD), to the glycocalyx on the surfaces of live cells when the antibody is conjugated to the enzyme's natural donor substrate GDP-fucose. Requiring no genetic modification, this method is fast and biocompatible with little interference to cells' endogenous functions. We applied this method to construct two antibody-cell conjugates (ACCs) using both primary cells and established cell lines, and the modified cells exhibited specific tumor targeting and resistance to inhibitory signals produced by tumor cells, respectively. Remarkably, Herceptin-NK-92MI conjugates, a natural killer cell line modified with Herceptin, exhibit enhanced activities to induce the lysis of HER2+ cancer cells both ex vivo and in a murine tumor model. Given the unprecedented substrate tolerance of the fucosyltransferase, this chemoenzymatic method offers a general approach to engineer cells as research tools and for medical applications.
Poster Abstract #31

CHEN ZHANG

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Probing the mechanisms by which septins regulate ORAI1 function

The STIM-ORAI-mediated calcium release-activated calcium channel, or CRAC channel, is a key source of calcium influx for intracellular Ca2+ signaling and effector function in T lymphocytes. STIM in the ER membrane senses depletion of ER calcium stores and moves to ER-plasma membrane (PM) junctions where it recruits PM ORAI channels and triggers store-operated Ca2+ entry. We have shown previously that septins 4 and 5 are crucial for efficient STIM1-ORAI1 cluster formation following store depletion. Septins were previously reported to specify PM barriers at certain sites and to serve as scaffolds that recruit signaling proteins, but their detailed role in calcium signaling remained elusive. We used live-cell super-resolution microscopy and single-molecule tracking to map ORAI1, STIM1, ER-PM junctions, and membrane-localized septin 4 in resting cells and store-depleted cells. We further investigated the trajectories of ORAI1 in septin 4/5-deficient cells or in cells where the interaction between ORAI1 and STIM1 was abolished by mutagenesis. Our data show that septins neither promote ORAI localization to junctions independent of STIM, nor do they specify corrals specifically surrounding junctions to confine ORAI. Rather, septins contribute to decreased ORAI1 mobility across the entire cell footprint after store depletion, slowing the escape of ORAI1 from junctions after they have been recruited there by STIM. Additionally, septins 4/5 are needed to maintain a normal complement of functional ER-PM junctions. Thus live-cell single-molecule tracking has allowed us to document two primary mechanisms by which septins 4/5 enhance STIM-ORAI signaling.
Poster Abstract #32

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Producing and Characterizing the Neutrophil Extracellular Trap (NET) Genome

Neutrophils are polymorphonuclear leukocytes of the phagocytic system acting as the first line of host immune response. As one of the most abundant types of white blood cells in most mammals (50-80%), neutrophils present key functions in pathogen clearance. Web-like chromatin structures, known as neutrophil extracellular traps (NETs) are produced by neutrophils for immune protection and infection control. NET formation is activated by innate immune receptors and intracellular signals leading to chromatin decondensation and NETosis, with involvement in potentiation of chronic inflammation and autoimmunity. The exact molecular composition of NETs has not been elucidated. In a step toward that goal, a NET isolation protocol has been developed and NET genomic sequencing has been performed. Our in vitro system aimed to differentiate HL-60 cells into neutrophils and to induce NET production. Kinetics studies with various concentrations of PMA (0.1-8000 nM) and time points (10min-8 hours) revealed a reliable induction of NETs after 4 hours at 1,320 nM PMA concentration. Morphological changes were monitored by Giemsa staining, DAPI staining, and 3D visualizations by tomographical imaging. To investigate the genomic sequence, HL-60, neutrophil, and NET DNA samples were isolated via centrifugation and resuspension steps followed by purity testing and were submitted for next-generation sequencing. The results demonstrated enrichment for NETs genome depths in specific regions with further bioinformatic analysis being conducted. Overall, we have designed a reliable model for NET production and characterization. Future analysis includes further understanding of the transcriptome and proteome compositions of NETs.
Poster Abstract #33

Jiang Li

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Elucidating the function of macrophage-derived Resistin-Like Molecule in Th2 immunity by RELMα-/- bone marrow chimeras and RELMα-reporter mice.

RELMα is a highly secreted protein in Th2 cytokine-induced inflammation including helminth infection and allergy. We previously showed that RELMα dampens Th2 immunity and inflammation to helminth Nippostrongylyus brasiliensis (Nb). RELMα is expressed by immune cells such as M2 macrophages, and by epithelial cells (EC), however, the functional impact of immune versus EC-derived RELMα is unknown. We generated bone marrow (BM) chimeras that were RELMα deficient (RELMα-/-) in BM or non BM cells and infected them with Nb. Non BM RELMα-/- chimeras had comparable inflammatory responses and Nb burdens to RELMα+/+ mice, however, both RELMα-/- and BM RELMα-/- mice had increased Nb-induced mucosal inflammation, correlated with accelerated Nb killing. CD11c+ lung macrophages were the dominant BM-derived source of RELMα, therefore, we employed a macrophage-Nb co-culture system to investigate whether RELMα regulates macrophage-mediated Nb killing. Compared to RELMα+/+ macrophages, RELMα-/- macrophages exhibited increased binding to Nb, and functionally impacted Nb viability. Collectively, these studies demonstrate that BM-derived RELMα is necessary and sufficient to dampen Nb immune responses and identify that one mechanism of action of RELMα is through inhibiting macrophage interaction and killing of Nb. Our findings suggest that RELMα acts as an immune brake that provides mutually beneficial effects for the host and parasite by limiting tissue damage and delaying parasite expulsion. For future studies to determine the importance of myeloid-derived RELMα and track RELMα + M2 macrophages, we have generated and characterized cell lineage-specific RELMα-/- mice and RELM α/Arginase1 reporter mice.
Poster Abstract #34

Xiaocui He and Zachary B Katz

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Interplay between PMCA1 pumps and CRAC channels through the lens of single molecule microscopy

Intracellular Ca2+ transients play a central role in signal transduction leading to diverse cellular responses, including a T cell’s response to antigenic stimuli. ORAI forms the calcium release-activated calcium channel (CRAC channel) and mediates store operated calcium entry (SOCE) as the key calcium influx mechanism in non-excitable cells, like T lymphocytes. Correspondingly, plasma membrane Ca2+ ATPases (PMCA) function to pump Ca2+ out of the cytosol to maintain a proper intracellular Ca2+ concentration. In this study, we use live-cell super-resolution microscopy and single-molecule tracking of ORAI1 and PMCA1 in response to ER Ca2+ store depletion and subsequent cytoplasmic Ca2+ influx. This provides novel detail on how CRAC channels and calcium pumps cooperate with each other to produce an effective calcium transient and return to homeostasis. By using the CRISPR/Cas9 method, we create a series of cell models: ORAI1 KO, PMCA1 KO, Hap2-PMCA1 KI and AID-mCherry PMCA1 KI cells which enable us to investigate the localization and mobility of ORAI1 and PMCA1 in different contexts.
Peptides of endogenous retrovirus Gag gene variants are not agonistic ligands for autoreactive T cells in non-obese diabetic mice

Endogenous retroviruses (ERV) have been isolated from the pancreatic islets of non-obese diabetic (NOD) mice. We previously demonstrated that a murine leukemia retrovirus–like ERV Gag protein was secreted together with exosomes by an insulinoma and by primary islet mesenchymal stem cells. Here, we further demonstrated that Gag is an effective antigen to stimulate IFN-gamma production by autoreactive T cells using recombinant Gag protein or virus-like particles (VLPs) expressing the Gag gene. However, candidate peptides of this Gag protein were not effective in stimulating the T cells. Upon analyses of ERV Gag genes expressed in the islets of prediabetic NOD mice, we were surprised to find multiple Gag gene variants with complete open-reading frames. These results suggested the expression of different endogenous proviruses, or alternatively, the detection of point mutations incurred during viral replication. A new batch of altered peptides was synthesized reflecting the variations among the Gag gene variants, and a few of them induced detectable amount of cytokine secretion from the T cells. But, the levels of IFN-gamma induced by the peptides were much lower than that induced by the Gag protein and VLPs, suggesting a low affinity activation. This weak agonistic feature of the Gag peptides was further confirmed by testing single diabetogenic clones, as well as by using MHC tetramers loaded with the peptides. The data suggest that ERV Gag or its gene variants may trigger an autoimmune response through a pathway that may not require direct presentation of viral peptides to autoreactive T cells.
Treating Idiopathic Thrombocytopenic Purpura with Bone Marrow Transplantation

Idiopathic Thrombocytopenic Purpura (ITP) is an autoimmune disease which causes the destruction of platelets. Patients who suffer from ITP can have excessive bleeding due to a lack of platelet coagulation. Current therapies to treat ITP such as splenectomies and steroid treatments do not guarantee lasting remission and there is currently no cure for this disease. The purpose of this study is to find a more effective and permanent therapy such as bone marrow transplantation (BMT) to treat ITP using a novel mouse model. BMT can be a risky procedure, so we improve it in mice by increasing the hematopoietic stem cell (HSC) homing efficiency to the bone marrow by upregulating the important chemotactic receptor, CXCR4, using compounds such as fluticasone propionate (Flonase). We found that glucocorticoids such as Flonase significantly increases CXCR4 on cultured murine HSCs, and our preliminary data suggests that HSCs double their bone marrow engraftment when treated with Flonase in competitive transplantations in wild type mice. We also found that Flonase slightly increases surface CXCR4 on HSC precursor cells, pre-HSCs, which supports Flonase efficacy. Second, we are creating an inducible ITP mouse model using CRISPR technology to test BMT as a possible therapy for ITP. This study can shed light on the application of BMT in ITP and a novel mouse model that can be used to test possible therapies that can have more lasting and less damaging effects than existing treatments for ITP.
Poster Abstract #37

Gen-Sheng Feng

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An efficient combination immunotherapy for liver cancer by remodeling innate and adaptive immunity

In dissecting molecular signaling events in HCC development using mouse models, we and others identified anti-oncogenic effects for a number of pro-oncogenic molecules. To interrogate the underlying mechanisms, we and others have taken a genetic approach to delete a target gene in hepatocytes by Albumin-cre, or in hepatocytes and Kupffer cells by Mx1-cre, which is induced by a synthetic dsRNA, polyIC. These experiments have provided mechanistic insights into hepatic cell-cell communication in driving liver tumorigenesis. Using this genetic approach with the Alb-cre and Mx1-cre systems, we analyzed the role of Shp2 in liver tumorigenesis. However, by including more stringent controls for the Mx1-cre inducible gene deletion, we have found that injection of polyIC at the pre-cancer stage robustly suppressed DEN-induced tumorigenesis in control (Shp2fl/fl), Shp2fl/fl:Alb-cre and Shp2fl/fl:Mx1-cre mice, independent of gene deletion. We extended this observation to Ptenfl/fl:Alb-cre mice, and found that polyIC had a similar inhibitory effect on tumors induced by Pten deficiency and associated NASH. Mechanistically, polyIC injection suppressed liver tumor initiation by reprogramming of macrophage polarization, and activation of NK cells and dendritic cells. Although neither polyIC nor anti-PD-L1 suppressed tumor progression, co-injection of the two reagents showed potent inhibition on HCC progression. In experiments originally designed to dissect mechanisms of HCC development, we have found a potent inhibitory effect of a synthetic dsRNA on liver cancer initiation. We have further developed a combination therapy for liver cancer, by systemic activation of both innate and acquired immune functions.
Using single cell RNA-sequencing to identify novel regulators of circulating and tissue-resident memory T cell fates

During infection, CD8+ T cells differentiate into effector cells that serve to clear the pathogen and long-lived memory cells that persist and provide robust immunity to reinfection. The memory T cell pool is heterogeneous, comprised of distinct subsets with unique functional capabilities and patterns of localization that together provide rapid and long-lasting protection. While central memory and effector memory T cells circulate throughout the blood and lymphoid or non-lymphoid tissues, respectively, another recently described memory T cell subset remains resident in tissues. Positioned at key barrier surfaces, tissue-resident memory T cells (Trm) coordinate rapid and potent immune responses that provide heightened protection against infection, and elicit effective anti-tumor immunity. Despite their key protective roles, the factors that control the development of Trm remain incompletely understood. Furthermore, heterogeneity within the Trm pool remains unexplored. Single-cell RNA sequencing (scRNA-seq) is an unbiased approach to reveal heterogeneity within cell populations that cannot be discerned by traditional methods, thereby identifying subsets that may have distinct functions or represent developmental intermediates. Here, we apply scRNA-seq to explore heterogeneity within tissue-resident and circulating CD8 T cells throughout the course of infection and to identify novel regulators of distinct T cell memory fates.
Detection and Characterization of antigen-specific CD8 T cells in the pancreas of donors with type 1 diabetes

Type 1 diabetes is a chronic autoimmune disorder characterized by the specific immune destruction of insulin-producing beta cells in the pancreas. Autoreactive CD8 T cells recognizing beta cell-derived peptides are prime candidates for causing such beta cell damage. Moreover, CD8 T cells predominantly infiltrate insulin-containing islets, indicating that insulin is an important potential driver of autoreactivity. To further study the distribution and localization of autoreactive T cells in both, endocrine and exocrine compartments, sections were stained using a preproinsulin tetramer and CD8. In addition, to define the immune phenotype of autoantigen-specific CD8 T cells we included the memory marker CD45RO. Overall, the goal is to study autoantigen-specific CD8 T cells in the exocrine and endocrine pancreas of donors with pre-diabetes and donors with type 1 diabetes to understand how their specificities and phenotype evolve with disease onset and progression. Preproinsulin-positive cells were found individually or clustered not only localized close to the islets but also present in the exocrine tissue. The distribution of preproinsulin-positive CD8 T cells was, however, highly variable between islets. In addition, the vast majority of preproinsulin positive cells were CD45RO positive indicating that these cells are indeed memory T cells. Our findings are of potential interest to the field, as they suggest that antigen presentation and encounter by CD8 T cells might occur even in patients with long-standing type 1 diabetes and as long as the antigen is present.
Birkir Reynisson

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Investigating the role of properties other than MHC binding for MHC class II antigen presentation

T-cells play an important role in the adaptive immune system in the fight against pathogens and cancer. A key step in T-cell activation is binding of the T-Cell Receptor (TCR) with a peptide-Major Histocompatability Complex (pMHC) complex on the surface of an antigen presenting cell. Peptide binding to form the pMHC complex is important and much effort has been spent towards modelling binding affinity of MHC towards peptides. Impressive model reliability has been achieved by applying neural networks to modelling pMHC formation [1]. Strides have been made to solve the related problem of peptide binding to MHC-II receptor [2], but the ability of said model to reliably identify epitopes remains unsatisfactory. A recent development has been to adapt models to be trained on Eluted Ligand Mass Spectrometry data. This data is encoded to include also surrounding sequences of ligands within the source protein (i.e. context), thus capturing the proteolytic signal of ligand processing [3]. Taking advantage of ligand context has shown promise in a narrow case of allele specific models, where such models showed superior performance to models trained only on MHC peptide binding affinity data. Here we present preliminary results of extending the context strategy to the pan-specific realm by retraining models on a broader collection of recent Eluted Ligand data gathered from the literature.

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Host fatty acids promote immune evasion by Streptococcus pneumoniae

Streptococcus pneumoniae is a global cause of mortality worldwide despite the availability of pneumococcal conjugate vaccines. Although its capsule and thick cell wall are one of the major virulence factors protecting it from host immune responses, an abundant glycolipid of the bacterial cell wall can be recognized by a unique subset of T lymphocytes: invariant natural killer T (iNKT) cells. Antigenic activity of this glycolipid depends on the inclusion of a particular fatty acid. We reveal that in fatty acid-rich environments, like in host tissues, S. pneumoniae senses the supply of nutrients and shuts down its endogenous fatty acid biosynthesis. Instead, a different host fatty acid gets incorporated into the bacterial cell wall thus creating glycolipids that do not stimulate iNKT cells anymore. A bacterial mutant impaired in fatty acid biosynthesis, and thus antigen production, induces decreased iNKT cell responses leading to an increased bacterial load in mice compared to wild-type bacteria. By depleting a specific free fatty acid in the host, bacterial burden could be strongly reduced. We propose that bacterial uptake of a host fatty acid and incorporation into membrane glycolipids, likely as a means to save metabolic energy, ultimately alters antigenicity for iNKT cells. This pathogen immune evasion mechanism is tied to the availability of an important nutrient and we propose that inhibiting the uptake of this nutrient by S. pneumoniae could have therapeutic potential.
Examining the role of a pro-autoimmune genetic risk variant, PTPN22 1858C>T in enhancing germinal center responses in a model of Type 1 Diabetes

Studies have reported an association between the minor allelic variant of PTPN22, R620W, and increased incidence of autoimmune disorders such as type 1 diabetes (T1D). To examine the mechanisms that contribute to this outcome, our lab introduced the murine ortholog of the human R620W allele, R619W, into the endogenous locus of Non-obese diabetic (NOD) mice that develop spontaneous T1D. NOD mice harboring this mutation (NOD 619W/W) demonstrated a similar phenotype seen in affected humans with accelerated onset of T1D and enhanced anti-islet autoantibodies (IAA). IAA affinity can often identify individuals at risk of developing diabetes. Germinal centers (GC) are lymphoid structures where affinity maturation occurs with the help of follicular helper T cells (Tfh) cells. An increase in circulating Tfh cells has been reported in newly diagnosed and pre-diabetic individuals, thus suggesting a role for GC reactivity in T1D. Similar to human observations, NOD 619W/W mice also show enhanced IAA and increased Tfh and GC B cell numbers before onset of T1D. A smaller ratio of GC suppressors (follicular helper Tregs, Tfr) to GC promoters was also observed. To examine the underlying mechanisms that promote GC responses in mice carrying the risk allele, we have been employing a GC in vitro system to examine early GC B cell responses. We have found that naive B cells sorted from NOD mice carrying the mutation developed more GC B cells. Our lab is currently examining the immunological pathways that enhanced germinal center responses in mice carrying the risk allele.
GPR183 activation contributes to colitis progression

There are currently 1.6 million individuals with inflammatory bowel disease (IBD) in the US. While IBD pathogenesis is multifactorial, the immune system plays a significant role. To date, anti-TNF therapies are among the most efficacious IBD treatments. However, anti-TNF therapy is only effective in approximately 50% of patients, representing an urgent need to identify novel regulators of IBD related inflammation. Previous GWAS studies focused on IBD have identified over 200 hundred genes with genetic variants that significantly associate with disease susceptibility. However, whether these genetic variants lead to differential gene expression during disease pathogenesis remains unquantified. By analyzing IBD patient colonic gene expression, and comparing it to known GWAS-associated genes, we identified GPR183 as a potentially novel regulator of intestinal inflammation. GPR183, a G protein-coupled receptor family member, promotes immune cell chemotaxis when activated by 7-alpha-25-hydroxycholesterol (7alpha25HC), a cholesterol derivative produced by cholesterol 25-hydroxylase (CH25H) acting with other enzymes. Our gene expression analysis of IBD patient colon biopsies revealed a significant upregulation of GPR183 and CH25H expression compared to healthy patients. Additionally, GPR183 levels are upregulated in patients that do not respond to anti-TNF. CH25H deficient mice exhibit less severe disease in a model of dextran sulfate sodium (DSS)-induced colitis. Further, small molecule inhibition of the 7alpha25HC, GPR183 interaction reduces disease severity during DSS treatment. Thus, our results suggest differences in the expression or regulation of GPR183 and its ligands could play a critical role in the pathogenesis of IBD. Supported by NIH grant P01 DK46763 and F32 AI140581.
Fantastic voyage: from hyperglycemia to cerebrovascular inflammation

Diseases characterized by cerebrovascular inflammation and impaired blood supply to the brain, present a major threat to human health. Such diseases are significantly more common among people with abnormally elevated levels of blood glucose. However, the relation between hyperglycemia and cerebrovascular inflammation is still missing. Formation of blood clots can disrupt brain homeostasis. Paradoxically, these clots may be promoted by a proximate feedback, such as immune cell hyper-activation. To understand the cellular events by which hyperglycemia influences blood clot formation, it is crucial to monitor the lesion site in real time. Toward this essential goal we apply a state-of-the-art in vivo imaging of cerebral blood flow in individual cerebral vessels in mice, concurrent with the delivery of amplified ultra-short laser pulses to induce microscopic lesions in the wall of a targeted microvessel (Nishimura et al. 2006 Nat Meth). We now show how elevated blood glucose affects the immediate and early inflammatory response to vascular injury. Our data demonstrate that delivery of minimal laser pulses, which hardly has effect under normal conditions, is sufficient under acute hyperglycemic conditions, to promote significant blood clots. We also show that platelet activation and blood clot formation lead to recruitment of neutrophils and initiation of cerebrovascular inflammation; hyperglycemia aggravates platelet activation and neutrophil recruitment as well as cerebrovascular rupture and blood extravasation. Streptozotocin (STZ)-induced type 1 diabetes mellitus also exacerbates cerebrovascular clot formation and inflammation in untreated, but not insulin-treated, mice. Finally, we provide evidence that hyperglycemia-induced ROS production causes platelet hyperactivation, leading to aggravated
MiR-409-3p and MiR-1896 Co-operatively Participate in IL-17-Induced Inflammatory Cytokine Production in Astrocytes and Pathogenesis of EAE Mice via Targeting SOCS3/STAT3 Signaling

Th17 cells and interleukin-17 (IL-17) have been found to play an important role in the pathology of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). Response to IL-17, reactive astrocytes accompany with immune cells infiltration and axonal damage in MS/EAE. However, the role and the regulatory mechanism of IL-17-activated astrocytes in inflammation and in the EAE process still remain largely unknown. Here, we elucidated that miR-409-3p and miR-1896, as co-upregulated microRNAs in activated astrocytes and in EAE mice, targeted suppressor of cytokine signaling proteins 3 (SOCS3). Overexpression of miR-409-3p or miR-1896 significantly reduced SOCS3 expression and increased phosphorylation of STAT3 as well as induced the inflammatory cytokines production (IL-1β, IL-6, IP-10, MCP-1 and KC), CD4+T cells migration and demyelination, in turn aggravating EAE development. Importantly, the effects of co-overexpression of miR-409-3p and miR-1896 in vitro or in vivo are strongly co-operative. In contrast, simultaneously silenced miR-409-3p and miR-1896 co-operatively ameliorates inflammation and demyelination in the central nervous system of EAE mice. Collectively, our findings highlight that miR-409-3p and miR-1896 co-ordinately promote the production of inflammatory cytokines in reactive astrocytes through the SOCS3/STAT3 pathway and enhance reactive astrocyte-directed chemotaxis of CD4+T cells, leading to aggravate pathogenesis in EAE mice. Co-inhibition of miR-409-3p and miR-1896 may be a therapeutic target for treating multiple sclerosis and neuroinflammation.

KEYWORDS: multiple sclerosis; experimental autoimmune encephalomyelitis; astrocytes;
Poster Abstract #46

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A novel enhancer orchestrates nuclear architecture to generate a diverse antigen receptor repertoire

The genome is organized into topologically associated domains (TAD) that enclose smaller subTADs. Here we identify and characterize an enhancer that is located in the middle of the V gene region of the immunoglobulin kappa light chain (Igκ) locus that becomes active preceding the stage at which this locus undergoes V(D)J recombination. This enhancer is a hub of long-range interactions connecting subTADs in the V gene region with the recombination center at the J genes. Deletion of this element results in a highly altered long-range interaction pattern across the locus and, importantly, affects individual V gene utilization locus-wide. These results indicate the existence of an enhancer-dependent framework in the Igκ locus, and further suggest that the composition of the diverse antibody repertoire is regulated in a subTAD-specific manner. This enhancer thus plays a structural role orchestrating the proper folding of the Igκ locus in preparation for V(D)J recombination.
Dual nanoparticle enhanced immunotherapy using TLR7 agonists, checkpoint inhibitor and ultrasound-guided histotripsy

Silica particles have been developed as a convenient drug carrier and ultrasound imaging contrast agent because of their ease of modification and long imaging time. Nano-sized version of the silica nanoshells (NS) was engineered with a small molecule toll-like receptor 7 (TLR7) agonist, 1V209, and displayed enhanced adjuvant activities in vitro and in vivo. TLR7 agonist and silica combination triggered NLRP3 inflammasome and released IL-1β to re-stimulate NF-κB pathway while neither unconjugated TLR7 agonist or silica shells produced IL-1β. An immunization study demonstrated that 1V209-NS conjugates increased a thousand-fold OVA-specific IgG antibodies in mice sera and skewed response to a Th1-mediated immunity compared to unconjugated TLR7 agonist. 1V209-NS conjugates were administered intratumorally into mice and silica shells have a tendency to prolong the agonist accumulation time and the localization of agonist promoted the infiltration of T cells in tumors. In this study, nano-sized and micro-sized silica shells were employed to enhance the immuno-therapeutic agents and ultrasound sensitivity. With silica shells, the three-prong method containing TLR7 agonist, checkpoint inhibitor, and microshells-assisted histotripsy showed that not only can the injected tumor be induced into remission, but an uninjected contralateral tumor can also be induced into remission (abscopal effect)
Poster Abstract #48

Matthew S. Tsai

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Utilizing single-cell RNA sequencing to investigate the cellular and molecular landscape of ulcerative colitis

The inflammatory bowel diseases (IBD), traditionally classified as Crohn’s disease (CD) or ulcerative colitis (UC), are chronic intestinal disorders characterized by dysregulated host immune responses to the gut microbiota in genetically susceptible hosts. The cellular and molecular underpinnings of the disease have been studied previously but remain incompletely understood. To elucidate the aberrant immune response in ulcerative colitis, we utilized a single-cell RNA sequencing approach to define molecular subtypes within the peripheral blood and intestinal tissue from patients with ulcerative colitis and healthy controls. We characterized a number of subsets within each of the major immune cell types and determined their tissue specificity, abundance, and gene expression in the healthy vs. disease states. We identified immune cell subtypes that were enriched or depleted in patients with ulcerative colitis as well as a number of genes in specific immune subtypes that were differentially expressed between the healthy vs. disease states. These new insights underscore the power and necessity of a single-cell approach in elucidating the pathogenesis of inflammatory bowel disease.
CTCF is necessary for CD8+ effector T cell differentiation

Naive T cell differentiation into effector and memory subsets in response to infection requires the coordination of numerous factors that regulate changes in gene expression to support proliferation and cell function. Chromatin accessibility and DNA looping influences transcription factor binding, facilitates enhancer-promoter interactions, and insulates genes. CCCTC-Binding Factor (CTCF) is a highly conserved protein known to be important in shaping the genome and partnering with transcription factors, but its role in CD8+ T cell differentiation has yet to be determined. Here, we show that the loss of CTCF in antigen-specific CD8+ T cells promotes formation of memory-precursor cells while repressing formation of terminal effector cells in response to Listeria infection. Furthermore, loss of CTCF expression represses the formation of effector memory T cells but promotes accumulation of tissue-resident memory T cells. Upon rechallenge, we found CTCF-deficiency in memory T cells impairs the development of secondary terminal effector cells. When comparing ChIP and HiC datasets, we found that terminal effector cells gain CTCF binding within topologically associated domains, often overlapping with H3K27ac peaks which marks active enhancers and promoters. Since genes near the overlap of CTCF and H3K27ac peaks have increased expression in terminal effector cells, we propose that CTCF promotes effector function by stabilizing promoter-enhancer interactions which influences the expression of key genes. Insights into the role of CTCF in CD8+ T cell differentiation during infection will hopefully contribute to the development of therapies which better utilize the T cell response.
CD4+ cytotoxic T cells in the experimental infection with Trypanosoma cruzi

Cytotoxic CD4+ T cells (CD4 CTLs) have been described in viral infections, autoimmunity and anti-tumor responses, both in man and mouse. CD4+ T lymphocytes play a key protective role during infection with the protozoan parasite Trypanosoma cruzi, the etiologic agent of Chagas disease (American trypanosomiasis). However, the role of CD4 CTLs in patients with Chagas disease remains unclear. Here, we found that experimental infection with T. cruzi induced the differentiation of CD4 CTLs, which are found in the spleen, gut and myocardium of infected mice. Flow cytometry analyses showed that most TCRαβ+CD4+GzB+ cells are CD8α- and NK1.1- and express the cytolytic marker 2B4 (CD244). Sorted spleen CD4+GzB+ cells express T-bet, ThPOK and Runx3 RNA and the kinetics of appearance of CD4 CTLs in the spleen follows the kinetics of IFN-γ-producing cells, peaking at around day 14 post-infection (pi.). At this pi time point, around 40% of CD4+ cells infiltrating the myocardium are GzB+ cells, whose degranulation was evidenced by the expression of CD107a. In addition, compared to controls, infected mice have higher percentages of GzB+CD4+ T cells in the small intestine, both in the lamina propria and in the intraepithelial compartment. We also demonstrated that CD4 CTLs display antigen (Ag)-specific cytotoxicity and that intrinsic IL-18R signaling in CD4+ T cells plays a critical role for an optimal CD4 CTL response. In conclusion, infection with T. cruzi induces the differentiation of Ag-specific CD4 CTLs, whose role in the resistance to infection is being presently investigated.

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Poster Abstract #51

GOOYOUNG SEO

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LIGHT-HVEM Signaling in Innate Lymphoid Cell Subsets Protects Against Enteric Bacterial Infection

Innate lymphoid cells (ILCs) are important regulators of early infection at mucosal barriers. ILCs are divided into three groups based on expression profiles, and are activated by cytokines and neuropeptides. Yet, it remains unknown if ILCs integrate other signals in providing protection. We show that signaling through herpes virus entry mediator (HVEM), a member of the TNF receptor superfamily, in ILC3 is important for host defense against oral infection with the bacterial pathogen Yersinia enterocolitica. HVEM stimulates protective IFNg secretion from ILCs, and mice with HVEM-deficient ILC3 exhibit reduced IFNg production, higher bacterial burdens and increased mortality. In addition, IFN production is critical as adoptive transfer of wild-type but not IFNg-deficient ILC3 can restore protection to mice lacking ILCs. We identify the TNF superfamily member, LIGHT, as the ligand inducing HVEM signals in ILCs. Thus HVEM signaling mediated by LIGHT plays a critical role in regulating ILC3-derived IFNg production for protection following infection.
Poster Abstract #52

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Modulating tumor hypoxia for anti-tumor immunity

While immunotherapy represents a remarkable option for cancer treatment, about 70% of treated individuals see their tumors progress. While tumor heterogeneity is an important rate limiting factor for a therapeutic response, hypoxia is a feature common to most solid tumors and is generally abundant in a tumor microenvironment. Anti-tumor immune cells, like their cancer cell counterpart, require enough nutrients and oxygen delivery to maintain their functionality. However, prior studies on hypoxia were mainly from in vitro experiments. To better understand how hypoxia can potentially regulate anti-tumor immunity in vivo, we utilized PHD2+/- mouse model whereby enhanced endothelial normalization in aberrant blood vessels leads to increased tumor oxygenation. By injecting well-defined mouse melanoma cell line called YUMMER into wild-type (WT) vs PHD2+/- mice, we evaluated how increased oxygenation can mediate anti-tumor immunity. We observed reduced tumor hypoxia and more tumor-infiltrating CD8+ and CD4+ T cells in PHD2+/- mice, accompanied by decreased infiltration of tumor-promoting myeloid cells. Furthermore, delayed tumor growth is seen in PHD2+/- mice. In addition, T cells in the more hypoxic tumors of wild-type mice exhibited significantly higher expression of inhibitory molecules characteristic of exhaustion and were less functional. Based on these findings, we administered anti-PD1 & anti-CTLA-4 combination therapy to PHD2+/- vs WT mice to see if oxygenation could affect response to immunotherapy. Tumor growth was significantly delayed in treated PHD2+/- mice as compared to their wild-type counterpart. Our studies therefore highlight that enhanced oxygenation could be an important modulator for an effective anti-tumor immune response.
miR-146a modulates autoreactive Th17 cell differentiation and regulates organ-specific autoimmunity

Autoreactive CD4 T cells that differentiate into pathogenic Th17 cells can trigger autoimmune diseases. Therefore, investigating the regulatory network that modulates Th17 differentiation may yield important therapeutic insights. miR-146a has emerged as a critical modulator of immune reactions, but its role in regulating autoreactive Th17 cells and organ-specific autoimmunity remains largely unknown. Here, we have reported that miR-146a–deficient mice developed more severe experimental autoimmune encephalomyelitis (EAE), an animal model of human multiple sclerosis (MS). We bred miR-146a–deficient mice with 2D2 T cell receptor–Tg mice to generate 2D2 CD4 T cells that are deficient in miR-146a and specific for myelin oligodendrocyte glycoprotein (MOG), an autoantigen in the EAE model. miR-146a–deficient 2D2 T cells induced more severe EAE and were more prone to differentiate into Th17 cells. Microarray analysis revealed enhancements in IL-6– and IL-21–induced Th17 differentiation pathways in these T cells. Further study showed that miR-146a inhibited the production of autocrine IL-6 and IL-21 in 2D2 T cells, which in turn reduced their Th17 differentiation. Thus, our study identifies miR-146a as an important molecular brake that blocks the autocrine IL-6– and IL-21–induced Th17 differentiation pathways in autoreactive CD4 T cells, highlighting its potential as a therapeutic target for treating autoimmune diseases.
Altered thymic differentiation and modulation of arthritis by invariant NKT cells expressing mutant ZAP70

Various subsets of invariant natural killer T (iNKT) cells with different cytokine productions develop in the mouse thymus, but the factors driving their differentiation remain unclear. Here we show that hypomorphic alleles of Zap70 or chemical inhibition of Zap70 catalysis leads to an increase of IFN-γ-producing iNKT cells (NKT1 cells), suggesting that NKT1 cells may require a lower TCR signal threshold. Zap70 mutant mice develop IL-17-dependent arthritis. In a mouse experimental arthritis model, NKT17 cells are increased as the disease progresses, while NKT1 numbers negatively correlates with disease severity, with this protective effect of NKT1 linked to their IFN-γ expression. NKT1 cells are also present in the synovial fluid of arthritis patients. Our data therefore suggest that TCR signal strength during thymic differentiation may influence not only IFN-γ production, but also the protective function of iNKT cells in arthritis.
Poster Abstract #55
Clarice Monteiro Rodrigues Santos

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The expansion of circulating IL-6 and IL-17-secreting follicular helper T cells is associated with neurological disabilities in neuromyelitis optica

Neuromyelitis optica (NMO) is an autoimmune inflammatory disease of the central nervous system (CNS) which aquaporin 4 (AQP4) antibody appears to play a central role in pathogenesis. The production of pathogenic antibodies by B lymphocytes depends on their productive interaction with follicular CD4+ T (TFH) cells, classically identified by the expression of CXCR5 and IL-21. Different subtypes of TFH cells can also produce different cytokines but little was known about TFH cells subsets in NMO.

For our study, whole blood from NMO patients (n=21) and healthy individuals (n=20) were briefly stimulated with PMA plus Ionomycin for 4 h and the frequency of different circulating TFH cell subsets was determined by flow cytometry. The plasma levels of cytokines were quantified by ELISA. The seropositivity for anti-AQP4 and anti-MOG antibodies was evaluated by cell-based assay (CBA).

In our study, the proportion of classical TFH cells (CXCR5+IL-21+CD4+) was higher in NMO patients in comparison with the control group. Among different cytokine-secreting TFH cell subsets, the percentage of those able to produce IL-6 or IL-17 was significantly higher in NMO patients presenting anti-AQP4 antibodies. Additionally, the plasma levels of IL-6 and IL-17, and the proportion of IL-6+ and IL-17+ TFH cells, were directly associated with disease activity. In contrast, NMO patients with higher proportion of IL-10+ TFH cell subsets showed a lower neurological disabilities score. Our findings suggest that the expansion of peripheral IL-6+ and IL-17+ TFH cells may be involved in NMO pathogenesis.
γδ T Cell Activation in Response to a Keratinocyte Wound Signature

Epidermal resident γδ T cells are key players in the wound healing response, providing growth factors and cytokines that promote keratinocyte proliferation following recognition of damaged self. These cells are known to be unresponsive in chronic wounds and as such represent a novel target for new wound healing therapies. In this study we seek to modulate the γδ T cell response in chronic wounds through the identification, characterization, and delivery of keratinocyte-expressed proteins that comprise a “wound signature” allowing γδ T cells to recognize and respond to epithelial damage. We have identified a set of target genes through RNA-seq analysis which are upregulated following wounding in mice and correspond to membrane-bound and secreted proteins available for interactions with neighboring cells. Candidate gene expression was measured following wounding of human skin tissue, and ICAM1, CRISPLD2, and HSPA8 were shown to also be significantly upregulated in human skin wound samples. In vitro assays indicate that Hspa8 and ICAM-1 are important for γδ T cell activation, and these proteins have been chosen for in vivo delivery. In order to deliver these candidate proteins into in vivo wound models we have developed a hydrogel-based delivery system with a highly tunable time frame of release. This system improves the retention of protein cargo at the wounds site post-wounding in mice and will be used to administer our candidate proteins into mouse models of non-healing wounds to assess effects on acceleration of tissue repair.
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LXR activity in Kupffer cells modulates inflammatory transcriptional programs

Liver X Receptors (LXRs), composed of LXRα and LXRβ, are ligand activated nuclear receptors that are important regulators of immune responses through their interaction with NF-κB. LXRs control gene expression involved in cholesterol efflux, lipid metabolism, and inflammation and can suppress inflammatory response genes in macrophage foam cells present in atherosclerotic lesions. LXRα is highly expressed in liver resident macrophages, called Kupffer cells, which are involved in clearing pathogens from the portal circulation, as well as initiating inflammatory responses. We sought to understand the LXR-dependent responses modulating cholesterol accumulation and inflammation in Kupffer cells. We found a decreased expression of cholesterol efflux and homeostatic genes in Kupffer cells of mice lacking LXRs throughout all tissues; however, little is known about the function of LXRs intrinsic to Kupffer cells. Using a novel Kupffer cell specific Cre mouse, we deleted LXRs in a Kupffer cell restricted fashion and observed an expansion of a new subset of F4/80 High, Cd11b Intermediate, Tim4 Low Kupffer cell population that is associated with fatty liver disease. Ongoing studies are focused on pharmacological activation of Kupffer cell LXR activity through a synthetic mimetic of the natural LXR ligand desmosterol. Preliminary results suggest that this desmosterol mimetic functions in a Kupffer cell specific manner in the liver and is predicted to dampen hepatic inflammation, lipid accumulation, and associated cardiovascular damage. Thus, our work suggests that pharmacological activation of LXRs in Kupffer cells may act therapeutically in patients with fatty liver disease and/or other inflammatory diseases of the liver.
Poster Abstract #58

Ibrahim M Sayed

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Dysregulation of Engulfment and Cell Motility Protein-1 (ELMO1) in the gut fuels Inflammatory Bowel Disease (IBD)

BACKGROUND: Luminal dysbiosis is ubiquitous in IBD, but how the microbes trigger pro-inflammatory cascades in the epithelial and phagocytic cells remains unknown. Here we investigated the role of ELMO1 in sensing and responding to IBD-associated microbes in both the gut epithelium and macrophages.

METHODS: Stem cell-based enteroid-derived monolayers (EDMs), prepared from WT, ELMO1−/− mice and IBD patients' colon biopsies, were differentiated in vitro to mimic the gut epithelium. EDMs infected with IBD-associated invasive E. coli-LF82 were analyzed for bacterial internalization, cytokine production and monocyte recruitment. ELMO WT and ELMO KO mice were treated with DSS; mice weight, colon length and colon histology score were measured.

RESULTS: In IBD samples, expression of ELMO1 was elevated in the colonic epithelium and in the inflammatory infiltrates, together with up regulation of pro-inflammatory cytokines, MCP-1 and TNF-α. ELMO1−/− murine EDMs displayed a significant reduction of bacterial internalization and MCP-1 production compared to WT EDM. MCP-1 recruited monocytes that required ELMO1 to engulf the bacteria and induce TNF-α production. ELMO WT mice showed more colitis than ELMO KO; mice weight and colon length were significantly lower in ELMO WT mice, while histology score was significantly higher in ELMO WT mice.

CONCLUSIONS: ELMO1−/− EDMs displayed a reduction in bacterial internalization, MCP-1 production and monocyte recruitment. ELMO1−/− monocytes showed a decrease in engulfment efficiency and TNFα response. DSS-treated ELMO KO mice showed less degree of inflammation. These findings raise the possibility that a dysregulated epithelial ELMO1→MCP-1 axis may serve as an upstream therapeutic target in IBD.
Poster Abstract #59

Miguel Tam

Cheng-Rui Li, Bertrand Yeung, Xinfang Zhao, Craig Monell, and Xifeng Yang

BioLegend, Inc

Standardized oligo barcode antibody conjugates for simultaneous proteomics and transcriptomics. TotalSeq™ reagents compatible with CITE-seq, REAP-seq, and similar workflows.

The CITE-Seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing) platform is a recent advance in single cell analysis, which is based on high-throughput single cell sequencing (scSeq) and combines measurements of cellular proteins and transcriptomes. This platform will potentially transform how complex cell populations (lineage differentiation or tumor infiltrating lymphocytes) are studied. Published data indicated that CITE-Seq analysis on cell surface marker expression are comparable to multi-color flow cytometry, but provides superior capacity in multiplexing. Currently, individual investigators use their choice of oligo barcodes for different protein markers, and no standardized barcodes or controls are available. Comparison of data from different studies will be difficult in the absence of such controls. The availability of standardized oligonucleotide barcode- labeled antibodies can enable reliable comparison of data across longitudinal and multi-site studies. In collaboration with the New York Genome Center, we assigned unique barcodes to several of our clones and optimized teh conjugation and purification process. In this study we detail our quality control parameters, and oligo barcode-conjugated monoclonal antibody products are further validated on the CITE-seq technology platform with BioLegend in-house data and other collaborators data.
Poster Abstract #61

Lara Labarta-Bajo

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CD8 T Cell-Induced-Anorexia Associates With Blooming Of An Immunosuppressive Bacterium During Chronic Viral Infection

The host’s co-existence with persistent pathogens is accomplished by adaptations of immune cells, including CD8 T cells, which undergo functional loss (or “exhaustion”) during chronic infections. To better understand host adaptations we compared the serum metabolome of mice infected with the persistent versus acute variants of lymphocytic choriomeningitis virus (LCMV), observing a striking overlap with a fasting metabolome profile in the former setting. Consistently, chronic (but not acute) LCMV infection induced a profound anorexic behavior evidenced by a dramatic reduction (and even cessation) in food consumption. Because diminished food consumption can affect intestinal bacteria, we next compared the gut microbiome after acute and chronic infections and revealed that Akkermansia muciniphila bloomed concomitantly to the anorexic behavior in chronically-infected mice. Remarkably, A. muciniphila overgrowth was recapitulated when acutely-infected mice were deprived from food while features of CD8 T cell exhaustion were reproduced after they were orally administered with A. muciniphila. Similarly, oral inoculation of A. muciniphila in LCMV-chronically-infected mice further deepened CD8 T cell exhaustion. Lastly, cell-depletion experiments revealed that both anorexia and A. muciniphila blooming were dependent on CD8 T cells after persistent infection. Our data suggest that CD8 T cells induce a profound anorexic behavior after chronic viral infection, favoring the overgrowth of intestinal bacteria (e.g. A. muciniphila) that contribute to CD8 T cell exhaustion. These findings may reflect a new paradigm in which changes in host eating behavior and the intestinal microbiome may be part of a CD8-T-cell negative feedback-loop that promotes adaptations during chronic infections.
Scavenger receptor CD36 promotes immunosuppression in CD8 T cells

Cytotoxic CD8+ T lymphocytes are critical for immunity against tumors, but often the number of tumor infiltrating lymphocytes (TILs) may be low or the TILs may be dysfunctional, thereby impeding immunosurveillance. Here we identify CD36 as a coinhibitory receptor that critically limits anti-tumor and other CD8 T cell-dependent chronic immune responses. We found in several mouse tumor models and chronic viral infection, CD8 T cells increase the expression of the scavenger receptor CD36. Our data show that CD36 expression is positively correlated to the levels of inhibitory receptors PD1 and Tigit in CD8 TILs. We also found that CD36-deficient CD8 T cells exhibit higher TCR signaling in vitro. More importantly, CD36-deficient CD8 TILs had considerably more robust anti-tumor activity and cytokine production than wild-type cells. These results define a key role for CD36 in regulating chronic CD8 T cell-dependent responses.
Identification of new T cell Epitopes in the Mold Aeroallegen Aspergillus fumigatus

Exposure and sensitization to mold is associated with asthma that does not respond to medication. Most Aspergillus fumigatus (Asp) allergens were identified based on their IgE binding properties. However asthma and allergy pathology has a significant T cell component. The T cell epitopes of Asp are not known. We took a comprehensive approach to identify new T cell antigens. Using our validated 2D-gel immunoproteomics approach, we probed for IgE/IgG reactivity of Asp allergic patient plasma to two Aspergillus extracts. We also mined the literature for known Asp allergens and antigens identified by other researchers. The peptides thus generated were then assessed for HLA binding. We identified a total of 2769 HLA binding peptides that were pooled into 146 pools roughly equivalent to the antigens. We expanded T cells from PBMCs of Asp reactive donors with two different Asp extracts and screened for cytokine production in response to these peptide pools. 40 of the tested pools had reactivity in at least 15% of donors. The most reactive pool from the known allergen category was Alkaline Protease 1 (alp 1). Alp 1 acts as a virulence factor in invasive aspergillosis and is involved in immune evasion. Our experiments also identified many new antigens about which little is currently known. Our work will generate a new Asp peptide “megapool” that can detect mold specific T cell responses directly ex vivo, which provides a tool for future studies that increase our understanding of the immunopathology associated with mold allergy and asthma.
Lipid talk: Host-helminth parasite interaction via endocannabinoids

More than 2 billion humans carry infectious parasites leading to chronic co-morbidities and growth retardation in children. Parasitic infections induce a T helper type 2 (Th2) immune response in the host to promote clearance which can lead to fibrosis if chronic. We recently showed that infection with the soil-transmitted nematode Nippostrongylus brasiliensis (Nb) induces overproduction of endocannabinoids (eCBs) in the host. Endocannabinoids are endogenous cannabis-like molecules that influence the development of obesity and appear to be anti-inflammatory, however their function in infection is unknown. The overproduction of eCBs in Nb-infected mice was observed throughout infection in the infected lung and intestine. Blockade of cannabinoid receptor 1 (CB1) via administration of AM6545 (10 mg/Kg/2mL), a peripherally restricted CB1 neutral antagonist, throughout Nb infection severely worsened weight loss and parasite burden of mice. The increase in number of worms was coupled to exacerbated inflammation in the lungs and intestines of AM6545 treated Nb-infected mice. Additionally, treatment of animals with AM6545 at day 4 post infection with Nb worsened fecal egg burden but did not alter food intake or recovery of weight loss. Strikingly, we also found that Nb produces its own eCBs which vary in concentration based on stage of development, and that the endocannabinoid system is present in many parasitic nematodes including those that infect humans. These findings suggest that the eCB system is active in several hookworm species, and that host and helminth endocannabinoids may have immune and anti-inflammatory functions.
Blimp1 functions as a molecular switch to prevent inflammatory activity in RORγt+Foxp3+ regulatory T cells

Foxp3+Treg play crucial roles in suppressing unwanted immune responses and prevent the development of autoimmune diseases. Specialized Treg subsets exist and are required to modulate different types of immune response. RORγt+Foxp3+Treg were recently described as peripherally-induced microbiota-specific Foxp3+Treg population required to control effector responses in the intestinal mucosa. RORγt+Foxp3+Treg express the cytokine IL10 and other molecules associated with regulatory function. Despite their expression of RORγt and other genes associated with the Th17 program, Foxp3+RORγt+Treg do not produce IL17 and have potent suppressor function under homeostatic conditions, but the mechanisms underlying their phenotype are poorly understood. Here, we show that the transcription factor Blimp1 is a crucial regulator of the RORγt+Foxp3+Treg subset. The intrinsic expression of Blimp1 in these cells was required to prevent production of the inflammatory cytokines IL17A and F. Direct binding of Blimp1 to the Il17 locus in Treg was associated with inhibitory histone modifications, but unaltered binding of RORγt. In the absence of Blimp1 the Il17 locus was activated, with increased occupancy of the co-activator p300 and abundant binding of the transcriptional regulator IRF4, which was required, along with RORγt, for IL17 expression in the absence of Blimp1. Despite their sustained expression of Foxp3, Blimp1/-/RORγt-/-IL17-producing Treg lost suppressor function and promoted intestinal inflammation, indicating that repression of Th17-associated cytokines by Blimp1 is a crucial requirement for RORγt+Treg function. These findings uncover a novel aspect of Blimp1’s role in Foxp3+Treg biology and shed light on the intricate mechanisms underlying Treg phenotypic stability.
Anti-inflammatory metabolite itaconate regulates mitochondrial metabolism and mitigates brain injury

Metabolic reprogramming drives cell and tissue functions. For example, the dynamic control of metabolites such as itaconate influences inflammation and infection. Immunoresponsive gene 1 (Irg1) expression is induced by inflammatory stimuli, and its enzyme product cis-aconitate decarboxylase (CAD) catalyzes the production of endogenous itaconate via mitochondrial tricarboxylic acid (TCA) intermediates. Here, we identify a metabolic regulatory network that links itaconate to alteration of mitochondrial metabolism that occurs in the context of immune response. We show that itaconate-infusion in mouse models of brain injury mitigates inflammation and improves overall outcome. Itaconate alters TCA cycle metabolism and substrate utilization in brain cells via inhibiting succinate dehydrogenase (SDH) activity and BCAA catabolism. This metabolic network links itaconate to distinct mitochondrial pathways and offers a potential therapeutic strategy for metabolic injury and inflammation.
Assessment of Global Chromatin Accessibility in Peripheral iNKT Cell Subsets

Invariant Natural Killer T (iNKT) cells are a type of innate-like T lymphocyte that both initiate and inhibit immune responses. iNKT cells differentiate into three effector cell subsets in the thymus, NKT1, NKT2, and NKT17, which closely resemble Th1, Th2, and Th17 CD4+ T cells, and ILC1, ILC2, and ILC3 innate lymphoid cells, respectively. Following egress from the thymus, iNKT cells localize to tissues throughout the body and most do not recirculate. The impact of tissue localization on the epigenetic landscape and transcriptome of each subset is not known. Here, we assessed global chromatin accessibility using ATAC-seq in iNKT cell subsets in thymus compared to peripheral tissues, including spleen, liver, and lung, where these cells provide vital immunity to bacterial infections. By comparing iNKT cell subsets within each tissue, we find over 5,000 differentially accessible regions of chromatin. This surprising number of differences is consistent with our previous analysis of the transcriptomes of thymic iNKT cell subsets. Interestingly, relatively limited differences in accessible chromatin were observed when comparing individual subsets taken from the thymus, spleen and liver. On the other hand, a unique set of accessible chromatin regions were identified in lung NKT cells. These lung-related sites include an AP-1 motif signature, which might be consistent with activation or enhanced tissue residency. Taken together, these data suggest that the regulatory landscape of iNKT cell subsets is highly impacted by subset choice and only minimally impacted by tissue localization, with the exception of those in the lung.

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Poster Abstract #68

Chia-Hao Lin

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Cell type-specific role of IL-27 in immune regulation in EAE

IL-27, a cytokine of IL-12 superfamily has been implicated as a viable therapeutic target for many human diseases, including multiple sclerosis (MS). Despite that the pro-inflammatory role of IL-27 has been well-documented, recent studies have pointed to IL-27 as an immunosuppressive cytokine in many disease models. Here we aim to systemically explore the biological impact of different cellular sources of IL-27 in immune regulation, specifically in the setting of experimental autoimmune encephalomyelitis (EAE), a most commonly used experimental model for human MS. Interestingly, while mice with myeloid cell-specific ablation of IL-27 developed more severe disease phenotypes upon EAE induction, conditional deletion of IL-27 in dendritic cells (DCs) led to attenuated pathology. Mechanistically, our data revealed that only myeloid cell- but not DC-derived IL-27 is required for optimal production of IL-10, an immunosuppressive cytokine that is know to be driven by IL-27. Thus, IL-27 from different cellular sources could influence the outcomes of EAE pathogenesis through playing distinct roles in regulating immune responses.
Discovery of a novel microtubule targeting agent as an adjuvant for cancer

The strategy of cancer immune therapies focuses on augmenting adaptive immune responses. Recently, innate immune stimulating agents are recognized as a potential counterpart of combination therapy with conventional immune therapies. However, innate immune activation does not sustain due to negative feedback system. We hypothesized that the compounds that prolong innate immune stimulation improve efficacy of cancer immunotherapy. Around 170,000 compounds were subjected to high-throughput screening using NF-κB reporter human monocytic THP-1 cells. Following the pilot, main and confirmation screens using Toll-like receptor ligand as primary stimulant, 4H-chromene-3-carbonitrile scaffolds was identified as a hit. We repurchased 23 of the most promising candidates. Compound #1 was most effective in stimulating the release of cytokines and chemokines from immune cells, including murine primary dendritic cells. Mechanistically, #1 inhibited tubulin polymerization, and its effect on immune cell activation was abolished in cells mutated in the beta-tubulin gene (TUBB) encoding the site where colchicine binds. Treatment with #1 resulted in mitochondrial depolarization followed by mitogen-activated protein kinase activation. Intratumoral injection of #1 delayed tumor growth in a murine syngeneic model of head and neck cancer. When combined with PD-1 blockade, tumor growth slowed in both injected and uninjected nodule in the contralateral flank, suggesting the treatment induced tumor specific adaptive immune responses. Thus, we identified a new class of tubulin de-polymerizing agent that acts both as an innate and an adaptive immune activating agent, and that limits solid tumor growth when used concurrently with a checkpoint inhibitor.
Neural stem cell induced regulatory T cells specific for neural self-antigens are derived from the exTreg pool

Transplantation of neural stem cells (NSCs) is a promising therapeutic strategy to treat neurodegenerative diseases, such as Multiple Sclerosis (MS), although the mechanism by which these cells promote tissue repair remains poorly understood. We report that human NSCs promote antigen specific T regulatory cells (Tregs) which activate endogenous repair pathways and promote remyelination in a murine model of MS. We observed remyelination, decreased neuroinflammation and an increase in central nervous system (CNS)-resident CD4+CD25+FoxP3+ Tregs in EAE mice receiving an intra-spinal transplant of human NSCs. Recovery was not a result of cell replacement since hNSCs underwent xenograft rejection. Importantly, ablation of Tregs abrogated histopathological improvement. hNSCs promoted Treg expansion in co-cultures in vitro. RAG2/-2D2+ (R2D2) TCR transgenic mice, which bear a TCR repertoire restricted to myelin oligodendrocyte glycoprotein (MOG) and neurofilament, lack CD25+FoxP3+ Tregs under homeostatic conditions. However, following exposure to MOG self-antigen, R2D2 mice developed CD25+FoxP3+Helios+ Tregs in cervical lymph nodes and the spinal cord. Additionally, an increase in Tregs was observed in co-cultures with wild type B6 and R2D2 splenocytes, but not with RAG2/-OT-II+ splenocytes, suggesting a role for self-antigen specificity. Utilizing an exTreg reporter mouse (FoxP3-YFP-Cre x loxPTdt) we discovered that hNSC-Tregs are derived from the exTreg pool. These findings support the hypothesis that exposure to self-antigens is important for the maintenance of FoxP3 in CNS-targeted Tregs.
Poster Abstract #71

Jihye Han

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The E3 Ligase VHL Controls Lung Inflammation by Regulating Epithelium-ILC2 Cross-talk

Group 2 innate lymphoid cells (ILC2) are a specialized subset of lymphoid effector cells that play an important role in allergic inflammation in response to host-derived cytokines and alarmins. Pulmonary epithelium play a critical role in the initiation of type 2 immunity by production of cytokines, such as TSLP, IL-25 and IL-33 after exposure to allergens. However, the epithelium-derived signals and their regulatory mechanisms that directly regulate ILC2 remain unclear. Here we report that conditional deletion of the E3 ubiquitin ligase VHL in lung epithelial secretory cells exhibited allergic inflammation specifically due to increased ILC2 producing IL-5 and IL-13 under steady state and during pulmonary inflammation. VHL deficiency also resulted in the accumulation of hypoxia inducible factor proteins (HIFs), but did not alter the expression of IL-33 and TSLP in the lung epithelium. Our findings indicate that VHL-HIF pathways control allergic inflammation in the lung by regulating ILC2, but detailed molecular mechanisms need to be identified.
**Poster Abstract #72**

Jennifer Dan

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Development of bacteriophage-specific immune responses in a lung transplant recipient receiving bacteriophage therapy for a multidrug resistant pneumonia

Background: Bacteriophage therapy is an alternative approach to treat multidrug resistant bacterial infections. As non-self proteins, bacteriophages are immunogenic with the ability to elicit phage-specific antibodies that could potentially affect subsequent rounds of bacteriophage therapy. Essential to the development of antigen-specific antibodies are T follicular helper (Tfh) CD4+ T cells, specialized CD4+ T cells whose function is to provide help to B cells to facilitate B cell differentiation and development of high affinity antibodies, critical processes of acquired immunity. Here, we describe the development of phage-specific CD4+ T cells and phage-specific antibodies in a bilateral lung transplant recipient with a multidrug resistant Pseudomonas aeruginosa infection that had not responded to antibiotics.

Methods: The patient received two cycles of intravenous and inhaled bacteriophage therapy separated by 3 weeks. Multiple phage products were used; this study explores responses to two of them. Blood was collected at weekly intervals. Using the Activation Induced Marker (AIM) assay to quantify antigen-specific CD4+ T cells, we quantified phage-specific circulating Tfh (cTfh) and memory CD4+ T cells. We also quantified total phage-specific IgG and neutralizing IgG.

Results: Phage-specific cTfh developed towards the end of the 1st cycle of therapy, peaking during the 2nd cycle of therapy. Peak development of phage-specific cTfh coincided with rising phage-specific total and neutralizing IgG.

Conclusions: A humoral immune response to phage therapy developed in this patient. After 2 cycles of therapy, the patient was alive and well. Whether phage-specific cTfh and IgG impact future bacteriophage treatment efficacy remains to be determined.
Precursors of human CD4+ cytotoxic T lymphocytes identified by single-cell transcriptome analysis

CD4+ cytotoxic T lymphocytes (CD4-CTLs) have been reported to play a protective role in several viral infections. However, little is known in humans about the biology of CD4-CTL generation, their functional properties and heterogeneity, especially in relation to other well-described CD4+ memory T cell subsets. We performed single-cell RNA-seq in over 9000 cells to unravel CD4-CTL heterogeneity, transcriptional profile and clonality in humans. Single-cell differential gene expression analysis revealed a spectrum of known transcripts, including several linked to cytotoxic and co-stimulatory function that are expressed at higher levels in the TEMRA (effector memory T cells expressing CD45RA) subset, which is highly enriched for CD4-CTLs, compared to CD4+ T cells in the central and effector memory subsets (TCM, TEM). Simultaneous T cell antigen receptor (TCR) analysis in single cells and bulk subsets revealed that CD4-TEMRA cells show marked clonal expansion compared to TCM and TEM cells and that the majority of CD4-TEMRA were dengue virus (DENV)-specific in subjects with previous DENV infection. The profile of CD4-TEMRA was highly heterogeneous across subjects, with four distinct clusters identified by the single-cell analysis. Most importantly, we identified distinct clusters of CD4-CTL effector and precursor cells in the TEMRA subset; the precursor cells shared TCR clonotypes with CD4-CTL effectors and were distinguished by high expression of the interleukin-7 receptor. Our identification of a CD4-CTL precursor population may allow further investigation of how CD4-CTLs arise in humans and thus could provide insights into the mechanisms that may be utilized to generate durable and effective CD4-CTL immunity.
Poster Abstract #74

William Pandori

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Toxoplasma gondii induces IL-1β production and pyroptosis-independent release from primary human monocytes through the Syk signaling pathway

Toxoplasma gondii, an obligate intracellular eukaryotic parasite, is estimated to infect one-third of the global population and is life-threatening in developing fetuses and immunocompromised individuals. Monocytes contribute to host defense against T. gondii infection by initiating a robust inflammatory response mediated by IL-1β release. However, the mechanisms by which IL-1β is produced by and released from T. gondii-infected monocytes are not fully defined. Previously our lab has shown that T. gondii infection of primary human monocytes activates the NLRP3 inflammasome, which processes IL-1β for release. We now find that T. gondii infection induces spleen tyrosine kinase (Syk) phosphorylation in primary human monocytes, and inhibition of Syk reduces IL-1β release. The Syk inhibitor R406 decreased parasite-induced IL-1β and NLRP3 transcripts and NF-kB subunit p65 phosphorylation. In addition, inhibition of CARD9-MALT1 and NF-kB signaling reduced pro-IL-1β production in T. gondii-infected cells, indicating that Syk functions upstream of NF-kB-dependent IL-1β transcript production. IL-1β is thought to be released primarily through inflammatory cell death called pyroptosis, which is driven by Gasdermin-D cleavage. Interestingly, cell viability assays indicate that T. gondii-infected monocytes did not undergo cell death and Gasdermin-D was not cleaved. Furthermore, Gasdermin-D knockout THP-1 cells released comparable amounts of IL-1β to wild-type THP-1 cells after T. gondii infection. Taken together, our data indicate that T. gondii induces a Syk-NLRP3-caspase-1 pathway of inflammasome activation and IL-1β release in primary human monocytes, which does not involve Gasdermin-D cleavage or pyroptosis. This research expands our knowledge of how innate immune cells regulate inflammation during parasite infection.
The immune response to a novel cross-protective influenza vaccine

Despite the annual public health burden of seasonal influenza and the continuing threat of a global pandemic posed by the emergence of highly pathogenic/pandemic strains, conventional influenza vaccines do not provide universal protection, and exhibit suboptimal efficacy rates, even when they are well-matched to circulating strains. In collaboration with scientists at FluGen Inc., we have investigated the immune response to a novel M2-deficient vaccine virus, (M2SR), that induces long-lasting cross-protective immunity against multiple influenza strains, including highly-pathogenic H5 strains, in mouse and ferret models. In addition, M2SR seems less susceptible to the effects of pre-existing immunity, than the live-attenuated influenza vaccine, FluMist, in a ferret model. The vaccine induced strong systemic and mucosal antibody responses and virus-specific T cell responses in a mouse model. Antibodies to HA1, HA2, NP and M1 proteins were produced in response to M2SR. Following heterologous challenge, significant numbers of IFN-γ–producing CD8 T cells, with effector or effector/memory phenotypes and specific for conserved viral epitopes were observed in the lungs of vaccinated mice. The frequency of T cells with this phenotype has previously been correlated with protection from symptomatic influenza in humans. Cell types that are associated with asthma, such as eosinophils and basophils were present in extremely low numbers in the inflammatory infiltrate. In addition, vaccination with M2SR reduced the number of neutrophils, a cell type that has been implicated in lung damage. Thus, our results, to date, suggest that M2SR promotes strong adaptive immunity, without inducing potentially damaging inflammatory responses.
Loss of the SWI/SNF complex tumor suppressor ARID1A results in cGAS-dependent activation of interferon stimulated genes

ARID1A is a dedicated subunit of the SWI/SNF chromatin remodeling complex and has been shown to be genetically inactivated in several types of cancers, suggesting that it has a tumor suppressor role. In this study, we found that loss of ARID1A results in the induction of interferon-stimulated genes, specifically those associated with the interferon-related DNA damage transcription signature (IRDS). This is not due to the direct repression of IRDS genes by ARID1A, but rather to cytosolic recognition of double stranded DNA (dsDNA) present in micronuclei. Indeed, we show that micronuclei are markedly increased following conditional deletion of ARID1A in mouse embryonic fibroblasts (MEFs). Moreover, we detected a delay in the G2/M stage of the cell cycle in ARID1A-deficient MEFs. These micronuclei were bound by Lamin B1 and stained positive for dsDNA, γH2AX and the cytosolic DNA sensor cGAS. ISG induction was dependent on the cGAS/STING/TBK1 pathway. These data suggest that ARID1A loss results in cell cycle delay following dsDNA breaks, leading to formation of micronuclei and activation of cGAS/STING-dependent antiviral innate immune response. Finally, using genomic data from the TCGA database, we found a significant upregulation of IRDS genes in ARID1A mutant colon and gastric cancers, compared to those with intact ARID1A, highlighting the relevance of our study. Our findings offer insight into how ARID1A loss contributes to processes that activate the immune response in cancer cells, which has implications for the tumor-immune microenvironment and immune checkpoint blockade therapy of ARID1A mutant cancers.
**Poster Abstract #77**

Hideyuki Takahashi

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PI3Kγ inhibition activates T cell memory and relieves T cell exhaustion

Tumor-associated macrophages promote immunosuppressive microenvironment in head and neck squamous cell carcinoma (HNSCC). We previously reported that macrophage PI3-kinase γ (PI3Kγ) controls a critical switch between immune stimulation and suppression during inflammation and cancer. In the present study, we investigated the effect of PI3Kγ inhibition on T cell immune response, especially on T cell memory and exhaustion status using mouse models of HPV+ HNSCC.

Pik3cg−/− mice that lacks PI3Kγ exhibited suppressed growth of implanted HPV+ HNSCC tumors. The proportion of T cells, especially CD8+ T cells significantly increased in tumors from Pik3cg−/− mice. CD8+ T cells from Pik3cg−/− tumors expressed significantly more granzyme B and less T cells exhaustion markers. The proportion of CD8+ effector memory T cells significantly increased in spleens from Pik3cg−/− mice. Moreover, mice that were implanted with both tumor cells and T cells from spleens of tumor-bearing Pik3cg−/− mice exhibited significant suppression of tumor growth. All mice that had previously cleared tumors dramatically suppressed tumor growth when re-challenged with tumor cells and remained cancer-free.

Pik3cg−/− mice showed more activated T cell immune response and T cell memory than WT, resulting significant suppression of tumor growth. These results suggest that PI3Kγ-targeted therapy might enhance the activity of checkpoint inhibitors through the activation of T cell immune response in patients with HNSCC.
Poster Abstract #78

Isaac Gonzalez

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IL-18 regulation of T-cell function in the epidermis

According to the Center for Disease Control (CDC), in 2015 more than 100 million U.S adults are now living with diabetes or prediabetes. Patients who suffer from obesity and diabetes have impaired immune function consequently leading to chronic non-healing wounds. Previous studies demonstrate epidermal T-cell numbers and function get reduced during diabetes and obesity. A major role of epidermal T-cells play in the immune system is their ability to produce cytokines and chemokines that orchestrate immune response during infections. Our study focuses on the role of Interleukin 18 (IL-18) in regulating epidermal T-cell function. Epidermal T-cell lines were extracted from wild-type mice and then stimulated with IL-18 and IL-18 with anti-CD3. As a result, we saw a significant increase in T-cell production across all categories of cytokines and chemokines. Which includes T helper 1 (TH1), T helper 2 (TH2), and T helper 17 (TH17) cytokines which all play a role in fighting antimicrobial and parasitic infections. Interleukin 18 receptor (IL-18R) is seen to be upregulated during diabetes and obesity which is hypothesized to be a compensatory mechanism demanding a higher immune response. However once epidermal T-cell were stimulated with IL-18 and or with anti-CD3, IL-18R was downregulated, which is hypothesized to a mechanism set in place to prevent overstimulation in the T-cell. The role that IL-18 plays in amplifying immune responses, along with understanding the mechanisms of IL-18R expression and repression, suggest the use of IL-18 as a therapeutic to aid in antimicrobial responses.
Poster Abstract #79

Jessica Sanchez

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Adaptive Immune Cells Infiltrate the Brain and Reduce Alzheimer’s Disease Pathogenesis

The innate immune system, particularly microglia, have been implicated in Alzheimer’s disease (AD) pathogenesis, however the role of the adaptive immune system remains poorly understood. To investigate the impact of adaptive immune cells on AD pathology, our lab generated a transgenic mouse model of immune deficient AD mice (RAG2-/-5xfAD). Interestingly, deletion of adaptive immune cells leads to a dramatic increase of amyloid beta plaques and an activated neuroinflammatory state of innate microglia with a decreased ability to phagocytose amyloid beta plaques. To recapitulate the adaptive immune system, bone marrow transplants (BMT) from GFP+ wild type (WT) mice into RAG2-/-5xfAD and RAG2-/- controls were conducted. Reconstituted Rag2-/-5xfAD mice showed a decrease in amyloid beta plaques, indicating a fundamental role for the adaptive immune system in AD pathology. To further investigate the role of T cells in AD, we performed BMT and T cell adoptive transfers into aged RAG2-/-5xfAD and RAG2-/- mice. Immunohistochemistry (IHC) and flow cytometry revealed an increase in GFP+ CD8+ T cells in the RAG2-/-5xfAD brain parenchyma. T cells are seen adjacent to microglia in the RAG2-/-5xfAD brain. Therefore, we hypothesize that infiltrating CD8+ T cells are interacting with microglia leading to the decrease of amyloid beta in the AD brain. Further exploration of the crosstalk between adaptive immune cells and innate immune cells needs to be conducted to determine the mechanism in which the immune system is influencing AD pathogenesis.
The Role of Non-Neuronal Acetylcholine and Cholinergic Lymphocytes in the Murine Lung and Airways During Influenza Infection

It has long been known that numerous cell types in various tissues are responsible for the production of non-neuronal acetylcholine (ACh), eliciting a multitude of responses. Although initially discovered in mammals in the spleens of horse and oxen before the realization of its role in the central and peripheral nervous systems, non-neuronal ACh has been greatly overshadowed by its more straightforward role as the primary neurotransmitter at the neuromuscular junction.

Current research suggests that many lymphocytes, specifically CD4 and CD8 T Cells, entering the airways of influenza-infected mice actively produce ACh, potentially driving the mechanism responsible for initiating tissue repair. ACh, acting on macrophages, triggers a polarization shift from classically activated, phagocytosing macrophages (M1 macrophages) to wound healing and tissue repair macrophages (M2 macrophages) in which release of pro-inflammatory cytokines halts, and the release of anti-inflammatory cytokines begins. This study shows that acetylcholine-mediated mechanisms are critical to recovery and overall survival post-influenza infection.

Infected cohorts treated with the potent choline re-uptake inhibitor, Hemicholinium-3 (HC3), showed an impaired ability to recover weight lost during infection, as well as a significant decrease in mortality when compared to vehicle control counterparts. While it is unclear whether this effect is due to the inhibition of ACh released by infiltrating cholinergic lymphocytes or other ACh sources, this mechanism has proven paramount in determining survival after infection.
Poster Abstract #81

Juliana Navia Pelaez

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AIBP reduces TLR4 dimerization in spinal microglia, astrocytes and neurons in a mouse model of chemotherapy-induced peripheral neuropathy

TLR4 knockout protects mice from allodynia in mono and poly neuropathy models, suggesting an important role of neuraxial TLR4 in persistent pain states. The first step in TLR4 activation, receptor homodimerization, occurs in cholesterol-rich lipid raft microdomains. We have demonstrated that apoA-I binding protein (AIBP) augments microglia cholesterol efflux, reduces lipid rafts and inhibits TLR4-mediated neuroinflammation. Intrathecal (i.t.) injection of AIBP reverses cisplatin-induced mechanical allodynia in mice, a model of chemotherapy-induced peripheral neuropathy (CIPN) for which no effective treatment exists. Here, we investigated whether TLR4 dimerizes in spinal cord (SC) and dorsal root ganglia (DRG) in CINP and whether AIBP can reverse it. Mice were treated with cisplatin (2.3 mg/kg; days 1 and 3) and on day 7 i.t. injected with saline or AIBP and sacrificed 24h later. Lumbar SC and DRG were dissociated, demyelinated, and cells stained for FlowCytometry analysis. Cisplatin-induced injury resulted in robust TLR4 dimerization in SC microglia and in DRG neurons and satellite cells. Lipid raft content was increased in DRG neurons and in SC microglia in response to cisplatin, suggesting lipid raft overabundance as a factor in facilitating TLR4 activation in response to DAMPs generated by tissue damage. Importantly, i.t. AIBP reduced lipid raft content and TLR4 dimerization in SC microglia and reduced TLR4 dimerization in DRG neurons and satellite cells. These results suggest that AIBP can selectively reduce lipid rafts in activated spinal cells and inhibit TLR4 dimerization in glia and DRG neurons, modulating neuroinflammation and nociception in CIPN.
Aerosolized agricultural dust exposures elicit potent lung inflammatory responses in mice

Agricultural workers, particularly individuals working in concentrated animal feeding operations (CAFOs), are at an increased likelihood to develop respiratory diseases such as chronic obstructive pulmonary disease, chronic bronchitis, and to experience exacerbations of asthma. Current models to study this phenomenon utilize intranasal or intratracheal routes of exposure to organic dust extracts. However, whether the pathology elicited by intranasal organic dust exposure is reflective of natural inhalation exposure to airborne organic dust has yet to be determined. In this study we employed an environmental chamber, able to control mass concentration and particle size, to test the immune response elicited by aerosolized extracts of dusts collected from swine CAFOs (DE). Similar to an intranasal exposure, we identified a significant increase in immune cells recruited to the lungs following seven days of continuous exposure to DE at a particle concentration of approximately 1.5 mg/m3 that reflects reported levels identified in CAFOs. Additionally, inflammatory cytokines TNFα and myeloperoxidase, were elevated in the bronchoalveolar lavage fluid of DE-exposed mice. Interestingly, we found a significant difference in the number of neutrophils recruited to the lungs between C57Bl/6 and Balb/C strains of mice used in this study. Furthermore, the lungs of DE-exposed chamber mice exhibited the development of cellular aggregates that were distinctive from the lung pathology associated with repetitive intranasal exposure to DE. Taken together these data indicate that aerosolized exposures to DE elicit differences in lung pathology compared with intranasal delivery methods, while mouse genotype also alters neutrophil recruitment in response to DE exposure.
**Poster Abstract #83**

Rebekah Julie Park

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Exosomes as a Communication Tool between the Lymphatic System and Bladder Cancer

The lymphatic system is a network of lymphatic vessels that carry the interstitial fluid, cells, lipids and other large molecules, collectively called lymph. Lymph contains lymphocytes and, thus, the lymph system plays an important role in our immune system. Lymph node metastasis is considered to have a major prognostic value, boasting characteristics such as tumor aggression and a worse cancer survival rate. Tumor-associated lymphatics drain the interstitial fluids, containing various signal molecules, cells, large molecules, and biological wastes, from tumors to the lymph nodes. Previously, we have reported three fluorescent lymphatic reporter animals. In this study, we have optimized the imaging pipeline to further validate that these reporter models are useful tools to allow direct visualization of the lymphatic networks on the surface of the bladder for the lymphatic researchers and urologists. We showed that two transgenic mouse models, Prox1-EGFP and Prox1-tdTomato, harbor bacterial artificial chromosome where EGFP and tdTomato reporter genes were inserted respectively under the promoter of Prox1 gene, which function as the master regulator of lymphatic development. We also found that bladder cancer cells secreted exosomes, which enhance the proliferation and migration of cultured lymphatic epithelial cells (LECs). Conditioned medium from cultured T24 bladder cancer cells (American Type Culture Collection) contained tumor-secreted exosomes, which promoted migration and proliferation of LECs. Collectively, these experimental results suggest that further studies may broaden our understand on a potential regulatory mechanism of tumor-derived exosomes in lymphatic systems using our well-established fluorescent lymphatic reporter animals.
Poster Abstract #84

Mara Gilardi

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Novel Syngeneic HNSCC Animal Models to Identify New Immune-oncology Strategies

Objective: Although, the new era of immune-checkpoint inhibitors has revolutionized patient treatments, only, the 20% has an improved survival. For this reason, there is a need of pre-clinical animal models with a fully functioning immune system recapitulating the complexity of the tumor microenvironment.

Methods: The treatment of C57/BL/6 mice with a synthetic tobacco carcinogen 4-nitroquinoline 1-oxide (4NQO) allowed us to establish two syngeneic cell lines (4MOSCC) isolated from tumors in the tongue. These cell lines implanted in new mice, generated highly immune infiltrated squamous cell carcinomas. The sequencing highlights that cells carried the tobacco-associated mutational signatures of patients. Extensive characterization of their cancer-immune environment was performed by flow cytometry, Nano-string, microscopy and RNAseq.

Results: 4MOSCC exhibit typical HNSCC histology and tobacco-related mutations, and are highly immune-infiltrated, similar to ~70% of human HNSCCs. 4MOSCC1 tumors respond to anti-PD-1 (20%) and displayed a complete response to anti-CTLA-4, both in a CD8 dependent manner. 4MOSCC2 tumors, failed to respond to both immune check point inhibitors. Intriguingly, a deep analysis of the lesions, associated the different responses to a diverse immune infiltration profiles. 4MOSCC2 but not 4MOSCC1 showed metastasis in cervical lymph-nodes.

Both syngeneic cell lines are immunogenic, as vaccination with irradiated cells promoted the rejection of subsequent re-challenge.

Conclusion: We develop and exploited a new syngeneic immunocompetent animal model recapitulating tobacco-induced HNSCC in the clinic and outcomes of immunotherapy, providing an infinite powerful tool to give insights the mechanisms within the tumor-immune niche to predict responses and to identify multimodality immunotherapies.
Cancer-driven changes link T cell frequency to muscle strength in patients with cancer

Background: Tumor growth can promote both the loss of muscle strength and induce immune deficiencies in cancer patients. Previously, our lab has shown that T-cell homeostasis attenuates muscle loss in mice. However, the relationship between T cell subsets and muscle function in cancer patients is unknown. The purpose of this study is to determine whether levels of circulating T cell subsets correlate with levels of muscle strength in cancer patients.

Methods: The relative frequency of circulating naïve, memory, and regulatory T cell subsets was quantified in cancer patients and matched controls in whole blood and PBMCs. Muscle strength was measured using hand grip strength (HGS), chest press strength (CPS), knee extension strength (KES), stair climb power (SCP); while the Karnofsky and the ECOG scales were used to assess functional impairment.

Results: Our data show that i) higher frequencies of naïve and effector memory CD8+ cells and lower frequencies of central memory cells correlate significantly with stronger HGS and greater CPS, ii) lower frequency of regulatory cells correlates with greater lean mass index, and iii) lower frequency of cells that express CD95, and higher frequency of cells that co-express CD197 and CD45RA, on T and non-T cells, correlates with greater SCP and KES. Moreover, the higher the expression of CD4 mRNA the greater the functional impairment in individual patients.

Conclusions: We have identified several significant correlations between levels of T cell and non-T cell populations and muscle strength, performance, and body composition in patients with cancer, but not in matched controls.
In-situ detection of IL-6 in the pancreatic tissue sections of type 1 diabetes

Type 1 diabetes (T1D) is a chronic autoimmune disease where insulin-producing beta cells are destroyed by the immune system. Networks of inflammatory cytokines are implicated in autoimmune responses and cytokine blockade has been successful in treating autoimmune diseases. Due to scarcity of human T1D pancreas tissues, knowledge about the cytokine milieu in human T1D is very limited. IL-6 is a pleiotropic cytokine, with varying effects on immune cells and in physiology of the islet cells. The aim of this project is to characterize and quantify the expression of IL-6 in the islets of the pancreatic tissue sections of T1D, obtained from the network of pancreatic organ donors (nPOD). We have optimized a multicolor immunofluorescence imaging strategy to precisely locate and quantify the expression of IL-6 in islet cell types. We will compare the levels of IL-6 expression between non-diabetic, pre-diabetic and T1D donors to obtain a snapshot of the immunological changes that occur during disease progression. The findings from this project will answer key biological questions regarding the role of IL-6 in the pathogenesis of T1D.
Competition between type 1 interferon and IL-27 determines the expansion of memory-like CD8+ T cells in chronic viral infection

In chronic LCMV infection, T cells are persistently exposed to antigen stimulation which results in T cell exhaustion. Exhausted CD8 T cells lose effector function and express inhibitory receptors such as programmed cell death (PD)-1.

Recent studies identified a population of memory-like CD8+ T cells termed follicular cytotoxic T cells (TFC) which provide the proliferation burst and effector function following anti-PD-1/PD-L1 therapy. Understanding the cellular and molecular mechanisms that regulate TFC expansion will be crucial for improving the efficacy of immunotherapy.

Here, we demonstrate that blockade of IFN-1 signaling promotes TFC expansion in an IL-27-and STAT1-dependent manner. We explored the cellular mechanism by which IL-27 signaling promotes proliferative potential and survival of TCF1+ CD8 T cells.

Using transcriptome analysis of TFC, we identified an IL-27 target gene Irf1 and directly demonstrated its requirement for TFC expansion.

Our results identify IL-27 as a new player in TFC expansion and show a contrasting role of IL-27 and IFN type I in regulating the expansion of TFC.
Identification of SHP-2 phosphorylation sites by mass spectrometry

The co-inhibitory pathway downstream of PD-1 regulates T cell activity during immune responses. PD1 engagement by its ligands PD-L1 or PD-L2 causes the phosphorylation of two tyrosines in its cytosolic domain. SHP-2 phosphatase binds these phospho-tyrosines with its dual N-terminal SH2 domains. The recruitment and activation of SHP-2 by PD-1 results in the dephosphorylation of T cell signaling molecules and thereby reduces T cell activity. SHP-2 has two known tyrosine phosphorylation sites in its C-terminal tail, which have been suggested to regulate its catalytic activity. Based on our work with other enzymes containing dual SH2 domains (e.g. ZAP-70) and their structural similarities, we postulate that additional tyrosines in SHP-2 can become phosphorylated and regulate its activity. Here, we establish an expression system for a phosphatase-dead SHP-2 mutant (cysteine 459 → serine). The recombinant SHP-2 was phosphorylated in vitro using the tyrosine kinase Lck. Subsequent mass spectrometry analysis identified novel phosphorylation sites of SHP-2. We are characterizing these potential regulatory sites in our ongoing studies.
Poster Abstract #89

Jiaji Yu

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Isolation and characterization of NY-ESO-1-specific T cell receptors restricted on multiple major histocompatibility complex (MHC) molecules

Tumor-specific T cell receptor (TCR) gene transfer enables specific and potent immune targeting of tumor antigens. Due to the prevalence of the HLA-A2 major histocompatibility complex (MHC) class I supertype in most human populations, the majority of TCR gene therapy trials targeting public antigens have employed HLA-A2-restricted TCRs, limiting this approach to those patients expressing this allele. For these patients, TCR gene therapy trials have resulted in both tantalizing successes and lethal adverse events, underscoring the need for careful selection of antigenic targets. Broad and safe application of public antigen-targeted TCR gene therapies will require: 1) selecting public antigens that are highly tumor-specific and 2) targeting multiple epitopes derived from these antigens by obtaining an assortment of TCRs restricted by multiple common MHC alleles. The canonical cancer-testis antigen, NY-ESO-1, is not expressed in normal tissues but is aberrantly expressed across a broad array of cancer types. It has also been targeted with A2-restricted TCR gene therapy without adverse events or notable side effects. To enable the targeting of NY-ESO-1 in a broader array of HLA haplotypes, we isolated TCRs specific for NY-ESO-1 epitopes presented by four MHC molecules: HLA-A2, -B07, -B18, and -C03. Using these TCRs, we pilot an approach to extend TCR gene therapies targeting NY-ESO-1 to patient populations beyond those expressing HLA-A2. Particularly, we adapted three of these TCRs into TCR engineered adoptive T cell therapy on NSG humanized mice xenograft tumor model.
Development of a Combination Zika and Chikungunya Virus-Like Particle Vaccine

The emergence and spread of the Chikungunya virus (CHIKV), and more recently, Zika virus (ZIKV) pathogens into human populations has posed a significant health threat throughout the world. These arthropod-borne viruses circulate globally in many of the same tropical and subtropical geographical regions where the Aedes aegypti mosquito vector resides. The current unmet medical need to control the circulation and prevalence of disease due to the pathogenesis of these viruses suggest a combination vaccine may be an attractive solution that could have public health, commercial, and economic advantages. PaxVax has developed a combination vaccine based on virus-like particles (VLPs) generated in HEK293 mammalian cells by transient transfection of expression plasmids encoding for ZIKV prME or CHIKV C-E3-E2-6K-E1 structural proteins. Each of the VLPs were purified by tangential flow filtration and anion exchange column chromatography. A dose response study was conducted in C57Bl/6 x Balb/c F1 hybrid mice using a dose titration of either ZIKV VLP, CHIKV VLP, or both VLPs formulated with aluminum hydroxide adjuvant to assess the immunogenicity of the single and combination vaccines. Serum samples from VLP-immunized mice were tested for the presence of CHIKV- and ZIKV-specific neutralizing antibodies using luciferase-based neutralization assays. The combination VLP vaccine elicited high neutralization titers for ZIKV and CHIKV, comparable to antibody titers achieved with either VLP vaccine alone. These results suggest that purified ZIKV and CHIKV VLPs can be combined into one vaccine, can generate neutralizing antibodies which are known to correlate with protection from infection.
Poster Abstract #91

Yeara Jo

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Genomic Analysis Of Bone Marrow Progenitors During Viral Infection Reveals Novel Dendritic Cell Regulators

Dendritic cells (DC) play a central role in immune responses and can be broadly subdivided into conventional (c) as well as plasmacytoid (p) DCs. Notably, we and others have described several adaptations of DCs and their progenitors during acute and chronic infections, including impaired DC development, maturation and altered cytokine production. To understand the mechanisms underlying such adaptations we determined the transcriptional and chromatin landscapes of bone marrow (BM) DC progenitors from lymphocytic choriomeningitis virus (LCMV) infected mice, via RNA-Seq and ATAC-Seq, respectively. Initial analysis indicated that infection induced multiple alterations in gene signatures, including type-I-interferon signaling and metabolic pathways, as well as changes in chromatin accessibility, which were more enriched within intergenic regions. We next used Taiji algorithm, which computes transcription factor (TF) binding motifs, chromatin accessibility and gene expression to predict the activity of TFs. This analysis predicted altered activity of 11 known DC regulators and 24 TFs with no previous connection to DC biology. Follow-up knock-down experiments revealed that Glucocorticoid Modulatory Element Binding Protein 1 (Gmeb1), which was predicted to exhibit increased activity in progenitors from LCMV-infected mice, suppressed DC development and maturation. On the other hand, Zinc Finger Protein 524 (Zfp524), which activity was predicted to be reduced in progenitors from LCMV-infected mice, promoted pDC cytokine production while inhibiting the same function in cDCs. These results highlight two novel TFs that regulate DC development and/or function, significantly deepening our understanding of DC biology and providing potential new targets for DC-based immunotherapies in infectious and non-infectious diseases.
Victor Yimin Du

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Fine-tuning natural killer cells to target T-cell resistant tumors

Tumor resistance to T-cell immunotherapies, such as checkpoint inhibitors, represents a formidable barrier to cancer treatment. A well-known mechanism of resistance is impairment of MHC-I antigen presentation resulting from B2m gene disruption in tumors. While CD8+ T cells are defective in such tumors, it is expected that natural killer (NK) cells should be functional according to the “missing self-hypothesis.” However, this immune population does not seem to sufficiently exert tumor control in vivo as MHC-I deficient tumors progress in cancer patients. To better understand the above conundrum, we utilized CRISPR/Cas9 to knock-out B2M in a well-defined murine melanoma cell line called YUMMER and implanted B2M-/− YUMMER into C57BL/6 mice to induce tumor formation. While we observed more NK cells in B2M-/− tumors relative to their B2M+ controls, flow cytometry and RNA-seq analyses revealed a more “immature-like” profile of B2M-/− tumoral NK cells, characterized by reduced production of Granzyme B and TNF-a, and higher expression of the inhibitory receptor NKG2A. In addition, B2M-/− tumors expressed higher levels of CD155, ligand for the inhibitory TIGIT molecule known to play a role in T-cell and NK cell exhaustion. Using anti-TIGIT therapy led to NK-mediated delayed growth of B2M-/− tumors. Such lag in tumor growth was also seen with cytokine treatment involving a constitutively active form of IL-18, in both the YUMMER model and a patient-derived xenograft mouse model incorporating adoptive transfer of autologous human NK cells. Our studies highlight implications for cancer targeting in the absence of effective CD8+ T cells.
Chronic Viral Infection Induces Metabolic Reprogramming of Plasmacytoid Dendritic Cells

Viruses that cause chronic infections, including Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), and Hepatitis C virus (HCV), infect more than 500 million people worldwide and represent a major health burden. Chronic viral infections suppress immune responses to promote their own persistence. Plasmacytoid Dendritic Cells (pDCs) are an innate cell type which are specialized to produce exceptionally high levels of type I interferon (IFN-I) in response to viral infection, however in the context of chronic viral infection they lose the capacity to produce IFN-I. The metabolic program of pDCs has previously been implicated to be crucial for their function, but it is unclear how chronic infection modifies pDC metabolism. We demonstrate that chronic infection stimulates metabolic reprogramming in pDCs. Specifically, chronic infection is associated with sustained suppression of respiratory metabolism in pDCs. Furthermore, we demonstrate that respiratory metabolism in pDCs is highly correlated to their ability to produce cytokine, and that byproducts of respiratory metabolism (mitochondrial derived reactive oxygen species) promote cytokine production in pDCs. These data suggest that chronic infection drives changes in pDC metabolism that could be associated with infection driven pDC exhaustion.
Circulating TFH cells in ARV-treated HIV-1-infected pregnant women

The frequency and function of follicular helper T cells (TFH) are affected in HIV-1 infection compromising protective humoral immune response. In contrast, the gestational hormones appear to favor maternal antibodies production and the impact of HIV-1 infection in TFH cells in pregnant women (PW) has not been evaluated so far. Here we investigated the impact of HIV-1 infection and antiretroviral (ARV) therapy on the frequency of circulating TFH cells (CXCR5+CD4+) in pregnant women (PW), using HIV-1-infected non-PW and healthy PW as control groups. Our results demonstrated that ARV significantly diminished the percentage of circulating TFH cells, as well as PD-1+ subset, only in HIV-1-infected PW. The HIV-1 treatment also reduced the proportion of TFH cells able to produce IL-6 but elevated the frequency of IL-21+ cell subset, mainly among PW. The in vivo anti-HBsAg IgG titers after ARV therapy was significantly higher in PW and was associated with IL-21+TFH frequency and plasma levels of estrogen. As compared with healthy PW, either anti-HBsAg IgG titers or percentage of IL-21-secreting TFH cells were lower in HIV-1-infected PW. However, anti-gp41 antibodies levels were not different in the plasma of HIV-1-infected PW and nPW after ARV therapy. Finally, the ENV antigen enhanced, in vitro, the percentage of TFH cells unable to produce IL-21 in PW. In summary, our results suggest that the introduction of ARV therapy is more efficient in recovering total IL-21-secreting TFH cells but not HIV-1-specific TFH cells in pregnant women.
Deciphering liver environmental signaling pathways for Kupffer cell identity

Functional specialization of tissue resident macrophages occurs through environmental signals controlling activity and/or expression of transcription factors. Kupffer cells are resident macrophages in the hepatic sinusoids and have critical roles in the innate immune response and iron metabolism. During times of hepatic stress, Kupffer cells are primarily self-maintained with some support from circulating monocytes. While the identity of Kupffer cells is maintained by the transcription factors LXRα (encoded by Nr1h3) and Spic, the mechanisms governing their activity remain unknown. Here, we predicted a communication network between interacting liver sinusoidal endothelial cells (LSEC) and Kupffer cells enables maintenance of Kupffer cell identity and promotes differentiation of circulating monocytes into functional Kupffer cells. We created a mouse expressing Clec4f driven Cre with improved specificity to Kupffer cells to establish a DTR mediated Kupffer cell depletion/repopulation model. Using the transcriptomes from LSECs and the transcriptomes and enhancer landscapes of Kupffer cells and repopulating macrophages, we hypothesized that TGF-β-Smad, Notch-Rbpj, and TLR4-NFκB signaling pathways coordinate the transcriptional identity of Kupffer cells. Restricted deletion of Smad4 in Kupffer cells revealed a requirement for this axis in regulating expression of Nr1h3 and LXRα target genes. Finally, we induced Kupffer cell-specific genes in bone marrow myeloid progenitors as well as circulating monocytes using predicted candidate molecules and found synergistic induction of Nr1h3, Spic, as well as other Kupffer cell specific genes. Our studies identified for the first time liver environmental signals for Kupffer cell identity through enhancer analysis.
Poster Abstract #96

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From vaccines to oncolytic viruses: multimeric soluble forms of CD40L and other TNFSF ligands

While blocking TNF SuperFamily (TNFSF) ligands like TNF forms the basis for important therapeutic drugs, progress has lagged on using these immunologically powerful proteins as positive agonists. The field was initially held back by the erroneous belief that TNFSF ligands (which can be cleaved from cell membranes as intact single trimers) act by simply trimerizing their receptors on responding cells. Instead, with few exceptions, their cognate receptors need to be clustered in the plasma membrane in order to induce signaling. Likewise, agonistic antibodies generally must bind to FcR on adjacent cells in order to cluster and thereby activate receptors for CD40L, GITRL, 4-1BBL, OX40L, TRAIL, FasL, and the TNF receptor on Tregs (TNFR2).

To solve this roadblock, we constructed fusion proteins between ACRP30 or surfactant protein-D (SPD) (which serve as self-assembling scaffold proteins) to arrive at 2- and 4-trimer soluble forms of TNFSF ligands. These fusion proteins have activity as vaccine adjuvants (SPD-CD40L, -GITRL, -CD27/CD70, -4-1BBL), anti-tumor agents (SPD-CD40L, -GITRL), growth factors for B cells ex vivo (SPD-CD40L), and most recently as part of oncolytic viruses for cancer (SPD-CD40L, -4-1BBL, -OX40L). Thus, multimeric soluble TNFSF ligands have now arrived as uniquely useful immunological agents.
c-Kit-dependent tissue resident macrophage progenitors drive cancer progression

Macrophages play a key role in promoting tumor growth and resistance to therapy. Here we show that Tissue Resident Macrophages (TRM) as well as Bone Marrow-Derived Macrophages (BMDM) play critical but unique roles in promoting tumor growth. TRM were recently shown to originate in the yolk sac or fetal liver during embryogenesis; these cells self-maintain in postnatal tissues independent of hematopoietic stem cells. We found that BMDM are CD11b+Gr1+F4/80loCX3CR1loCCR2+ and are recruited to tumors in a CCR2-dependent manner. In contrast, CD11b+Gr1−F4/80hiCX3CR1hiCCR2− TRMs accumulate in tumors independently of the trafficking receptor CCR2. Gene expression and functional studies indicate that tumor-derived TRM are highly proliferative, immune suppressive and distinct from BMDM. We show that TRM develop from c-Kit/c-KitL− dependent TRM progenitors that are abundant in tumors but not in normal tissues; purified progenitors form macrophages and potently stimulate tumor growth when adoptively transferred into mice. Tumor cells induce the expansion of TRM progenitors by secreting Stem Cell Factor (SCF/c-KitL). Notably, in vitro and in vivo proliferation of TRM progenitors and tumor growth are significantly inhibited by SCF and c-Kit inhibitors, including a novel, allosteric dual inhibitor of cKit and CDK8/19 that dramatically suppresses tumor growth by targeting both TRM and tumor cells. As cKit inhibitors synergize with other immune therapy regimens to suppress tumor growth, our studies identify cKit as a valuable target for immune therapy of solid tumors.
NFkB-RelB is known as the effector of the NFkB non-canonical signaling pathway critical for lymph node organogenesis and B-cell survival. However, RelB-deficient mice, and the newly identified human patients (due to variant RelB-Y397 stop codon) suffer not only from immune deficiency but debilitating inflammation, indicating another major function of RelB that remains unknown. To characterize this function, we took an unbiased approach by transcriptomic profiling of various immune and tissue cell types. Remarkably, we found dramatic hyper-expression not of NFB target genes, as previously suggested, but of type I interferon stimulated genes (ISGs) in both human and mouse fibroblasts and dendritic cells, whose wildtype counterparts are known to express high amounts of RelB. We hypothesized, that elevated interferon production and interferon-induced chemokine expression, such as the T-cell chemoattractant Ccl5, may underlie the massive lymphocyte infiltration of key organs and the reported T-cell dependency of the inflammatory pathology in the mouse. We tested this hypothesis by blocking type I IFN signaling pharmacologically and genetically in the mouse and observed a complete reversion of the phenotype. Interestingly, while compound IFNAR-deficiency rescue the mouse lethality, IFN expression remained elevated, indicating that RelB functions as a gatekeeper of type I interferon. This function does not involve non-canonical signaling. Ongoing studies address the underlying molecular mechanism, new therapies for the patients would be just on the horizon.
Lyve1-derived hematopoietic stem cells are intrinsically different in their lineage output than other HSCs

While iPSCs offer the potential for an unlimited source of patient HSCs, the generation of transplantable HSCs from iPSCs has yet to be achieved. This suggests that a better understanding of how HSCs arise naturally in the course of embryonic development is needed. The first primitive waves of hematopoiesis arise from the embryonic yolk sac (YS), but definitive waves arise later and produce the first HSCs. Which tissues produce definitive hematopoiesis, and thereby HSCs, remains unclear. Using a Lyve1-Cre lineage tracing reporter, we previously published that Lyve1-Cre marks definitive hematopoietic cells that arise primarily in the YS, and show that about one-third of adult HSCs are derived from Lyve1-expressing precursors. Our central hypothesis is that Lyve1-derived cells are a significant source of adult HSCs. We have found that Lyve1-derived cells contribute to each definitive wave of embryonic hematopoiesis. Lyve1-derived adult HSCs contribute to cells in all of the major blood lineages. Interestingly, there seems to be a bias within the lymphoid branch. This bias, which is frequently maintained upon transplantation, does not appear during the developmental stages of these cells implying that these Lyve1-derived lymphocytes respond differently to peripheral signals. Taken together, this data suggests that Lyve1-derived cells give rise to engraftable, multipotent, and long term self-renewing HSCs that have functional properties distinct from HSCs that arise from other tissues. We interpret these results to support the notion that the extra-embryonic YS is an HSC niche in the embryo and contributes to adult hematopoiesis.
Metabolomics analysis of chronic versus fatal infection with LCMV

DBA/1J and DBA/2J are high related (<6% at SNP level) inbred strains of mice that, despite their genetic similarity, display dramatically different outcomes after LCMV Clone 13 (LCMV-13) infection. DBA/1 mice show extreme susceptibility to hemorrhagic fever-like signs with abnormal platelet function, immune-mediated damage and 100% fatality at day 6-7 post-infection, while DBA/2 mice survive and develop a chronic infection characterized by T-cell exhaustion. We used a liquid chromatography-mass spectrometry-based untargeted metabolomics approach to investigate the different phenotypes of these viral infections. Plasma metabolites were extracted from samples collected from DBA/1 and DBA/2 mice at days 0 and 5 after infection with LCMV-13. Data was analyzed using the program XCMS and features that were significantly different between the sample sets were searched against the METLIN database. Initial analysis has identified a variety of distinct compounds that differed significantly between DBA/1 and DBA/2 mice. Most notably was 3-Indolepropionic acid (IPA) which declined from Day 0 to Day 5 in both groups but was 15.6-fold lower in the DBA/1 (lethal infection) than in the DBA/2 group (chronic infection) at Day 5. IPA is a metabolite that is produced by the gut microflora and has been shown to protect against oxidative stress. Preliminary data from in vitro T-cell proliferation assays show that increased IPA may suppress antigen-specific T-cell proliferation during LCMV-13 infection. Our results suggest that the microbiome may play an important role in the host response to viral infection via production of indole metabolites such as IPA that influence T-cell function.
Deubiquitinase CYLD controls the plasticity of Treg cells by regulation of Scinderin expression

Regulatory T (Treg) cells are indispensable for the maintenance of immune tolerance and the prevention of autoimmunity, however, how Treg cells retain their immune suppressive function remains unclear. Here, we report that deubiquitinase CYLD plays a critical role in the maintenance of Treg cells. Conditional Foxp3-specific CYLD knockout mice showed severe pulmonary inflammation due to preferential migration of Treg cells into the lung and increased IL4 production compared to control mice, which was reversed by the deletion of IL-4. Interestingly, Foxp3 expression by CYLD-deficient Treg cells was not stable in presence of WT Treg cells under non-inflammatory conditions. The instability of Foxp3 was dependent on the autocrine expression of IL-4 by CYLD-deficient Treg cells, suggesting the autocrine production of IL-4 regulates the competitive fitness of Foxp3+ Treg cells. Furthermore, genome-wide microarray analysis unveiled that Scinderin, a member of the actin-binding gelsolin family, was highly upregulated by the NF-B signaling pathway in CYLD-deficient Treg cells and was essential for mounting a T helper type 2 (Th2) immune response through enhanced MAPK signaling pathways. Scinderin was able to bind to MEKs, and further accelerate MAPK signaling cascades by promoting MEK/MAPK interaction. Both MAPK activation and IL-4 production from CYLD-deficient Treg cells were abrogated by Scinderin knockdown or knockout, suggesting that Scinderin is responsible for IL-4 induction by regulation of the MAPK pathway. Our findings indicate that CYLD is essential to maintaining Treg function by preventing the conversion of Treg cells into Th2-like effector cells through regulating Scinderin expression.
Poster Abstract #102

Gajender Aleti

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Fusobacterium nucleatum induced inflammation

The next frontier in the study of microbial-immune relationships is to integrate the molecular information within the dynamic states of the habitat. Microbial dysbiosis is influenced by dominant pathobionts and their secreted molecules, which induce disease. Fusobacterium nucleatum (Fn), has been highly associated with chronic inflammation and cancer. Despite the clinical relevance of Fn, the molecular mechanism of its secretory proteins (designated here as FusoSecretome) in controlling microbial ecology and in modulating innate immune response is not well understood. Fn is a heterogeneous species with four recognized subspecies (animalis, nucleatum, polymorphum and vincentii), whose strain specific differences in virulence is also mostly unknown. Our hypothesis is Fn dominant phenotype expresses a specific secretome signature to subvert the innate immune functions.

We computationally identified several candidate virulence proteins based on the presence of host molecular mimicry elements (sequence or structural resemblance of molecules of the host and the microbe), and protein interactions between the secretome of Fn and the human proteins. Preliminary results from our integrative multi-omics approaches validate the expression of some of the novel virulence genes employed in Fn-immune cell cross talk in oral and systemic diseases. Elucidation of these novel virulence proteins will help us to further dissect the composition of Fn secretome in the context of the microbiome and the innate immune cells. This study will contribute to the development of better disease biomarkers and new therapeutics for suppression of systemic diseases.
Poster Abstract #103

Gregory Fonseca

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Using an attention neural network to define the combinations of transcription factors employed by macrophages in a panel of 12 cytokine responses

Macrophages are effectors of the innate and adaptive immune system, in part, through sensing host and non-host extracellular signals. This results in signaling cascades that terminate on transcription factors (TF) binding to the DNA. TFs collaborate to modify the cellular transcriptional profile to appropriately respond to a specific signal. DNA binding of TFs can be inferred by the presence of their DNA binding motif at sites of open chromatin. Our previous work using logistic regression machine learning suggests that several dozen TFs work together to activate the cells enhancer landscape with some combination of 3-4 acting at each specific enhancer. As such, each signal can result in thousands of possible TF combinations. Here, we present an attention neural network to define the combinations of TFs at enhancers in an panel of 12 macrophage modifying cytokines on C57Bl/6 bone marrow derived macrophages. We defined differences in open chromatin using ATAC-seq and enhancer activation using ChIP-seq of H3K27 acetylation. We have found that TF motifs tend to appear in defined groups, suggesting a predictable genetic structure in use by each specific signal. Using this data, we can begin to make predictions of the cytokines present in vivo during disease. Further, knowing the distinct TF combinations employed in response to cytokines, we can devise signal specific artificial TF response elements which may be used to modify cell responses.
Using Math to Resist Microbe-driven Colorectal Cancer

Initiation and progression of colorectal cancer (CRC) is fueled by genetic, epigenetic and environmental factors, e.g., inflammation triggered by cancer-associated microbes. The anaerobic bacteria Fusobacterium nucleatum (Fn) has emerged as one such microbe. Using mathematics (Boolean network analysis) and an organoid-based model system, here we show that Fn induces changes in gene expression, i.e., de-differentiation, epithelial-mesenchymal transition (EMT), loosening of cell-cell contacts and a unique pro-inflammatory program that are invariably seen during adenoma initiation and progression in the colon. An asymmetric analysis of normal and adenoma-derived transcriptomes revealed invariant Boolean relationships between AMPKα2 (PRKAA2), the proinflammatory cytokine (IL-8), the leaky tight-junction (TJ) protein Claudin-2, markers of differentiation [CHGA, CA1, MS4A12] and EMT/stemness [CEMIP, LGR5]. Infecting human and mouse EDMs (WT and APC min/+) with Fn led to collapse of the epithelial barrier and recapitulated the gene expression changes. Mechanistically, one of the earliest events is reduction in the metabolic master-regulator, AMPK and consequently, suppression of the tumor-suppressive stress-polarity signaling (SPS)-pathway it orchestrates. The SPS-pathway, as determined by the extent of AMPK-dependent phosphorylation of the polarity scaffold GIVis enhanced in patients on Metformin and silenced during adenoma-to-carcinoma progression in humans. Pharmacologic activation of the SPS-pathway with either Metformin or an AMPK-specific activator fortifies epithelial TJs and resists the proinflammatory, EMT and de-differentiation programs that are induced by Fn. Findings reveal that loss of epithelial polarity is an early event in CRC initiation and that activation of the SPS-pathway with AMPK agonists is a promising strategy for cancer chemoprevention.
A Boolean Network of Inflammatory Bowel Disease Reveals a Novel Barrier-Protective Therapeutic Target

Our gut barrier separates trillions of luminal microbes from our immune system. Although a leaky gut barrier has been implicated in the initiation and progression of inflammatory bowel disease (IBD), barrier-protective therapies are yet to emerge. We carried out asymmetric analysis of transcriptomic datasets using Boolean logic and built a network of invariant relationships between genes and chart disease path(s) from healthy to disease. These paths unexpectedly revealed disruption of epithelial tight junctions (TJs) as an initiating event and predicted PRKAB1 (b1 subunit of AMP-kinase) as one of the major targets with a known mechanism of action, favorable expression pharmacology, and modulatable by potent agonists whose activation may reset the network from a diseased to healthy state.

Using organoid-derived monolayers from murine and human colon we show that selective pharmacologic activation of PRKAB1 protects TJs against stress-induced collapse when exposed to stressors such as IBD-associated invasive microbes and ameliorates DSS-induced colitis in mice. We found that PRKAB1 is indeed suppressed in the epithelium of patients with IBD. To our surprise, the TJs were disrupted in diseased organoid-derived monolayers, indicating an intrinsic defect in the IBD-epithelium; activation of PRKAB1 could repair defective TJs in organoid-monolayers generated from IBD-afflicted patients. These findings provide proof-of-concept for a novel network-based drug discovery approach in IBD that were overlooked using traditional approaches.
Poster Abstract #106

Ricardo da Silva Antunes

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Urinary Peptides As a Novel Source of T Cell Allergen Epitopes.

Mouse allergy is associated with allergic rhinitis and asthma, posing a serious public health concern. Urine is a major source of mouse allergens, as mice spray urine onto their surroundings, where the proteins dry up and become airborne. Here, we tested whether mouse urine and in particular oligopeptides that are abundant in mouse urine may contribute to mouse allergic T cell response. Over 1,300 distinct oligopeptides were detected by mass spectrometry analysis of the urine low molecular weight fraction. A pool of 225 unique oligopeptides was tested for its capacity to elicit T cell reactivity in mouse allergic donors. Reactivity was detected in about 95% of donors and predominantly associated with IL-5. Peptides from non-urine related proteins such as epidermal growth factor or collagen, accounted for the highest response. Peptides derived from major urinary proteins were the main T cell targets from kidney or urine related sources. Further analysis of enrichment of 4-1BB expressing cells demonstrated that peptide pool-specific reactivity can be detected directly ex vivo in mouse allergic but not in non-allergic donors and revealed a bone fide memory T cell phenotype that confirmed a Th2 polarization. Finally, we found that not all the low molecular weight reactivity is accounted by the identified peptides and that gamma delta T cells are also involved in the responsiveness to components of mouse urine. Overall, these data suggest that mouse urine-derived oligopeptides are a novel target for mouse allergy-associated T cell responses, which may contribute to immunopathological mechanisms in mouse allergy.
PSGL-1 signaling intrinsically alters differentiation and glycolysis of T lymphocytes following TCR activation

P-selectin glycoprotein ligand-1 (PSGL-1) is an adhesion molecule expressed on the surface of CD4+ and CD8+ T cells and was recently identified as an immune checkpoint inhibitor. Our studies aim to better understand the role of PSGL-1 signaling in T cell responses. Under in vitro helper T cell (TH)-subset inducing conditions, PSGL-1+/- OT-II CD4+ T cells demonstrated greater development of IL-17A+ T cells in TH17-skewing conditions, with reduced IFNγ+ cells under both TH0 and TH1 conditions. In a B16-OVA tumor model, we observed increased IL-13 producing intratumoral TH2 CD4+ T cells in PSGL-1/-/- mice, demonstrating inherent PSGL-1-dependent regulation of TH-subset differentiation in vitro and in vivo. In addition, in vitro activation of PSGL-1/-/- OT-II CD4+ T cells by TCR ligation with a sub-optimal dose of anti-CD3 resulted in increased cell recovery and T cell activation compared to wild-type OT-II CD4+ T cells by 72 hr, indicating that PSGL-1-deficiency leads to greater sensitivity to activation. In support of this, we recovered more PSGL-1/-/- T cells than wild-type after adoptive transfer of in vitro-activated CD4+ T cells into RAG/-/- host mice. In assessing metabolic changes, we identified that both PSGL-1/-/- CD4+ and CD8+ T cells display increased glycolysis after 72 hours of in vitro activation compared to wild-type T cells. Moreover, PSGL-1/-/- CD8+ T cells have increased glycolysis with both sub-optimal and optimal levels of anti-CD3 stimulation. Taken together, these data show that PSGL-1 signaling has a fundamental cell-intrinsic and immediate role in regulating the development of T cell effector responses.
Therapeutic blockade of CD3-mediated signaling ameliorates re-activated memory Th2-driven allergic airway inflammation

Allergic asthma is an inflammatory disease of the lungs characterized by Type 2 helper T cell (Th2)-driven immune responses to inhaled allergens. Memory Th2 cells are a major driving force behind asthma, secreting cytokines such as IL-4, IL-5, and IL-13 and persisting long after allergen encounter. The selective removal of pre-existing memory Th2 cells could be a key process resulting in enhanced tolerance. One such method could involve targeting CD3 through the use of blocking monoclonal anti-CD3 antibodies, which have been shown to successfully induce tolerance in animal models of autoimmune diseases and have shown promise in clinical trials. We tested blocking CD3 complex signaling in a HDM model of allergic inflammation to determine if we could induce airway tolerance. Importantly, pre-sensitized C57BL/6J mice displayed strongly ablated lung inflammation and Th2 responses when treated at the time of memory T cell reactivation with F(ab’)2 anti-CD3 antibody. This treatment was found to specifically inhibit HDM-specific CD4+ T cell proliferation and Th2 cytokine production. Interestingly, mice treated with anti-CD3 also showed an increased frequency of Treg cells, improving the Treg:Th2 cell ratio in favor of airway tolerance. Additionally, a significant reduction in long-lasting memory Th2-driven airway inflammation was observed even when therapeutic anti-CD3 antibody was given several weeks later during a tertiary challenge with allergen. These data suggest that blocking signaling mediated by the CD3 complex could be employed to inhibit memory recall responses to complex allergens thus ameliorating the development of airway inflammation.
Regnase-1 controls development and peripheral differentiation of B cells

Regnase-1 is an emerging regulator of immune responses with established roles in post-transcriptional control of inflammatory cytokines and T-cell response. Interestingly, Regnase-1 mRNA is highly expressed in follicular B-cells but is downregulated in germinal center B cells, indicating its potential regulatory role in B cell-mediated immune responses. However, the role of Regnase-1 in B cells has not been studied. We tested B cell-specific role of Regnase-1 using conditional knockout mice bearing a floxed Regnase-1 gene with Cd79a-dependent Cre. Upon ablation of Regnase-1 at the pro-B cell stage, these mice, at 8 weeks, developed striking immunopathogenic features including lymphoid hypertrophy, disrupted B cell follicular architecture, immune cell infiltration in the liver, 10 times elevated serum antibody levels, compared to the control mice. Moreover, the frequency of Cd11c and T-bet expressing B cell population, previously described as ABCs was 6-fold higher. Furthermore, to assess the role of Regnase-1 in peripheral differentiation of B-cells, we utilized an inducible deletion mouse model and studied T cell dependent B cell response. Acute deletion of Regnase-1 in mature naïve B-cells led to a heightened germinal center response with an increased frequency of centroblasts and elevated serum immunoglobulin levels. Whole transcriptome analysis of Regnase-1 deficient B cells revealed altered molecular pathways, such as, IL-1, TNF-R2, IL-18, IL-33, and NFkB signaling pathways and several potentially novel regulators of B cell response. In summary, our findings show an essential role of Regnase-1 in B-cells and provide key insights towards understanding novel pathways of post-transcriptional regulation of B cell-mediated immunity.
**Poster Abstract #110**

Erin Armentrout

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T cell-specific deletion of Blimp1 (Prdm1) leads to altered intestinal microbiota and IFNγ and IL17-independent inflammation

Blimp-1 is a zinc finger-containing transcription factor expressed in several cell lineages, including B and T lymphocytes. Genome Wide Association Studies (GWAS) have linked polymorphisms in the gene encoding Blimp-1 (PRDM1) to several chronic inflammatory conditions, including Inflammatory Bowel Disease (IBD). T cell-specific deletion of Blimp-1 in mice (Blimp-1CKO mice) results in spontaneous development of severe colitis associated with the accumulation of IL17 and in some instances, IFNγ-producing CD4+T cells. However, the mechanisms underlying the development of chronic inflammation in Blimp-1CKO mice are not fully understood. Here we show that genetic deletion of IFNγ or IL17A in Blimp-1CKO mice does not rescue their inflammatory phenotype. Moreover, neutralization of IL17A and IL17F in IFNγ/Blimp-1 double knockout mice also failed to prevent intestinal inflammation, indicating that Th1 and Th17 may not be the prominent effectors of inflammation in this model. Analysis of the intestinal microbiota (16S and ITS sequencing) revealed decreased bacterial (but not fungal) diversity in Blimp-1CKO mice as early as 3 weeks of age, before symptoms of colitis were detectable. This decrease suggests that changes in the microbiota in these mice are not secondary to development of intestinal inflammation. Current experiments in the lab focus on determining if Blimp-1CKO mice intestinal microbiota could promote intestinal inflammation when transplanted in germ-free mice. We anticipate that elucidating the mechanisms underlying development of IBD in the Blimp-1CKO mice may reveal novel molecular pathways regulating T cell-mediated chronic inflammatory diseases.
Poster Abstract #111

Gislaine Martins

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Dynamics of Blimp-1 expression in vivo as revealed by a novel knock in reporter mice

The transcription factor Blimp-1 (B-lymphocyte-induced maturation protein-1/PRDI-BFI/Prdm1), plays crucial roles in the terminal differentiation of several different cell lineages and regulates functional aspects of hematopoietic cells, including T lymphocytes. Blimp’s direct target genes in effector (Teff) and regulatory (Treg) T cells include functional molecules such as the cytokines IL17 and IL10. Mice with T cell-specific deletion of Blimp-1 spontaneously develop chronic intestinal inflammation that resembles human IBD. Despite Blimp-1’s abundant expression in the immune system, the mechanisms regulating its expression in vivo are not understood. Studies of Blimp-1 expression in vivo have been hindered by the limited availability of reagents. Here, we describe novel Blimp1-reporter [tandem dimer (td) Tomato-Prdm1 knock-in mouse, Blimp1tdTomato] mice, in which an internal ribosomal entry sequence (IRES) and tdTomato were “knocked-in” in the Prdm1 gene 3’UTR. This resulted in expression of tdTomato under control of endogenous Prdm1 regulatory regions without disruption of the endogenous gene, thus faithfully reporting Blimp-1 expression in both lymphoid and non-lymphoid tissues. Flow cytometry and two-photon microscopy analysis of dual reporter Blimp1tdTomato/Foxp3GFP mice revealed previously unappreciated differential expression of Blimp-1 in Teff and Treg cells at different sites, including environmental surfaces, in which Blimp1 expression was at least partially associated with the presence of an intact microbiota. In addition, we found that Blimp-1 expression in Teff and Treg cells is differentially regulated by TGF in vivo. Further evaluation of the mechanisms regulating Blimp1 expression in T cells in different tissues is currently underway and will be discussed at the meeting.
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Robust Immunological Tolerance Induced by CD22 Targeted Antigenic liposomes and PLGA Rapamycin Nanoparticles Delays the onset of Autoimmune Disease in K/BxN arthritis model

Current treatments for unwanted antibody responses are limited and mostly rely on broadly immunosuppressive drugs compromising overall immunity. Therefore, induction of antigen specific tolerance is highly desirable in these scenarios. Ongoing immune responses require B and T cell tolerance, so nanoparticles that can achieve both are of interest. Earlier we have shown that co-presentation of an antigen with glycan ligands of the inhibitory co-receptor CD22, a sialic acid binding immunoglobulin-like lectin (Siglec), on Siglec-engaging tolerance-inducing antigenic liposomal nanoparticles (STALs) leads to robust antigen specific B cell tolerance to protein antigens in naïve mice through inhibition of B cell activation. In addition, other groups have shown that administration of free antigen with PLGA rapamycin nanoparticles results in robust antigen specific T cell tolerance in naïve mice through increased production of regulatory T cells. However, neither nanoparticle alone can induce both robust B cell and T cell tolerance in naïve or sensitized animals. Here we illustrate that co-administration of STALs with PLGA-R NPs induced tolerance that sustained multiple challenges as compared to administration of either nanoparticle alone. Further, co-delivery of GPI-LP-CD22L and PLGA-R NPs delayed onset of disease for 11 weeks in the K/BxN arthritis model. Together these data show synergy between B cell-tolerizing STALs and T cell-tolerizing PLGA-R NPs and establish a NP platform to induce antigen specific tolerance in disease models.
Poster Abstract #113

Laura Jimenez

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Evaluating LKB1 as a Driver of B cell Activation and Anti-Tumor Responses

Immune defense requires coordination of innate and adaptive immune system, including B cells that generate antibodies against pathogens or tumor antigens. Antigen triggered B cell activation and differentiation into high affinity antibody secreting plasma cells can result from immune synapse formation between B cells and antigen presenting cells (APCs). B cells use PAR polarity proteins to establish immune synapses with APCs and coordinate antigen uptake and processing. We identified LKB1, a member of the PAR protein family, is required for B cell activation and differentiation into plasma cells but its role downstream of antigen stimulation, immune synapse formation and anti-tumor immunity is unknown. My studies reveal activated B cells relocalize LKB1 to sites of immune synapse formation where LKB1 may function to promote assembly of polarity complexes required for synapse formation. Interestingly loss of LKB1 activity promotes mislocalization of polarity complexes, spontaneous B cell activation, formation of giant germinal centers and secretion of proinflammatory cytokines and chemokines. Similar inflammatory profiles are known to recruit cytotoxic T lymphocytes into solid tumors. These studies strongly suggest LKB1 inactivation regulates immune synapse formation to promote B cell activation, germinal center formation and potentially infiltration of T cells in the tumor microenvironment. We are currently utilizing syngeneic tumor models to examine the role of B cell specific LKB1 in promoting anti-tumor immunity. Together, these studies will assess LKB1 as a therapeutic target for modulating B cell responses in the context of cancer.
CRTC2 Inactivation is Required for Antibody Secreting Cell Survival During a Humoral Immune Response

Antibody secreting cells (ASCs) are the effector population of the B cell lineage and are required for humoral immunity. Transition from an activated B cell to an ASC requires complete transcriptional reorganization of gene programs. Previously, we showed in vitro with isolated human tonsil B cells that CRTC2, a transcriptional co-activator of CREB, regulated this transitional process by controlling germinal center (GC) exit and ASC differentiation. Specifically, the expression of a constitutively active and nucleus-localized form of CRTC2 (CRTC2-AA) in stimulated naïve human B cells, maintained expression of GC genes and inhibited the production of immunoglobulins. To study the in vivo role of CRTC2 in B cell development and immune responses, we generated a lymphocyte specific CRTC2-AA transgenic (TG) mouse model. In this model, we show that although CRTC2 is constitutively active at all stages of B cell development, early B and T lymphocytes develop normally. However, differentiation of mature B cells into ASCs is defective, resulting in reduced ASC numbers and antibody titers. In vitro differentiation of CFSE labeled naïve B cells into ASCs revealed that the defect in differentiation was not due to a developmental block, but due to an increase in cell death in mature TG ASCs. To identify differentially expressed genes during the course of differentiation, we sorted CFSE labeled cells by FACS into an early-division, late-division, and ASC population and performed RNA-Sequencing. Coupled with CRTC2 ChIP-Seq data-sets, we plan to identify important CRTC2 regulated genes that are critical for survival during ASC differentiation and maturation.
Defining a CRTC2 inactivation gene expression program regulating antibody secreting cell fate.

Effective humoral immunity against pathogens requires the production of high affinity antibodies. Immunoglobulin (Ig) production is regulated by the development of antibody secreting cells (ASCs), generated through intricate transcriptional regulation following B cell activation. We previously detailed an ATM-LKB1 signaling axis following DNA damage during Ig remodeling, responsible for inhibition of germinal center B cell (GC B) proliferation and commitment to ASC formation. Activation of ATM-LKB1 signaling results in downstream inactivation of CRTC2, a CREB transcriptional co-activator. Dysregulation of CRTC2 inactivation resulted in maintenance of key GC B associated genes and repression of ASC formation in primary human tonsillar B cells. However, little is known of the physiologic consequence of CRTC2 inactivation in ASC development. Using a murine model of T cell-dependent B cell activation, we performed a coupled bioinformatics approach to identify transcriptional modules controlled by CRTC2. Integrated ChIP- and RNA-sequencing of naïve and stimulated GC B cells reveal a CRTC2 inactivation program resulting in decreased transcription of BCR and TLR4 signaling, and unanticipated upregulation of endoplasmic reticulum stress response and proteasome genes, consistent with GC B exit and commitment to ASC differentiation. Epigenomic and chromatin accessibility mining of publicly available datasets identifies coordinate binding of CRTC2 targets with key GC B regulators ETS1 and PU.1 at sites of active Pol II and H3K4me3 transcriptional activity. Together, these data provide investigatory insight into the role of CRTC2 inactivation in regulating transcriptional transition from GC B self-renewal to ASC production.