The 59th Midwinter Conference of Immunologists at Asilomar

January 25 -28, 2020

Asilomar Conference Grounds, Pacific Grove, California

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Chairpersons

The Dan H. Campbell Memorial Lecture

Saturday, January 25, 8:00 PM

The Chapel Auditorium

Lora Hooper

UT Southwestern Medical Center

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The 2020 Midwinter Conference of Immunologists

gratefully acknowledges the following contributors:

The American Association of Immunologists
Amgen
BioLegend, Inc.
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Bristol -Myers Squibb
Burroughs Welcome Fund
Cellular Immunology (Elsevier)
Genentech, Inc.
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The 2020 Midwinter Conference of Immunologists at Asilomar
Pacific Grove, California (USA) www.midwconfimmunol.org

The 60th Midwinter Conference of Immunologists will be held January 23-26, 2021

CONFERENCE SCHEDULE

All Sessions: The Chapel Auditorium

Saturday, January 25th		
4:00 pm	**Registration**	
8:00 pm	***The Dan H. Campbell Memorial Lecture***	
9:00–11:00 pm	***Reception in the Nautilus Room***	
Sunday, January 26th		
8:30–12:00 Noon	**Session I**	**Immunity at the Host-Pathogen Interface**
4:00– 6:00 pm	**Poster Session**	***Fred Farr Forum and Kiln Room***
7:30–10:00 pm	**Session II**	**Tissue Immunity and B Cells**
10:00–11:00 pm	***Reception***	***Fred Farr Forum and Kiln Room***
Monday, January 27th		
8:30 – 12:00 Noon	**Session III**	**Innate Immunity: Activation & Effector Functions**
4:00 – 6:00 PM	**Oral Presentations**	***The Chapel Auditorium***
7:30 – 10:00 PM	**Session IV**	**Evolution & Intelligent Design in Immunity**
10:00–11:00 PM	***Reception***	***Fred Farr Forum and Kiln Room***
Tuesday, January 28th		
8:30–12:00 Noon	**Session V**	**Lymphocyte Activation, Residence, Function**
Saturday through Monday	**Posters on Display**	***Fred Farr Forum and Kiln Room***

CONFERENCE PROGRAM

SESSION I

Sunday Morning
8:30 AM to Noon

Immunity at the Host-Pathogen Interface
Chairperson: Katrin Mayer-Barber

Speakers:

Jörn Coers, Duke University
"Recognition of Cytosolic Bacteria by Guanylate Binding Proteins"

Christopher Hunter, University of Pennsylvania *(for Sarah Stanley)*

Katrin Mayer-Barber, National Institutes of Health
"An Unanticipated Role for Eosinophils in Host Resistance against *Mycobacterium Tuberculosis"*

Elina Zuniga, University of California, San Diego
"Adaptations During Long-Term (Host-Pathogen) Relationships"

Two short presentations chosen from abstracts

Tajie Harris, University of Virginia
"Gasdermin-D-dependent IL-1α release from microglia promotes protective immunity during chronic T. gondii infection"
Gretchen Diehl, Baylor College of Medicine
"Early life selection of gut microbiota specific T cells

Sunday Afternoon
4:00 – 6:00 PM

POSTER SESSION and informal discussion groups.

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

Tissue Immunity and B Cells
Chairperson: Jakob von Moltke

Speakers:

Frederick Alt, Harvard University
"Fundamental Roles of Chromatin Loop Extrusion in V(D)J and IgH Class Switch Recombination"

Sarah Gaffen, University of Pittsburgh
"At the Crossroads of IL-17 Signaling"

Ari Molofsky, University of California, San Francisco
"Stromal Crosstalk with Type 2 Immunity at Tissue Adventitial Niches"

Jakob von Moltke, University of Washington
"Tuft Cell-derived Leukotrienes Drive Rapid Activation of Type 2 Immunity in the Small Intestine"

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

Innate Immunity: Activation & Effector Functions
Chairperson: Mary O'Riordan

Speakers:

Catherine Blish, Stanford University
"Defining Protective Human Natural Killer Cell Responses to Infection"

Igor Brodsky, University of Pennsylvania
"Guarding the Guardians: RIPK1 and the Detection of Pathogen Manipulation of Immune Signaling"

Veit Hornung, University of Munchen
"TLR8 is a Sensor of RNase T2 Degradation Products"

Mary O'Riordan, University of Michigan
"Cellular Stress Pathways Shape Innate Anti-Microbial Defenses"

Two short presentations chosen from abstracts

Kirk Jensen, University of California, Merced
"Nfkbid-dependent B cell responses to T. gondii - new interactions revealed by a genetic screen"
Malay Haldar, University of Pennsylvania
"Tumor-derived retinoic acid promote intratumoral monocyte differentiation into immunosuppressive macrophages"

Monday Afternoon
4:00 – 6:00 PM

ORAL POSTER PRESENTATIONS

SESSION IV **Evolution & Intelligent Design in Immunity**

Monday Evening ***Chairperson: Nels Elde***
7:30 -10:00 PM

Speakers:

Brenda Bass, University of Utah
"Distinguishing Self and Non-self dsRNA in Vertebrates and Invertebrates"

Nels Elde, University of Utah
"Visualizing Immune Responses in Zebrafish"

Neil King, University of Washington
"Combining Computational Protein Design and Immunology to Create Novel Vaccines, Therapeutics, and Research Tools"

Russell Vance, University of California, Berkeley
"How Anti-viral Responses go Wrong: Regulation of Type I Interferons during Tuberculosis"

Award Presentations to Graduate, Postdoctoral, and Young Investigators

Poster Awards:

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

Oral Presentation Awards:

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

SESSION V **Lymphocyte Activation, Residence, Function**

Tuesday Morning ***Chairperson: Ananda Goldrath***
8:30-12:00 Noon

Speakers:

Jason Cyster, University of California, San Francisco
"Cues guiding B cell responses"

Ananda Goldrath, University of California, San Diego
"Programming Tissue Residency by to Enhance Immunity to Infection and Tumors"

Joan Goverman, University of Washington
"How Myelin-Specific CD8 T Cells Contribute to CNS Autoimmunity"

Terri Laufer, University of Pennsylvania
"An Intestinal Niche for Regulatory T Cells"

2020 Midwinter Conference of Immunologists

Oral Poster Presentation Session

***Monday, January 27, 2020: 4.00-6.00pm**
Chapel Auditorium
Moderators: Tajie Harris and Kirk Jensen

Name:	**"Abstract Title"**
Jackie Carozza	"Extracellular cGAMP is a cancer cell-produced immunotransmitter that promotes anti-cancer immunity" (#18)
Wei Hu	"Reversal of Fatal Autoimmunity by Regulatory T cells" (#54)
Keenan Lacey	"Investigating the host-pathogen interactions during nosocomial methicillin resistant Staphylococcus aureus pneumonia" (#66)
Bo Liu	"Unc93b1 recruits syntenin-1 to control TLR7 signaling and prevent autoimmunity" (#73)
CJ Cambier	"Host-pathogen lipid interactions influence mycobacterial pathogenesis" (#17)
Nina Serwas	"A novel endocytic mechanism used for antigen transfer from peripheral to immune cells" (#109)
A. Palaferri Schieber	"Vitamins in Host Defense Against an Enteric Pathogen" (#93)
Frank Soveg	"Membrane-targeting of oligoadenylate synthetase 1 primes antiviral activity" (#113)

Recognition of Cytosolic Bacteria by Guanylate Binding Proteins

Jörn Coers
Duke University

The surface exposed O-antigen segment of lipopolysaccharide (LPS) provides nonspecific barrier functions, while the lipid A portion anchors LPS in the outer membrane of gram-negative bacteria. Human guanylate binding protein-1 (hGBP1) colocalizes with intracellular gram-negative bacterial pathogens, promotes activation of the lipid A sensor Caspase-4, and blocks actin-driven dissemination of the enteric pathogen *Shigella.* The underlying molecular mechanism for hGBP1's diverse antimicrobial functions is unknown. I will present mostly unpublished data in support of a model in which hGBP1 acts as a LPS-binding surfactant that dissolves the rigidity of the O-antigen layer and thereby exerts pleiotropic effects on the functionality of gram-negative bacterial cell envelopes.

Relevant publication on the topic from my lab:

Pilla, D., Hagar, JA., Haldar, AK., Mason, AK., Ernst, RK., Yamamoto M., Miao, EA., Coers, J. Guanylate binding proteins promote caspase-11-dependent pyroptosis in response to cytoplasmic LPS. Proc Natl Acad Sci U.S.A. 2014 Apr 22;111(16):6046-51. PMID: 24715728; PMC4000848.

Finethy, R., Luoma, S., Orench-Rivera, N., Feeley, EM., Haldar, AK., Yamaoto, Y., Kanneganti, TD., Kuehn, MJ., Coers, J. Inflammasome activation by bacterial outer membrane vesicles requires guanylate binding proteins. mBio 2017 Oct 3;8(5). pii: e01188-17. PMID: 28974614

Feeley, EM.*, Pilla-Moffett, D.*, Zwack, EE., Piro, AS., Finethy, R., Kolb, JP, Martinez, J., Brodsky, IE., Coers, J. Galectin-3 directs antimicrobial Guanylate Binding Proteins to vacuoles furnished with bacterial secretion systems

Piro, AS., Hernandez, D., Luoma, S., Feeley, EM., Finethy, R., Yirga, A., Frickel, EM., Lesser, CF., Coers, J. Detection of cytosolic *Shigelle flexneri* via a C-terminal triple-ariginine motif of GBP1 inhibits actin-based motility. mBio 2017 Dec 12;8(6) pii: e01979-17. PMID: 29233899

An Unanticipated Role for Eosinophils in Host Resistance against *Mycobacterium Tuberculosis*

Katrin D. Mayer-Barber

National Institutes of Health

Earl Stadtman Investigator, Chief, Inflammation and Innate Immunity Unit Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)

Mycobacterium tuberculosis (*Mtb*) is the leading cause of mortality worldwide due to a single infectious agent. *Mtb* resides in pulmonary macrophages, neutrophils and other phagocytes, which limit bacterial growth. The development of effective vaccines and host directed therapies (HDT) against *M. tuberculosis (Mtb)* infection requires a detailed understanding of the cellular basis of protective adaptive and innate immunity[1]. Considerable progress has been made in our understanding of protective adaptive immunity, yet relatively little is known about the contribution of specific subsets of innate effector cells. Particularly, the biological relevance of granulocytes like neutrophils and eosinophils is poorly understood. The innate inflammatory response is a prime target for HDT and manipulation of granulocytes could have major inflammatory and immunoregulatory implications for host resistance [2,3,4]. Although eosinophils can have similar effector functions to neutrophils, including an overlapping repertoire of granular contents capable of limiting bacterial growth, their role in *Mtb* infection is largely unknown. I will be present our current unpublished work in which we have carefully characterized the granulocytic immune response to *Mtb* in human, non-human primate and murine models of TB disease. Unexpectedly, we found that eosinophils, both in non-human primates and mice, are the first granulocytes to respond to Mtb infection by recruitment and sequestration to the infected airways. Eosinophils were significantly increased in the bronchoalveolar lavage fluid of *Mtb*-infected rhesus macaques compared to pre-infection samples, providing evidence that eosinophils are recruited early to the lungs during *Mtb* infection. We observed an early recruitment of eosinophils into the lungs after Mtb infection in mice and, most importantly, survival after *Mtb* infection was significantly reduced in eosinophil-deficient mouse strains and bacterial loads were increased. In vitro, eosinophils from healthy human donors exposed to *Mtb* released inflammatory cytokines, degranulated and expressed surface proteins that are associated with activation and migration and eosinophils were abundantly present in lung tissue samples from TB patients undergoing resection surgery. Taken together, these data argue for a previously unrecognized protective role of eosinophils in host resistance against Mtb infection.

[1] Mayer-Barber KD*, Andrade BB, Oland SD, Amaral EP, Barber DL, Gonzales J, Derrick SC, Shi R, Kumar, NP, Wei W, Yuan X, Zhang G, Cai Y, Babu S, Catalfamo M, Salazar AM, Via LE, Barry III CE and Sher A. *Host-directed therapy of tuberculosis based on interleukin-1 - type I interferon crosstalk.* Nature, 2014 July 3rd; 511 (7507)

[2] Mayer-Barber KD*, Andrade BB, Barber DL, Hieny S, Feng CG, Caspar P, White S, Gordon S, Sher A. *Innate and Adaptive Interferons Suppress IL-1α and IL-1b Production by Distinct Pulmonary Myeloid Subsets during Mycobacterium tuberculosis Infection.* Immunity, 2011 Dec 23;35(6):1023-34.

[3] Mayer-Barber KD*, Barber DL, Shenderov K,White SD, Wilson MS, Cheever A, Kugler D, Hieny S, Caspar P, Núñez G, Schlueter D, Flavell RA, Sutterwalla FS, Sher A. *Cutting edge: Caspase-1 Independent IL-1beta production is critical for host resistance to Mycobacterium tuberculosis and does not require TLR signaling in vivo.* Journal of Immunology 2010 Apr 1;184(7):3326-30.

[4] Bohrer AC, Tocheny C, Assmann M, Ganusov VV and Mayer-Barber KD. *Cutting edge: IL-1R1 mediates host resistance to Mycobacterium tuberculosis by trans-protection of infected cells.* Journal of Immunology. 2018, ji1800438. doi: 10.4049/jimmunol.1800438.

This work was supported by the intramural research programs of NIAID, NIH.

Adaptations During Long-Term (Host-Pathogen) Relationships

Elina Zuniga

University of California San Diego

Chronic infections represent a major biomedical problem and are characterized by a long-term equilibrium between the pathogen and the immune system. Such equilibrium is enabled by adaptations of immune cells that attenuate selected immune functions to minimize immunopathology while keeping the pathogen in check. Similar immune-adaptations may be detected, often in a transient manner, early after acute infections but are only sustained and may only impact pathogen persistence during chronic infections. We are interested in studying the mechanisms underlying immune cell adaptations in the context of chronic infections to unveil new basic biology of the immune system and potentially unlocking new therapeutic strategies for immunotherapies. I will present our recent work related to adaptations of Dendritic Cells and their progenitors in the context of acute and chronic viral infections.

1. Macal M, Jo Y, Dallari S, Chang A, and **E. Zúñiga.** Self-Renewal and Toll-like Receptor Signaling Sustain Exhausted Plasmacytoid Dendritic Cells during Chronic Viral Infection. 2018. Immunity. 48(4):730-744.

2. Loureiro ME, Fernandes A, Radoshitzky S, Chi X, Dallari S, Marooki N, Lèger P, Foscaldi S, Sharma S, López N, de la Torre JC, Bavari S and **E. Zúñiga**. DDX3 Is Exploited By Arenaviruses To Suppress Type I Interferons And Favor Their Replication. 2018. Plos Pathogen. *2018. 14(7).*

3. M Macal, G.M. Lewis, S Kunz, RA. Flavell, J. Harker and **E. Zúñiga**. Plasmacytoid Dendritic Cells Are Productively Infected and Activated through TLR-7 Early After Arenavirus Infection. Cell Host & Microbe. 2012. 11(6):617-30.

4. **E Zúñiga**, L Liou, L Mack, M Mendoza and MBA Oldstone. Persistent virus infection inhibits type I interferon production by plasmacytoid dendritic cells to facilitate opportunistic infections. Cell Host & Microbe. 2008. 16 (4):374-86.

Fundamental Roles of Chromatin Loop Extrusion in V(D)J and IgH Class Switch Recombination

Frederick W. Alt

Harvard University

Zhaoqing Ba[1], Xuefei Zhang[1], Jiangman Lou[1], Zhuoyi Liang, Hongli Hu[1], Sai Luo[1],, Nia Kyristis[1], Eddie Dring[1], Kyong-Rim Kieffer-Kwon[2], Muhammad S. Shamim[3,], Aviva Presser Aiden[3,], Erez Lieberman Aiden[3], Rafael Casellas[2], Yu Zhang[1,4], and Frederick W. Alt[1]

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[2]Lymphocyte Nuclear Biology, NIAMS, NIH, Center of Cancer Research, NCI, NIH, Bethesda, MD
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RAG endonuclease initiates IgH and IgL variable region exon assembly from V, D, and J segments from its location within a chromosomal V(D)J recombination center (RC). We find that IgH RC-bound RAG linearly scans chromatin propelled past it by loop extrusion allowing it to, thereby, locate distal DNA target substrates. The RC also serves as a dynamic extrusion impediment, allowing passing loop extrusion impediments, including CBE anchors, transcription, and dCas9 binding to have prolonged interactions with the RC and, thereby, focus RAG to targets focally within impeded regions. This loop extrusion process is critical for aligning IgH D and J segments for RAG cleavage and deletional orientation-specific joining and is similarly involved in IgL variable region exon assembly. To further elucidate loop extrusion-mediated RAG scanning, we employed an auxin-inducible degron (AID) to conditionally destroy either cohesion-component Rad21 or CTCF in G1-arrested *v-Abl* pre-B lines, which undergo IgH and IgL V(D)J recombination. AID-tagged Rad21 and CTCF were acutely degraded without impacting cell viability and effects on loop domain formation and RAG scanning were measured by sensitive 3C-HTGTS and HTGTS-Rep-seq assays. These studies strongly support cohesin-mediated loop extrusion as a driving force for RAG scanning of chromatin across the entire (3Mb) *Igh* and *Igκ* loci. Finally, we find that, during the independent IgH class switch recombination (CSR) process, chromatin loop extrusion synapses regulatory elements, long switch regions and DSBs within them to achieve the previously enigmatic mechanism by which *Igh* organization *in cis* promotes orientation-specific CSR DSB joining.

Jain, S., Ba, Z., Zhang, Y., Dai, H-Q and Alt, F.W. (2018) CTCF-binding elements mediate accessibility of RAG substrates during chromatin scanning. **Cell** 174, 102-116, doi: 10.1016/j.cell.2018.04.035. PMC6026039

Zhang, X., Zhang, Y., Ba, Z., Kyritsis, N., Casellas, R. and Alt, F.W. (2019) Fundamental roles of chromatin loop extrusion in antibody class switching. **Nature.** 575. 385-389. doi: 10.1038/s41586-019-1723-0. PMC6856444.

Zhang, Y., Zhang, X., Ba, Z., Liang, Z., Dring, E.W., Hu, H., Lou, J., Kyritsis, N., Zurita, J., Shamim, M.S., Presser Aiden, A., Lieberman Aiden E. and Alt, F.W. (2019) The fundamental role of chromatin loop extrusion in physiological V(D)J recombination. **Nature.** 573, 600-604. doi: 10.1038/s41586-019-1547-y. PMC In Process.

At the Crossroads of IL-17 Signaling

Sarah L. Gaffen
University of Pittsburgh

Fungal infections are a serious threat to public health, but our understanding of immunity to fungi lags far behind that of other organisms. Even today there are no licensed vaccines to any fungal microbes. Oropharyngeal candidiasis (OPC, oral thrush) is an opportunistic infection of the oral mucosa caused by the dimorphic commensal fungus *Candida albicans*. Immunity to OPC is highly dependent on Th17 cells, and IL-17 and IL-22 produced by these cells contribute non-redundantly to antifungal immunity via distinct downstream signaling pathways. Strikingly, both cytokines act on non-hematopoietic cells to control OPC, specifically cells of the oral epithelium. The oral mucosa is a stratified non-keratinizing tissue composed of a proliferative basal epithelial layer (BEL) that undergoes a program of differentiation to generate a post-mitotic suprabasal epithelial layer (SEL). Sloughing of the SEL is an integral part of an antifungal immune strategy to control fungal invasion, but the role of Type 17 cytokines in this process is not well defined. We found that IL-22 and IL-17 receptors are required in anatomically distinct locations. Whereas loss of IL-22RA1 or STAT3 in the oral BEL causes susceptibility to OPC, IL-17RA is needed dominantly in the SEL. Transcriptional profiling linked IL-22/STAT3 to oral epithelial cell proliferation and survival, but also, unexpectedly, to an IL-17-driven gene signature. Mechanistically, IL-22 signals on the BEL to replenish the IL-17RA-expressing SEL, thereby restoring the ability of the oral epithelium to respond to IL-17 and mediate antifungal events such as neutrophil recruitment and β-defensin production. Consequently, IL-22 signaling in BEL 'licenses' IL-17 signaling in the oral mucosa, revealing spatially distinct yet cooperative activities of Type 17 cytokines in the oral mucosal immunity.

References

1. Li X, Bechara R, Zhao J, McGeachy M, Gaffen SL. IL-17 receptor based signaling and implications for diseases. *Nature Immunol.* 2019; 20:1594-1602.
2. Verma AH, Richardson JP, Zhou C, Coleman BM, Moyes DL, Ho J, Huppler AR, Ramani K, McGeachy MJ, Mufazalov MA, Waisman A, Kane LP, Biswas PS, Hube B, Naglik JR, Gaffen SL. Oral epithelial cells orchestrate innate Type 17 responses to *Candida albicans* through the virulence factor Candidalysin. *Science Immunol*, 2017; 2:eaam8834.
3. Conti HR, Bruno VM, Childs EC, Daugherty S, Hunter JP, Mengesha BG, Saevig DL, Hendricks MR, Coleman BM, Brane L, Solis N, Cruz JA, Verma AH, Garg AV, Hise AG, Richardson JP, Naglik JR, Filler SG, Kolls JK, Sinha S, Gaffen SL. IL-17RA signaling in oral epithelium is critical for protection against oropharyngeal candidiasis. *Cell Host & Microbe*, 2016; 20:606-617.
4. Conti HR, Gaffen SL. IL-17 and *Candida albicans* infections. *J Immunol*, 2015; 195:780-788.

Stromal Crosstalk with Type 2 Immunity at Tissue Adventitial Niches

Ari Molofsky
University of California, San Francisco

Mobilization of type 2 immune responses is critical to both physiologic tissue remodeling and allergic pathology. However, whether there are physical tissue niches that mediate type 2 immunity is not well described. We use quantitative 3D-imaging, single cell transcriptomics, and genetic models to define tissue niches of group 2 innate lymphoid cells (ILC2), critical instigators of type 2 immunity. We identify a dominant adventitial 'cuff' niche around intermediate to large vessels and other tubular structures, present in the lung, adipose, and most other tissues. ILC2s, as well as additional subsets of tissue-resident hematopoietic cells, localize to these sites. ILC2s are most intimately associated with fibroblast-like adventitial stromal cells that are functionally distinct and sufficient to support ILC2 survival and activation, acting via TSLP, IL-33, and additional mechanisms. We describe the impact of lymphocyte-derived cytokines, such as IL-13, on adventitial stromal cell immunologic functions. Using models of type 2 allergic inflammation, we find that ILC2 niches are dynamically altered during inflammatory challenge. We determine the functional tradeoffs of ILC2 *niche relocalization* in mounting protective IFN□-driven type 1 immune responses. Together our data support a model where ILC2s engage in a bi-directional conversation with micro-anatomically and functionally distinct stromal cells, and other niche components, creating type 2-biased tissue outposts that tightly regulate tissue allergic immunity.

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2. Dahlgren, M. W. *et al.* Adventitial Stromal Cells Define Group 2 Innate Lymphoid Cell Tissue Niches. *Immunity* **50**, 707-722.e6 (2019).

3. Molofsky, A. B. *et al.* Interleukin-33 And Interferon-g Counter-Regulate Group 2 Innate Lymphoid Cell Activation During Immune Perturbation. *Immunity* **43**, 161–174 (2015).

4. Vainchtein, I. D. *et al.* Astrocyte-derived Interleukin-33 promotes microglial synapse pruning during brain development. *Science (80-.).* **359**, 1269–1273 (2018).

Tuft Cell-derived Leukotrienes Drive Rapid Activation of Type 2 Immunity in the Small Intestine

Jakob von Moltke
University of Washington

John W. McGinty[1], Hung-An Ting[1], Tyler Billipp[1], Danish Kahn[1], Hong-Erh Liang[2,3,4], Nora Barrett[5], Ichiro Matsumoto[6], Richard M. Locksley[2,3,4], Jakob von Moltke[1*]

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Helminths, allergens, and certain protists all induce a type 2 immune response, but the underlying mechanisms of immune activation remain poorly understood. In the small intestine, chemosensing by epithelial tuft cells results in activation of group 2 innate lymphoid cells (ILC2s), which in turn drive increased tuft cell frequency. This feed-forward circuit is essential for remodeling of intestinal physiology and helminth clearance. ILC2 activation requires tuft cell-derived IL-25, but whether additional signals regulate the circuit is unclear. Here we show that tuft cells secrete cysteinyl leukotrienes to rapidly activate type 2 immunity following helminth infection. Leukotrienes cooperate with IL-25 to activate ILC2s, and tuft cell-specific ablation of leukotriene synthesis attenuates type 2 immunity and delays helminth clearance. Conversely, leukotrienes are dispensable for the small intestinal type 2 immune response activated by tuft cell sensing of protist- and/or bacteria-derived succinate. Our findings identify a novel tuft cell effector function and suggest contextual regulation of tuft-ILC2 circuits within the small intestine.

Nadjsombati MS*, McGinty JW*, Lyons-Cohen MR, Jaffe JB, DiPeso L, Schneider C, Miller CN, Pollack JL, Nagana Gowda GA, Fontana MF, Erle DJ, Anderson MS, Locksley RM, Raftery D, **von Moltke J**. Detection of succinate by intestinal tuft cells triggers a type 2 innate immune circuit. 2018 Immunity. 49(1):33-41

von Moltke J, O'Leary CE, Barrett NA, Kanaoka Y, Austen F, Locksley RM. Leukotrienes provide an NFAT-dependent signal that synergizes with IL-33 to activate ILC2s. 2017 J Exp Med. 214(1):27-37.

von Moltke J, Ji M, Liang H-E, Locksley RM. Tuft cell-derived IL-25 regulates an intestinal ILC2 – epithelial response circuit. 2016 Nature. 529(7585):221-5

Defining Protective Human Natural Killer Cell Responses to Infection

Catherine A. Blish

Stanford University

Natural killer (NK) cells are effector cells of the innate immune system that are equipped to recognize and rapidly eradicate infected cells. Healthy cells escape NK cell killing because major histocompatibility complex class I proteins on healthy cells engage NK cell inhibitory receptors. In the context of infection, down-regulation of MHC class I molecules can activate NK cells. In addition, upregulation of stress-induced ligands on infected cells can trigger engagement of activating receptors on NK cells. What is less clear is exactly how NK cells distinguish particular infections, and how these pathways are influenced by the mechanisms that pathogens have evolved to evade NK cell surveillance. For instance, are there particular activating receptors that are involved in the recognition of a specific virus, such as HIV? Or are there virus-specific mechanisms to engage NK cell inhibitory receptors to promote escape? How do the receptors involved in NK cell recognition differ between pathogens? The goal is to better define the mechanisms of NK cell recognition of pathogens including HIV, dengue virus, influenza virus, and tuberculosis.

Background references:

1. Wilk AJ and **Blish CA**. Diversification of human NK cells: lessons from deep profiling. *J Leukoc Biol,* 2018 Jan 19. Doi: 10.1002/JLB.6RI0917-390R. PMCID: PMC6133712
2. Horowitz A, Strauss-Albee DM, Leipold M, Kubo J, Nemat-Gorgani N, Dogan OC, Dekker CL, Mackey S, Maecker H, Swan GE, Davis MM, Norman PJ, Guethlein LA, Desai M, Parham P, and **Blish CA**. Genetic and environmental determinants of human NK cell diversity revealed by mass cytometry. *Science Translational Medicine* 2013 5:108ra145; DOI: 10.1126/scitranslmed.3006702. PMCID: PMC3918221
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Guarding the Guardians: RIPK1 and the Detection of Pathogen Manipulation of Immune Signaling

Igor Brodsky

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Microbial pathogens inject virulence factors into target host cells in order to establish infection. These effector proteins disrupt critical cellular signaling pathways, but can also serve to alert the host to the presence of unscheduled or inappropriate enzymatic activities, thereby triggering anti-pathogen defenses via a process termed 'effector-triggered immunity.' The bacterial pathogen *Yersinia* disrupts NF-κB and MAPK signaling by inhibiting IKK and thus blocking essential pathways of immune defense. This bacterial virulence activity induces macrophage cell death via a pathway involving cell extrinsic apoptosis. *Yersinia*-induced apoptosis required the kinase activity of receptor-interacting protein kinase 1 (RIPK1), a central regulator of the distinct cell fates of cell death, and NF-κB signaling. Targeted disruption of RIPK1 kinase activity prevented *Yersinia*-induced cell death, and revealed that in vivo, *Yersinia*-induced apoptosis is critical for containment of bacteria in mesenteric lymph node granulomas, control of systemic bacterial burdens, and host survival. This apoptotic response provides a cell-extrinsic signal that promoted optimal innate immune cytokine production and antibacterial defense, demonstrating a key role for RIPK1-kinase induced apoptosis in overcoming *Yersinia* blockade of immune signaling. Intriguingly, IKK normally restrains RIPK1-induced cell death in response to microbial stimuli or inflammatory cytokines, revealing a circuit for the activation of effector triggered immunity in response to pathogen blockade of innate immune signaling.

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TLR8 is a Sensor of RNase T2 Degradation Products

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TLR8 is among the highest-expressed pattern-recognition receptors in the human myeloid compartment, yet its mode of action is poorly understood. TLR8 engages two distinct ligand binding sites to sense RNA degradation products, although it remains unclear how these ligands are formed *in cellulo* in the context of complex RNA molecule sensing. Here, we identified the lysosomal endoribonuclease RNase T2 as a non-redundant upstream component of TLR8-dependent RNA recognition. RNase T2 activity is required for rendering complex single-stranded, exogenous RNA molecules detectable for TLR8. This is due to RNase T2's preferential cleavage of single-stranded RNA molecules between purine and uridine residues, which critically contributes to the supply of catabolic uridine and the generation of purine-2′,3′-cyclophosphate-terminated oligoribonucleotides. Thus-generated molecules constitute agonistic ligands for the first and second binding pocket of TLR8. Together, these results establish the identity and origin of the RNA-derived molecular pattern sensed by TLR8.

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Cellular Stress Pathways Shape Innate Anti-Microbial Defenses

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Infection of phagocytes by bacterial pathogens provokes a multi-faceted host response that is shaped by cellular stress. The IRE1 ER stress sensor, activated by Toll-like Receptor (TLR) signaling, modulates both pro-inflammatory and anti-microbial functions. We find that IRE1 triggered by infection with methicillin-resistant *Staphylococcus aureus* (MRSA) programs induction of mitochondrial reactive oxygen species (ROS). Although mitochondria-derived antimicrobial effectors like reactive oxygen species (mROS) aid in bacterial killing, it is unclear how these effectors reach bacteria, like MRSA, that reside within the phagosome. We show in macrophages that IRE1-induced mROS are delivered to bacteria-containing phagosomes via mitochondria-derived vesicles (MDVs). MDV generation requires the mitochondrial stress response factor Parkin and contributes to mH_2O_2 accumulation in bacteria-containing phagosomes. Accumulation of phagosomal H_2O_2 was dependent on the mitochondrial matrix enzyme superoxide dismutase-2, which is also delivered to phagosomes by MDVs. Sod2 depletion compromises mH_2O_2 production and bacterial killing. Thus, mitochondrial redox capacity enhances macrophage antimicrobial function by delivering mitochondria-derived effector molecules into bacteria-containing phagosomes. Additionally, we find that MRSA infection of neutrophils also activates IRE1. In PMNs, IRE1 stimulates mROS production, and acts as a key regulator controlling production of neutrophil extracellular traps (NETs). IRE1 regulated NETosis through mROS-dependent and -independent mechanisms. Our data highlight a central role for cellular stress and IRE1 in promoting host anti-microbial defenses.

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Distinguishing Self and Non-self dsRNA in Vertebrates and Invertebrates

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Viruses produce double-stranded RNA (dsRNA) during infection, and viral dsRNA serves as a pathogen-associated molecular pattern, or PAMP, to initiate an immune response. dsRNA is also encoded and expressed in animal cells. Since dsRNA-binding proteins (dsRBPs) are not sequence specific, how do cells discriminate cellular from viral dsRNA? We seek to understand mechanisms by which dsRBPs mediate “self” versus “non-self” discrimination.

An exciting discovery in recent years is that one of the functions of the ADAR family of RNA editing enzymes is to convert adenosines to inosines in cellular dsRNA to mark it as self, allowing it to be distinguished from viral dsRNA. Indeed, in the absence of ADAR1 p150, mammals trigger a massive interferon response to their own dsRNA. Despite clear differences between vertebrate and invertebrate immune responses, this role for ADARs is conserved, and the invertebrate *C. elegans* triggers an antiviral RNAi response in the absence of its ADAR RNA editing enzymes.

Invertebrates lack an interferon pathway and instead rely on Dicer to cleave viral dsRNA. In invertebrates, self versus non-self discrimination is also enabled by recognition of dsRNA termini. For example, when *Drosophila* Dicer-2 encounters a dsRNA with a blunt terminus, presumed to mimic a terminus of viral dsRNA, an optimal reaction ensues, whereby the helicase domain enables ATP-dependent, processive cleavage of the dsRNA. In contrast, dsRNA with 3’ overhanging termini, which mimic termini of cellular dsRNAs, promote a suboptimal, distributive cleavage. To understand this discrimination, we used cryo-electron microscopy to solve structures of *Drosophila* Dicer-2 alone, and in complex with blunt dsRNA and an ATP analog. While the Platform-PAZ domains have been considered the only Dicer domains that bind dsRNA termini, unexpectedly, we found the helicase domain is required for binding blunt, but not 3’ overhanging, termini. We also observed that blunt dsRNA is locally unwound and threaded through the helicase domain in an ATP-dependent manner.

Despite a well conserved helicase domain, studies to date indicate mammalian Dicer cannot distinguish termini, and further, an ATP dependence has not been observed. Whether mammalian Dicer is ever involved in an antiviral response is controversial, and it remains possible that acquisition of the interferon pathway in vertebrates allowed Dicer to specialize for miRNA processing. Our ongoing studies are aimed at understanding in vitro biochemical differences between vertebrate and invertebrate Dicers that may explain their different functions in vivo.

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Visualizing Immune Responses in Zebrafish

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The discovery of new viruses currently outpaces our capacity for experimental examination of infection biology. To better couple virus discovery with immunology, we genetically modified zebrafish to visually report on virus infections. After generating a strain that expresses green fluorescent protein (GFP) under an interferon-stimulated gene promoter, we repeatedly observed transgenic larvae spontaneously expressing GFP days after hatching. RNA sequencing comparisons of co-housed GFP-positive and GFP-negative zebrafish revealed a naturally occurring picornavirus that induced hundreds of antiviral defense genes not observed following immunostimulatory treatments or experimental infections with other viruses. Among the many genes induced by picornavirus infection was a large set encoding GTPase of immunity-associated proteins (GIMAPs). The GIMAP gene family is massively expanded in fish genomes and may also play a crucial role in antiviral responses in mammals, including humans. We subsequently detected zebrafish picornavirus in publicly available sequencing data from seemingly asymptomatic zebrafish in many research institutes and found that it altered gene expression in a previous study of zebrafish development. These data also revealed tissue tropism and identified the clonal CG2 strain as notably vulnerable to picornavirus infection. Our study describes a naturally occurring picornavirus that elicits strong antiviral responses in zebrafish and provides new strategies for simultaneously discovering viruses and their impact on vertebrate hosts.

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Combining Computational Protein Design and Immunology to Create Novel Vaccines, Therapeutics, and Research Tools

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Recent advances in computational protein design have enabled the predictive design of novel protein structures and self-assembling protein nanomaterials with atomic-level accuracy. These capabilities open up new possibilities for generating novel vaccines, therapeutics, and research tools with structures and functions tailored to specific applications. Applications in immunology are particularly promising, as the ability of the immune system to learn, adapt, remember, and amplify in response to specific stimuli can make designed proteins powerful interventions. I will discuss recent work in this area from my group, including the design of novel nanoparticle vaccines for enhanced potency and breadth as well as proteins intended to activate the innate immune system by targeting specific innate immune receptors and pathways.

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How Anti-viral Responses go Wrong: Regulation of Type I Interferons during Tuberculosis

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Mycobacterium tuberculosis is the causative agent of tuberculosis (TB), a severe disease that kills more humans every year than any other single infectious disease. Despite this, *M. tuberculosis* exhibits highly variable pathogenicity, and many humans infected with *M. tuberculosis* fail to develop symptoms. A major outstanding question is what explains the variable outcomes of *M. tuberculosis* infection in humans. Mechanistic insights into this question require genetically tractable animal models. We have taken advantage of the longstanding observation that C3H mice are more susceptible to infection with *M. tuberculosis* than the more commonly used B6 mouse strain. A recessive locus on mouse chromosome 1 that at least partly explains the susceptibility of C3H mice was previously identified (Pan et al, 2005) and named the *Supersusceptibility to tuberculosis 1* (*Sst1*) locus. We found that the TB susceptibility of B6 mice harboring the $Sst1^S$ locus from C3H mice was due to an exacerbated type I interferon response. Type I interferons are typically considered to be anti-viral cytokines, and prior studies in mice have demonstrated detrimental effects of anti-viral type I interferon responses during infections with several bacterial species, including *M. tuberculosis*. In addition, studies in humans have shown that progression to active TB disease is often preceded by and/or correlates with induction of type I interferons. However, the reason(s) for the detrimental effects of type I interferons during TB have been unclear. Our results indicate that interferon-dependent induction of a soluble antagonist of interleukin-1 (IL-1), called IL-1 receptor antagonist (IL-1Ra), is a dominant factor mediating the susceptibility of B6.$Sst1^S$ mice to *M. tuberculosis*. In this presentation, I will discuss our latest results, including our efforts to identify the causative gene within the *Sst1* locus that controls TB susceptibility.

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Cues Guiding B Cell Responses

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Lymphoid tissues provide crucial organizing grounds for cellular interactions needed to initiate and sustain humoral immune responses. This includes supporting the germinal center (GC) response, sites where antigen-reactive B cells undergo antibody diversification and affinity maturation. In a long-running project, we have been defining the molecular cues involved in organizing GCs and confining cells within these structures. This has included our recent identification of a novel metabolite, S-geranylgeranyl-L-glutathione (Ggg) as a ligand for the GC-expressed receptor P2RY8. Our ongoing studies on how Ggg-P2RY8 signaling confines GC B cells and Tfh cells will be discussed. The signaling requirements for B cell selection and differentiation in GCs are complex, involving inputs from both the BCR and helper T cells. We have recently established that the TNFR family member, HVEM, on GC B cells engages the ITIM-containing receptor BTLA on Tfh cells to restrain the amount of T cell help delivered. The HVEM-BTLA system functions to enhance the stringency of B cell selection in the GC. A summary of this project will be provided. We have also examined requirements for GC B cell differentiation into memory B cells. Using an in vivo CRISPR-based knockdown screening approach, we have identified several transcription factors that are needed for efficient generation of memory B cells, including the homeobox transcription factor Hhex. Our ongoing work on this project will be presented.

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Programming Tissue Residency by to Enhance Immunity to Infection and Tumors

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Tissue-resident memory $CD8^+$ T cells (T_{RM}) are optimally positioned at common sites of pathogen exposure, where they elicit rapid and robust antiviral immune responses. However, the molecular signals controlling tissue residency and homeostasis of T_{RM} remain unclear. Exploiting a dual-screening platform integrating computational and RNAi *in vivo* screening approaches, we have identified transcriptional regulators of T_{RM} differentiation. This strategy revealed numerous transcription factors with indispensible roles in T_{RM} differentiation and homeostasis. Further, we show that tumor infiltrating lymphocytes (TIL) share a core T_{RM} transcriptional signature, and that these factors can control TIL residency. Furthermore, enhanced anti-tumor function by HIF activity in T cells is mediated by the enrichment of T_{RM}-like TIL. These results provide novel insight into the biology of T cell residency, which could be leveraged to enhance vaccine efficacy or adoptive therapy treatments against cancer.

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How Myelin-Specific CD8 T Cells Contribute to CNS Autoimmunity

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Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS). Although CD4 T cells are implicated in MS pathogenesis and have been the main focus of MS research using the animal model experimental autoimmune encephalomyelitis (EAE), substantial evidence from patients with MS points to a role for CD8 T cells in disease pathogenesis. We previously identified MHC class I-restricted murine CD8 T cells specific for myelin basic protein (MBP) and developed a T cell receptor transgenic model based on this specificity. Using this model, we showed that the MBP-specific CD8 T cells could initiate CNS autoimmunity, and that the MBP epitope recognized by the CD8 T cells is presented in the CNS when EAE is initiated by CD4 T cells. Our recent studies investigated whether naïve MBP-specific CD8 T cells recruited to the CNS during CD4 T cell-initiated EAE engaged in determinant-spreading and influenced disease. Surprisingly, we found that recruitment of MBP-specific CD8 T cells exacerbated brain but not spinal cord inflammation. Although 8.8 CD8 T cells were recruited to and activated within both the brain and spinal cord, 8.8 CD8 T cells accumulated over time only in the brain. A higher frequency of 8.8 CD8 T cells exhibiting an activated phenotype was also observed in the brain compared to the spinal cord. Presentation of the MBP epitope in the CNS was largely restricted to monocytes and monocyte-derived cells in both the brain and spinal cord; however, the frequency of these cell types presenting the MBP epitope was unexpectedly higher in the brain. Infiltration of MBP-specific CD8 T cells enhanced the production of reactive oxygen species by these cell-types only in the brain and this enhancement required FasL expression by the 8.8 CD8 T cells. These data suggest that myelin-specific CD8 T cells contribute to the pathogenesis of CD4 T cell-initiated EAE via a FasL-dependent mechanism that preferentially promotes lesion formation in the brain. In contrast, EAE initiated by MBP-specific CD8 T cells rather than CD4 T cells is dependent on CD8 T cell expression of IFNg and perforin but not FasL. Thus, CD8 T cells utilize distinct mechanisms to initiate versus exacerbate EAE.

An Intestinal Niche for Regulatory T Cells

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The intestine is a highly compartmentalized organ. Differences in physiology, environmental factors, and the expression of homing receptors of the white blood cells trafficking to unique anatomic and functional regions shape the immune cell composition of each intestinal compartment. Intestinal regulatory T cells (Tregs) are required to maintain tolerance toward food antigens and commensals. We have, therefore, focused on the cellular and molecular pathways that regulate the localization and maintenance of intestinal Tregs.

Using the K14 transgenic mouse model lacking peripheral TCR-MHCII interactions, we previously demonstrated that thymically generated Tregs could enter to the siLP of weanlings to fill and maintain an intestinal niche independently of antigen (Ag)-specific signals[1]. Similar to WTs, K14 siLP Tregs display an effector phenotype. Effector Tregs are known to be largely peripheral tissue-resident that may require continued TCR and costimulatory signals (such as ICOS) for their maintenance. In contrast, we found that in the absence of antigen-specific signals, B7-1/B7-2–CD28/CTLA-4 (but not ICOS-ICOSL) pathways maintain intestinal Tre proliferation and numbers. Our results suggest that a population of thymically-derived effector Tregs depend on costimulatory signals for maintenance in the siLP independently of Ag-specific signals.

We used three-dimensional tissue clearing and confocal microscopy to determine the anatomic distribution of Tregs in the small intestine and colon of WT and K14 mice. In both settings, and in primary human samples, Tregs are found in two distinct compartments: homogeneously spread within villi **(villi Tregs),** and tightly clustered in isolated lymphoid follicles **(ILFs Tregs).** ILFs localize at the base of individual intestinal villi and contain a cluster of B cells surrounded by dendritic cells. Accordingly, our imaging studies shows that inside of ILFs, Tregs are in close proximity to B cells and dendritic cells. Depletion studies suggest that DCs, but not B cells, maintain Treg numbers. Considering that in ILFs, B cells are the most abundant cell population and that ILF Tregs seems to be in close contact with B cells, is plausible to hypothesize that B cells inside of ILF are important to sustain Tregs numbers. Surprisingly, we observed that costimulation blockade with CTLA4-Ig, leads to a significant loss of villi Tregs, while ILF Tregs persist. This differential response suggests that villi Tregs and ILF Tregs have different requirements for their maintenance.

In conclusion our results suggest a model of intestinal Treg compartmentalization in which ILF Tregs are maintained independently of both antigen-specific signals and B7-dependent pathways and villi Treg proliferation is sustained by an APC through B7-1/B7-2–CD28/CTLA-4 pathway independently of antigen-specific signals.

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