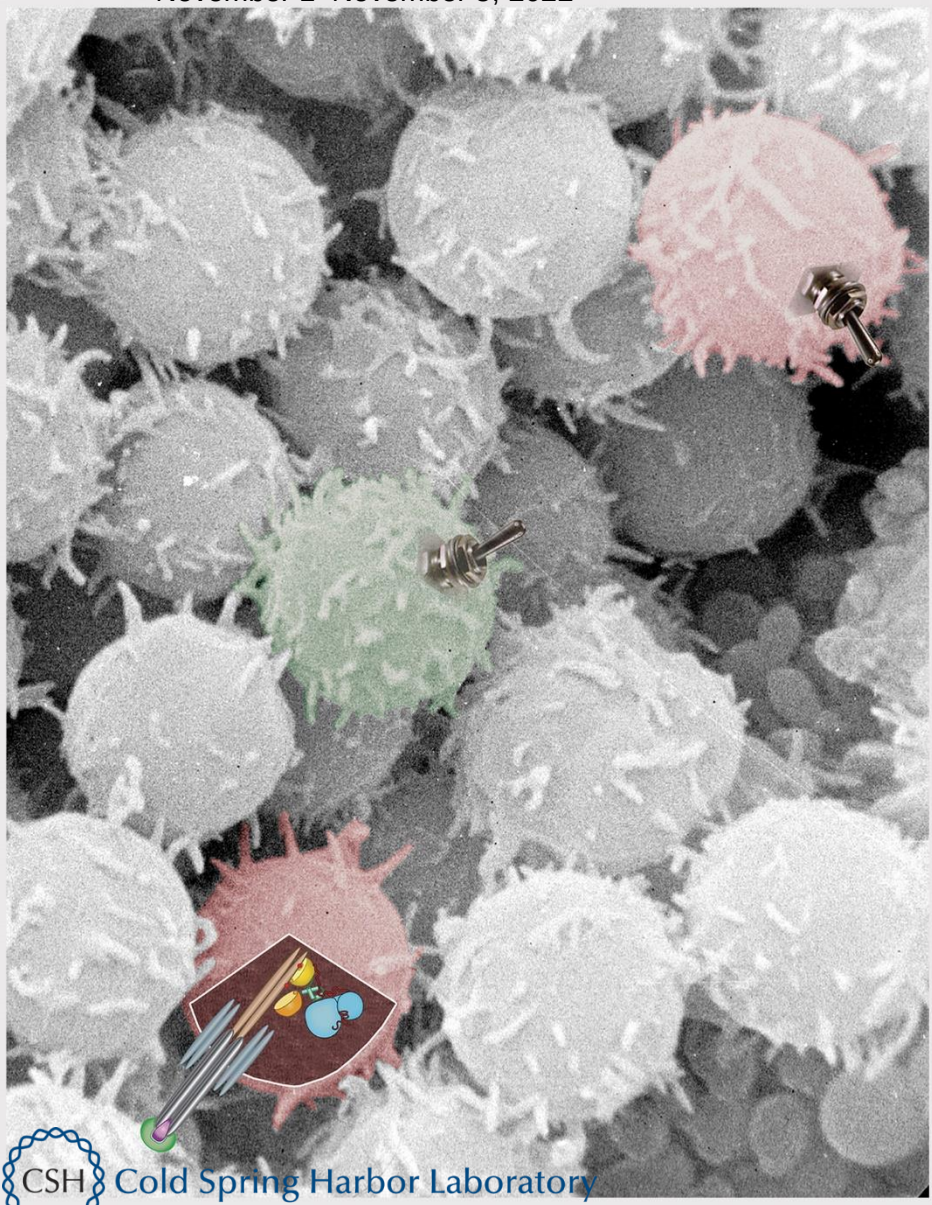
Abstracts of papers presented
at the 2022 meeting on

GENE EXPRESSION & SIGNALING IN THE IMMUNE SYSTEM

November 1–November 5, 2022



CSH Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2022 meeting on

GENE EXPRESSION & SIGNALING IN THE IMMUNE SYSTEM

November 1–November 5, 2022

Arranged by

Jon Kagan, *Harvard Medical School*
Ellen Rothenberg, *California Institute of Technology*
Alexander Rudensky, *HHMI / Memorial Sloan Kettering Cancer Center*
Arthur Weiss, *HHMI / University of California San Francisco*

This meeting was funded in part by the **National Institute of Allergy and Infectious Diseases,** a branch of the **National Institutes of Health.**

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GENE EXPRESSION & SIGNALING IN THE IMMUNE SYSTEM
Tuesday, November 1 – Saturday, November 5, 2022

Tuesday	7:30 pm – 10:30 pm	**1** Regulation of Gene Expression
Wednesday	9:00 am – 12:00 pm	**2** Differentiation
Wednesday	2:00 pm – 5:00 pm	**3** Signaling at the Membrane
Wednesday	5:00 pm	*Wine & Cheese Party*
Wednesday	7:30 pm – 10:30 pm	**Poster Session I**
Thursday	9:00 am – 12:00 pm	**4** Intracellular Signaling
Thursday	2:00 pm – 5:00 pm	**5** Intercellular Communication in 4D
Thursday	7:30 pm – 10:30 pm	**Poster Session II**
Friday	9:00 am – 12:00 pm	**6** Host:Microbe Interactions
Friday	2:00 pm – 5:00 pm	**7** Immune Responses
Friday	6:00 pm	*Cocktails and Banquet*
Saturday	9:00 am – 12:00 pm	**8** Tissue-Immune Communications (Immunophysiology)

All times shown are US Eastern: Time Zone Converter

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

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PROGRAM

TUESDAY, November 1—7:30 PM

SESSION 1 REGULATION OF GENE EXPRESSION

Chairperson: **Ellen Rothenberg,** California Institute of Technology, Pasadena

WEDNESDAY, November 2—9:00 AM

SESSION 2 DIFFERENTIATION

Chairperson: **Marco Colonna,** Washington University School of Medicine, St. Louis, Missouri

WEDNESDAY, November 2—2:00 PM

SESSION 3 SIGNALING AT THE MEMBRANE

Chairperson: **Art Weiss,** HHMI, University of California, San Francisco

WEDNESDAY, November 2—5:00 PM

Wine & Cheese Party

WEDNESDAY, November 2—7:30 PM

POSTER SESSION I

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THURSDAY, November 3—9:00 AM

SESSION 4 INTRACELLULAR SIGNALING

Chairperson: **Jon Kagan,** Boston Children's Hospital / Harvard Medical School, Massachusetts

THURSDAY, November 3—2:00 PM

SESSION 5 INTERCELLULAR COMMUNICATION IN 4D

Chairperson: **Susan Schwab,** NYU School of Medicine, New York

Secondary cues selectively rewire potent, broad-acting antiviral defenses triggered by interferons
Tyron Chang, Sruthi Chappidi, Dustin C. Hancks.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas. 32

A coronin 1-dependent kin-to-kin density-sensing pathway defines T cell population size
Tohnyui Ndinyanka Fabrice, Christelle Bianda, Haiyan Zhang, Rajesh Jayachandran, Julie Ruer-Laventie, Mayumi Mori, Despina Moshous, Geoffrey Fucile, Alexander Schmidt, Jean Pieters.
Presenter affiliation: University of Basel, Basel, Switzerland. 33

Non-coding IL-2RA variation imbalances T cell feedback circuitry to shape autoimmune disease risk
Dimitre R. Simeonov, Kyemyung Park, Jessica Cortez, Arabella Young, Zhongmei Li, Vinh Nguyen, Jennifer Umhoefer, Alyssa C. Indart, Jonathan M. Woo, Mark S. Anderson, John S. Tsang, Harikesh S. Wong, Ronald N. Germain, Alexander Marson.
Presenter affiliation: QIMR Berghofer, Herston, Australia; NIH, NIAID, Bethesda, Maryland; Ragon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts. 34

THURSDAY, November 3—7:30 PM

POSTER SESSION II

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FRIDAY, November 4—9:00 AM

SESSION 6 HOST:MICROBE INTERACTIONS

Chairperson: **Kathy McCoy,** University of Calgary, Canada

Immunology of long COVID
Akiko Iwasaki.
Presenter affiliation: Yale University School of Medicine, New Haven, Connecticut. 35

FRIDAY, November 4—2:00 PM

SESSION 7 IMMUNE RESPONSES

Chairperson: **Alexander Rudensky,** Memorial Sloan Kettering Cancer Center, New York, New York

I will survive—S1P signaling in naive T cell survival
Susan R. Schwab, Dhaval Dixit, Victoria M. Hallisey, Yi Sheng Zhu, Martyna Okuniewska, Jordan Axelrad, Jerry E. Chipuk.
Presenter affiliation: New York University, New York, New York. 42

Priming of innate antiviral immunity by actin cytoskeleton remodeling
Dhiraj Acharya, Rebecca Reis, Meta Volcic, GuanQun Liu, May K. Wang, Bing S. Chia, Rayhane Nchioua, Rüdiger Groß, Jan Münch, Frank Kirchhoff, Konstantin M. Sparrer, Michaela U. Gack.
Presenter affiliation: Cleveland Clinic, Port St. Lucie, Florida; The University of Chicago, Chicago, Illinois. 43

Mechanism of Th2 fate instruction by CD301b$^+$ migratory cDC2s
Naoya Tatsumi, Yosuke Kumamoto.
Presenter affiliation: Rutgers New Jersey Medical School, Newark, New Jersey. 44

The transcription factor ELF4 tunes the inflammatory potential of CD4$^+$ T cells
Sam J. Olyha, Molly L. Bucklin, Paul M. Tyler, Timothy J. Maher, Carrie L. Lucas.
Presenter affiliation: Yale University School of Medicine, New Haven, Connecticut. 45

A prostaglandin E2-dependent subset of IL-1β+ macrophages fuels pathogenic inflammation and immune escape in pancreatic cancer
Renato Ostuni.
Presenter affiliation: San Raffaele Institute, Milan, Italy. 46

Physiological roles of transcription factor Bcl11b in T cell fate determination
Tom Sidwell, Maria L. Quiloan, Ellen V. Rothenberg.
Presenter affiliation: California Institute of Technology, Pasadena, California. 47

FRIDAY, November 4—6:00 PM

COCKTAILS and BANQUET

SESSION 8 TISSUE-IMMUNE COMMUNICATIONS (IMMUNOPHYSIOLOGY)

Chairperson: **Diane Mathis,** Harvard Medical School, Boston, Massachusetts

POSTER SESSION I

New approaches to study the function and distribution of histone modifications in murine immune cells
Dughan Ahimovic, Andrew W. Daman, Michael Bale, Alexia Martinez de Paz, Steven Z. Josefowicz.
Presenter affiliation: Weill Cornell Medicine, New York, New York.
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Large-scale single-cell RNA sequencing of healthy and inflamed terminal ileal samples yields insights into Crohn's disease biology
Tobi Alegbe, Monika Krzak, Dennis L. Taylor, Mennatallah Ghouraba, Michelle Strickland, Vivek Iyer, Guillaume Noell, Gareth Jones, Moritz Przybilla, Kimberley Ai Xian Cheam, Daniele Corridoni, Miles Parkes, Rebecca E. McIntyre, Tim Raine, Carl A. Anderson.
Presenter affiliation: Wellcome Sanger Institute, Hinxton, United Kingdom.
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Binary promoter switches in transcription factors—A model for the digital programming of cell fate determination
Stephen K. Anderson, Hongchuan Li, Ahasanur Rahman, Paul W. Wright, Aharon G. Freud.
Presenter affiliation: Frederick National Laboratory for Cancer Research, Frederick, Maryland; National Cancer Institute, Frederick, Maryland.
View Poster 56

HIC1 interacts with FOXP3 multi-protein complex—A novel mechanism to regulate human regulatory T cell differentiation and function
Syed Bilal Ahmad Andrabi, Kedar Batkulwar, Santosh Bhosale, Robert Moulder, Meraj Khan, Tanja Buchacher, Mohd Moin Khan, Omid Rasool, Ubaid Ullah Kalim, Riitta Lahesmaa.
Presenter affiliation: University of Turku, Turku, Finland.
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Solid phase synthesis of mature peptidoglycan fragments
Madison V. Anonick, Siavash Mashayekh, Catherine Grimes.
Presenter affiliation: University of Delaware, Newark, Delaware.
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Assessing the role of gut microbial metabolites in immune modulation of multiple sclerosis
Martina Antonini, Federico Montini, Annamaria Finardi, Denise Drago, Luca Massimino, Federica Ungaro, Annapaola Andolfo, Cristina Airoldi, Roberto Furlan, Vittorio Martinelli, Marika Falcone.
Presenter affiliation: IRCCS San Raffaele Scientific Institute, Milan, Italy.
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TAP dysfunction in dendritic cells enables noncanonical cross-presentation for T cell priming
Gaëtan Barbet, Priyanka Nair-Gupta, Michael Schotsaert, Adolfo García-Sastre, Julie Magarian Blander.
Presenter affiliation: Rutgers University/CHINJ, New Brunswick, New Jersey.
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Role of cleavage and polyadenylation factor (CFIm25) in macrophage differentiation
Atish Barua, Sremoyee Mukherjee, Claire Moore.
Presenter affiliation: Tufts University, Boston, Massachusetts.
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Drug resistant *Mycobacterium tuberculosis* suppresses host immune responses through type I interferon signaling
Suhas Bobba, Nicole C. Howard, Shibali Das, Mushtaq Ahmed, Nargis Khan, Ignacio Marchante, Linrui Tang, Shyamala Thirunavukkarasu, Luis B. Barreiro, Barun Mathema, Joaquin Sanz, Maziar Divangahi, Shabaana A. Khader.
Presenter affiliation: Washington University School of Medicine, St. Louis, Missouri.
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Setd3-regulated B cell activation and differentiation
Maegan Bunjamin, Kelvin Nai Yao Lam, Xiangfu Guo, Wenting Zhao, I-Hsin Su.
Presenter affiliation: Laboratory of Molecular Immunology and Cell Signalling, Singapore.
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Immune cell expression of the IFNAR1 receptor is regulated by an intronic transposon
Carmen A. Buttler, Edward B. Chuong.
Presenter affiliation: University of Colorado Boulder, Boulder, Colorado.
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Epigenetic regulation of iNKT cell development
Salomé Carcy, Katie Papciak, Semir Beyaz, Hannah V. Meyer.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
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A multi-enhancer hub at the Ets1 locus is required for T cell function
Aditi Chandra, Sora Yoon, Michael F. Michieletto, Naomi Goldman, Emily K. Ferrari, Maria Fasolino, Leonel Joannas, Barbara Kee, Jorge Henao-Mejia, Golnaz Vahedi.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania.
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Typhoid toxin sorting and exocytic transport from *Salmonella* typhi-infected cells
Shu-Jung Chang, Yu-Ting Hsu, Yun Chen, Yen-Yi Lin, Maria Lara-Tejero, Jorge Galan.
Presenter affiliation: Yale University, New Haven, Connecticut; National Taiwan University, Taipei, Taiwan.
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Regulation of heterogeneous gene expression in medullary thymic epithelial cells
Sarah R. Chapin, Aybüke Garipcan, Onur Eskiocak, Sara Goodwin, Semir Beyaz, Hannah Meyer.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
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The role of EMT transcription factor ZEB2 in fetal hematopoiesis
Jing Chen, Xiao Huang, Theresa L. Murphy, Kenneth M. Murphy.
Presenter affiliation: Washington University in St. Louis, Saint Louis, Missouri.
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Epigenetic memory of COVID-19 in innate immune cells and their progenitors
Jin-Gyu Cheong, Arjun Ravishankar, Siddhartha Sharma, Christopher N. Parkhurst, Duygu Ucar, Steven Z. Josefowicz.
Presenter affiliation: Weill Cornell Medicine, New York, New York.
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An integrated Treg transcription factor network reveals context-specific roles for FoxP3
Kaitavjeet Chowdhary, Juliette Leon, Deepshika Ramanan, Diane Mathis, Christophe Benoist.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts.
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A retrotransposon-derived IFNAR2 splice variant functions as an interferon decoy receptor
Giulia I. Pasquesi, David M. Simpson, Lily Nguyen, Atma Ivancevic, Holly Allen, Keala Gapin, Isabella Horton, Qing Yang, Arturo Barbachano-Guerrero, Cody Warren, Benjamin G. Bitler, Sara L. Sawyer, Edward B. Chuong.
Presenter affiliation: University of Colorado Boulder, Boulder, Colorado.
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Characterization of the function of TIF1γ in the control of T regulatory cells
Eugenio Contreras-Castillo, Jose Luis Ramos-Balderas, Paula Licona-Limón.
Presenter affiliation: Cell Physiology Institute, National Autonomous University of Mexico, Mexico City, Mexico.
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IKK-α-dependent histone phosphorylation regulates IFNγ-inducible transcription
Andrew W. Daman, Alexia Martinez de Paz, Dughan Ahimovic, Michael Bale, Chenyang Jiang, Steven Josefowicz.
Presenter affiliation: Weill Cornell Medical College, New York City, New York.
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Role for Lck Y192 in modulating auto-regulatory Lck-Zap70 kinase loop and regulates TCR signaling
Hassan Damen, Denis-Claude Roy, Vibhuti Dave.
Presenter affiliation: University of Montreal, Montreal, Canada.
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Role of *Ikzf3* as a driver mutation in precursor B cell acute lymphoblastic leukemia
Bruno Rodrigues de Oliveira, James Iansavitchous, Heidi Rysan, Wei Cen Wang, Rodney P. DeKoter.
Presenter affiliation: Western University, London, Canada.
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Conditional depletion of BRD4 in microglia leads to a partial resistant to EAE pathology
Anup Dey, Matthew Butcher, Ryoji Yagi, Anne Gegonne, Jingfang Zhu, Dinah Singer, Keiko Ozato.
Presenter affiliation: National Institute of Child Health and Human Development, Bethesda, Maryland.
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Understanding the role of aberrant expression of SP140 splice variant in disease
Adam Dziulko, Edward Chuong.
Presenter affiliation: CU Boulder, Boulder, Colorado.
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BCL6-dependent TCF-1^{+} progenitor cells maintain effector and helper CD4^{+} T cell responses to persistent antigen
Yu Xia, Katalin Sandor, Joy A. Pai, Bence Daniel, Saravanan Raju, Renee Wu, Sunnie Hsiung, Chun Chou, Robert D. Schreiber, Kenneth M. Murphy, Ansuman T. Satpathy, Takeshi Egawa.
Presenter affiliation: Washington University School of Medicine, St. Louis, Missouri.
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Genetic determinants of alternative splicing in stimulated iPSC-derived macrophages enhance the understanding of immune-mediated disease risk.
Omar El Garwany, Nikolaos I. Panousis, Andrew Knights, Natsuhiko Kumasaka, Maria Imaz, Alice Barnett, Lorena B. Vilarino, Anthi Tsingene, Celine Gomez, Daniel J. Gaffney, Carl A. Anderson.
Presenter affiliation: Wellcome Sanger Institute, Hinxton, United Kingdom.
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Z-DNA underlies the target specificity of Aire by promoting poising of transcriptional promoters
Yuan Fang, Christophe Benoist, Diane Mathis.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts; Harvard University, Cambridge, Massachusetts.
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Investigating the role of an intragenic tandem amplification of Pax5 in B cell development and leukemia
Anna Fedl, Sabine Jurado, Markus Jaritz, Kimon Froussios, Salwan Roumaia, Daniela Kostanova-Poliakova, Sabine Strehl, Meinrad Busslinger.
Presenter affiliation: Research Institute of Molecular Pathology, Vienna, Austria.
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To each their own—Distinct pathways to type 2 immunity for disparate allergen classes
Xinyi Feng, Caroline Perner, Caroline L. Sokol.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts.
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OTUD4 balances the proteasomal degradation and nuclear accumulation of the NF-κB transcription factor c-REL
Eslam Katab, Gonca Cetin, Anushree J. Kumar, Jana Zecha, Maike Kober-Hasslacher, Daniel Kovacs, Katja Steiger, Ritu Mishra, Wilko Weichert, Nicole Wimberger, Julia Mergner, Mikel Azkargorta, Felix Elortza, Bernhard Kuster, Daniel Krappmann, Florian Bassermann, Marc Schmidt-Supprian, Vanesa Fernández-Sáiz.
Presenter affiliation: Technical University Munich, School of Medicine, Munich, Germany; Center for Translational Cancer Research, Munich, Germany; Max Planck Institute of Biochemistry, Martinsried, Germany.
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Virus-host cross-transcriptional regulation
Xing Liu, Ted Hong, Kyle Pedro, Anna Berenson, Sreeja Parameswaran, Kevin Ernst, Jared Sewell, Luis Agosto, Andrew Henderson, Matthew Weirauch, Juan Fuxman Bass.
Presenter affiliation: Boston University, Boston, Massachusetts.
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ESCRT-dependent STING degradation curtails steady-state and cGAMP-induced signaling
Matteo Gentili, Bingxu Liu, Malvina Papanastasiou, Deborah Dele-Oni, Marc A. Schwartz, Rebecca J. Carlson, Aziz Al'Khafaji, Karsten Krug, Adam Brown, John G. Doench, Steven A. Carr, Nir Hacohen.
Presenter affiliation: Broad Institute of MIT and Harvard, Cambridge, MA, USA, Cambridge, Massachusetts.
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Transcriptional regulation of the thymus master regulator *Foxn1*
Noam Kadouri, Tal Givony, Shir Nevo, Joschka Hey, Shifra Ben Dor, Golda Damari, Bareket Dassa, Jan Dobes, Dieter Weichenhan, Marion Bähr, Michelle Paulsen, Rebecca Haffner-Krausz, Marcus A. Mall, Christoph Plass, Yael Goldfarb, Jakub Abramson.
Presenter affiliation: Weizmann Institute of Science, Rehovot, Israel.
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Intrinsically disordered domain of transcription factor TCF-1 is required for T cell developmental fidelity
Naomi Goldman, Aditi Chandra, Abhijeet Patel, Ashley Vanderbeck, Ivan Maillard, Golnaz Vahedi.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania.
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Computational modeling of STAT activation features that predict gene expression profiles
Neha Cheemalavagu, Karsen E. Shoger, Yuqi M. Cao, Brandon A. Michalides, Samuel A. Botta, James R. Faeder, Rachel A. Gottschalk.
Presenter affiliation: University of Pittsburgh, Pittsburgh, Pennsylvania; Center for Systems Immunology, Pittsburgh, Pennsylvania.
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***Roseomonas mucosa* adheres to human keratinocytes and supports homeostasis via novel interaction with TLR5**
Portia Gough, Ian Myles.
Presenter affiliation: National Institute of Allergy and Infectious Disease, Bethesda, Maryland.
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SARS-CoV-2 infection and mRNA vaccine prime distinct memory CD4$^+$ T cells
Sophie L. Gray-Gaillard, Sabrina Solis, Grace Ciabattoni, Marie I. Samanovic, Amber R. Cornelius, Ellie N. Ivanova, Sergei B. Koralov, Mark J. Mulligan, Ramin S. Herati.
Presenter affiliation: New York University Grossman School of Medicine, New York, New York.
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Tooth autoimmunity—From broken central tolerance to broken teeth
Yael Gruper, Anette S. B. Wolf, Liad Glanz, František Špoutil, Yonatan Herzig, Yael Goldfarb, Goretti A. Novaliches, Jan Dobeš, Tomáš Wald, Blanka Mrázková, Mihaela Cuida Marthinussen, Marine Besnard, Carole Guillonneau, Knut E.A. Lundin, Radim Osicka, Jan Procházka, Eystein S. Husebye, Jakub Abramson.
Presenter affiliation: Weizmann Institute of Science, Rehovot, Israel.
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Deciphering the role of the C-terminal domains of *Pax5 in vivo*
Sarah Gruenbacher, Markus Jaritz, Louisa Hill, Meinrad Busslinger.
Presenter affiliation: Research Institute of Molecular Pathology (IMP), Vienna, Austria.
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Intercellular transfer within the tumor microenvironment
Kaito A. Hioki, Daniel J. Ryan, Iris Thesmar, Adam C. Lynch, Leonid A. Pobezinsky, Elena L. Pobezinskaya.
Presenter affiliation: University of Massachusetts Amherst, Amherst, Massachusetts.
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The PD-1/PD-L1 signaling axis regulates stem-like progenitor CD8+ T cell differentiation
Jyh Liang Hor, Edward C. Schrom, Ronald N. Germain.
Presenter affiliation: NIAID, NIH, Bethesda, Maryland.
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The divergence of PD-1^{+}CXCR5^{+}CD4^{+} T cell differentiation and stage-specific regulation of GC-Tfh cells
Ryan J. McMonigle, Fangming Zhu, Andrew R. Schroeder, Braxton D. Greer, Edahi Gonzalez-Avalos, Diego O. Sialer, Yin-Hu Wang, Kelly M. Chandler, Adam Getzler, Emily R. Brown, Changchun Xiao, Olaf Kutsch, Matthew E. Pipkin, Hui Hu.
Presenter affiliation: University of Alabama at Birmingham, Birmingham, Alabama.
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Identification of TXP as a molecule involved in antigen cross-presentation
Moe Ikegawa, Takumi Kawasaki, Taro Kawai.
Presenter affiliation: Nara Institute of Science and Technology, Ikoma, Japan.
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Examining transcription factor-cofactor complexes in various T cell states
Melissa Inge, Matthew Lawton, Andrew Emili, Wilson Wong, Trevor Siggers.
Presenter affiliation: Boston University, Boston, Massachusetts.
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Germinal center expansion but not plasmablast differentiation is proportional to peptide-MHCII density via CD40-CD40L signaling strength
Zhixin Jing, Mark J. McCarron, Michael L. Dustin, David R. Fooksman.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York.
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Signaling and antigen-presenting bifunctional receptors for understanding and engineering immunity
Paul Zdinak, Sanya Arshad, Jessica Torrey, Lyubov Kublo, Salome Martinez, Eduardo Zarate-Martinez, Rashi Ranjan, Louise Hicks, Alok V. Joglekar.
Presenter affiliation: University of Pittsburgh, Pittsburgh, Pennsylvania.
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Early responder memory CD4$^+$ T cells modulate heterologous disease outcome
Nikolas Rakebrandt, Nima Yassini, Anna Kolz, Michelle Schorer, Katharina Lambert, Celine Rauld, Zsolt Balazs, Michael Krauthammer, Jose M. Carballido, Anneli Peters, Nicole Joller.
Presenter affiliation: University of Zurich, Zurich, Switzerland.
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NC2 represses the production of immunostimulatory RNAs
Tristan X. Jordan, Benjamin R. tenOever.
Presenter affiliation: New York University Grossman School of Medicine, New York, New York.
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Splicing of ultraconserved poison exon elements in RNA binding proteins reprograms the T effector immune response
Timofey A. Karginov, Antoine Ménoret, Nathan K. Leclair, Karthik Chandiran, Jenny E. Suarez-Ramirez, Keaton Karlinsey, Patrick A. Murphy, Adam J. Adler, Beiyan Zhou, Linda S. Cauley, Olga Anczuków, Anthony T. Vella.
Presenter affiliation: UConn Health, Farmington, Connecticut.
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IL-2 rescues impaired T cell function upon loss of Itk via metabolic reprogramming
Zenia Kaul, Julio Gomez-Rodriguez, Dominic Golec, Julie Reilley, Rafael J. Arguello, Pamela L. Schwartzberg.
Presenter affiliation: NIAID, NIH, Bethesda, Maryland.
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Ablation of cDC1 development by tumor-derived IL-6 through C/EBPβ induction in DC progenitors.
Sunkyung Kim, Jing Chen, Feiya Ou, Stephen T. Ferris, Tian-Tian Liu, Ray A. Ohara, Renee Wu, William E. Gillanders, Theresa L. Murphy, Kenneth M. Murphy.
Presenter affiliation: Washington University in St. Louis, School of Medicine, Saint Louis, Missouri.
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Zbtb20 transcription factor defines innate-like Tregs essential for intestinal homeostasis
Agata K. Krzyzanowska, Rashade A. Hayens II, Damian Kovalovsky, Karen L. Edelblum, Lynn M. Corcoran, Hsin-Ching Lin, Arnold B. Rabson, Lisa K. Denzin, Derek B. Sant'Angelo.
Presenter affiliation: Child Health Institute of New Jersey, New Brunckwick, New Jersey; Rutgers Graduate School of Biomedical Sciences, New Brunckwick, New Jersey.
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Epitranscriptomic shaping of signal transduction controls the development, activation and survival of T cells
Taku Ito-Kureha, Giulia Cantini, Annalisa Marsico, Julian König, Vigo Heissmeyer.
Presenter affiliation: Ludwig-Maximilians-Universität in Munich, Planegg-Martinsried, Germany.
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Large scale nascent RNA analysis of human peripheral leukocyte shows the divergence of immune and stress-responsive transcriptional programs
Seungha A. Lee, Aileen K. Kimm, Eunyoo Kim, Samantha S. Kim, Hojoong Kwak.
Presenter affiliation: Cornell University, Ithaca, New York.
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Identifying novel methylation substrates of SETD3 methyltransferase in immune cells
Nai Yao Lam, Maegan Bunjamin, Alexander Ludwig, Bin Wu, I-Hsin Su.
Presenter affiliation: Laboratory of Molecular Immunology and Cell Signalling, Singapore.
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How faithful are T cell memories?
Hyun Jae Lee, Shihan Li, Michael Bramhall, Cameron G. Williams, Jessica A. Engel, Megan S. Soon, Marcela L. Moreira, Irving Barrera, Evan Murray, Fei Chen, Ashraful Haque.
Presenter affiliation: University of Melbourne, Melbourne, Australia.
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Cytokine-driven integrin expression controls CD8 T cell homeostasis
Can Li, Jung-Hyun Park.
Presenter affiliation: Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
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Control of Foxp3 induction and maintenance by sequential histone acetylation and DNA demethylation
Jun Li, Beisi Xu, Minghong He, Xinying Zong, Trevor Cunningham, Cher Sha, Yiping Fan, Richard Cross, Jacob H. Hanna, Yongqiang Feng.
Presenter affiliation: St. Jude Children's Research Hospital, Memphis, Tennessee.
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A hepatocyte specific interferon-inducible long non-coding RNA, HPN-AS1, controls the induction of type I IFN
Yu-Hsuan Lee, Yu-Chih Lin, You-Jia Chen, Helene Minyi Liu.
Presenter affiliation: National Taiwan University, Taipei City, Taiwan.
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Spatial maps of T cell receptors and transcriptomes reveal distinct immune niches and interactions in the adaptive immune response
Sophia Liu, Bryan Iorgulescu, Shuqiang Li, Mehdi Borji, Irving A. Barrera-Lopez, Vignesh Shanmugam, Haoxiang Lyu, Julia W. Morriss, Zoe N. Garcia, Evan Murray, David A. Reardon, Charles H. Yoon, David A. Braun, Kenneth J. Livak, Catherine J. Wu, Fei Chen.
Presenter affiliation: Harvard University, Boston, Massachusetts; Harvard-MIT, Cambridge, Massachusetts; Broad Institute of MIT and Harvard, Cambridge, Massachusetts.
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Ablation of cDC2 development by triple mutations within the *Zeb2* enhancer
Tian-Tian Liu, Sunkyung Kim, Theresa L. Murphy, Kenneth M. Murphy.
Presenter affiliation: Washington University in St. Louis, School of Medicine, St. Louis, Missouri.
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POSTER SESSION II

A single amino acid substitution in the adaptor LAT accelerates TCR proofreading kinetics and alters T cell selection, maintenance, and function
Wan-Lin Lo, Miriam Kuhlmann, Zhongmei Li, Alexander Marson, Dietmar Zehn, Arthur Weiss.
Presenter affiliation: University of Utah School of Medicine, Salt Lake City, Utah.
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Defective tolerogenic response to commensal LPS in the prediabetic phase promotes gut inflammation and loss of gut barrier integrity in autoimmune diabetes
Marta Lo Conte, Martina Antonini, Vittoria Palmieri, Marika Falcone.
Presenter affiliation: Division of Immunology, Transplantation and Infectious Diseases, Vita-Salute San Raffaele University, Milan, Italy.
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Methods for screening candidate causal regulatory variants in human T cells by CRISPR ribonucleoprotein
Michael H. Lorenzini, Karthyayani Sajeev, Graham McVicker.
Presenter affiliation: Salk Institute for Biological Studies, La Jolla, California; University of California, San Diego, La Jolla, California.
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Mild respiratory SARS-CoV-2 infection can cause multi-lineage cellular dysregulation and myelin loss in the brain
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Presenter affiliation: Yale University, New Haven, Connecticut.
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Presenter affiliation: Cambridge Institute for Medical Research, Cambridge, United Kingdom
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Presenter affiliation: Rockefeller University, New York, New York.
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Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
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Presenter affiliation: Rutgers RWJ Medical School, New Brunswick, New Jersey; Rutgers Graduate School of Biomedical Sciences, New Brunswick, New Jersey.
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Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
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Presenter affiliation: Center for Immunology and Inflammatory Diseases, Boston, Massachusetts; Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts; Harvard Medical School, Boston, Massachusetts.
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Presenter affiliation: Yale University School of Medicine, New Haven, Connecticut.
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Presenter affiliation: Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany.
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Presenter affiliation: UMass, Amherst, Massachusetts.
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Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts; Broad Institute of Harvard and MIT, Cambridge, Massachusetts; Harvard Medical School, Boston, Massachusetts.
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Presenter affiliation: The Rockefeller University, New York, New York.
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Presenter affiliation: Laboratory of Molecular Immunology and Cell Signaling, School of Biological Sciences, Singapore.
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Presenter affiliation: University of Hong Kong, Hong Kong; Centre for Translational Stem Cell Biology, Hong Kong.
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Presenter affiliation: Division of Rheumatology, University of California, San Francisco, San Francisco, California.
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Presenter affiliation: New York University Grossman School of Medicine, New York, New York.
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Presenter affiliation: Howard Hughes Medical Institute, Ludwig Center, Memorial Sloan Kettering Cancer Center, New York, New York.
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Presenter affiliation: Texas A&M University Health Science Center, Bryan, Texas.
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Presenter affiliation: University of Oklahoma Health Science Center, Oklahoma City, Oklahoma.
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Presenter affiliation: NIA, Baltimore, Maryland.
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Presenter affiliation: BC Children's Hospital Research Institute, Vancouver, Canada; BC Cancer Research Center, Vancouver, Canada; University of British Columbia, Bioinformatics, Canada.
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Presenter affiliation: MRC London Institute of Medical Sciences, London, United Kingdom.
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Natalia Duque-Wilckens, Dimitri Joseph, Nidia Maradiaga, V Srinivasan, Yeh Szu-Ying, Eric Nestler, Hari Subramanian, Adam J. Moeser, Alfred J. Robison.
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Presenter affiliation: Instituto de Medicina Molecular João Lobo Antunes, Lisbon, Portugal; H&TRC Health&Technology Research Center, ESTeSL, Lisbon, Portugal.
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Presenter affiliation: Southern Medical University, Guangzhou, China; Zhengzhou University, Zhengzhou, China.
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Presenter affiliation: Laboratory of Clinical Immunology and Microbiology (LCIM), NIAID, NIH, Bethesda, Maryland.
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Presenter affiliation: School of Biomedical Sciences, Hong Kong, China.
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Dongyan Song, Xiaoli S. Wu, Osama E. Demerdash, Kenneth Chang, Keith Rivera, Darryl Pappin, Christopher Vakoc, Nicholas K. Tonks.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Stony Brook University, Stony Brook, New York.
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Felipe Valença-Pereira, Ryan Sheridan, Kent Riemondy, Tina Thorton, Brad Barret, Qian Fang, Gabriela Paludo, Claudia Thompson, Patrick Collins, Mario Santiago, Eugene Oltz, Mercedes Rincon.
Presenter affiliation: University of Colorado School of Medicine, Aurora, Colorado.
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Presenter affiliation: University of Washington, Seattle, Washington.
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CONTROL OF STIMULUS-DEPENDENT RESPONSES IN MACROPHAGES BY SWI/SNF CHROMATIN REMODELING COMPLEXES

Jingwen Liao[1,2], Nasiha S Ahmed[1], Jovylyn Gatchalian[1], Josephine Ho[1], Mannix J Burns[1], Diana C Hargreaves[1]

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In response to microbial ligands and cytokines, macrophages induce a cascade of events resulting in a transcriptional response important for macrophage activation, cytokine release, and the adaptive immune response. This highly coordinated program is executed by stimulus-regulated transcription factors (SRTFs). The binding and activity of SRTFs is in turn restricted to cognate binding sites within 'cis-regulatory elements' made accessible by the action of myeloid lineage-determining transcription factors (LDTFs) working in conjunction with epigenetic regulators. Epigenetic regulators can additionally contribute to gene-specific regulatory requirements that manifest in differences in gene induction kinetics, selectivity, and resolution. Among these, the mammalian SWI/SNF chromatin remodeling complexes exhibit genetic requirements in innate immune cell differentiation and response to inflammation, but the mechanisms by which SWI/SNF complexes control site-specific opening and closing of gene regulatory elements in responding macrophages are unknown. SWI/SNF complexes are a family of polymorphic ATP-dependent chromatin remodeling complexes assembled around a core ATPase, either BRG1 or BRM. These macromolecular machines are thought to utilize energy derived from ATP hydrolysis to remodel nucleosomes on chromatin to create accessible regions. Until recently, the SWI/SNF complex was thought to exist as two main variants: the ARID1A-containing canonical BAF complex (cBAF) and the Polybromo-associated BAF (PBAF) complex. The discovery of the bromodomain-containing protein 9 (BRD9)-associated BAF complex, also known as the ncBAF or non-canonical BAF complex, provides further evidence of compositional heterogeneity within the SWI/SNF complexes in mice and humans. We show that SWI/SNF complex subunits contribute to complex targeting and function through domains that mediate specific interactions with binding partners, TFs, and features on chromatin. Further, using newly developed epigenetic inhibitors targeting subunits unique to particular SWI/SNF complex variants, we demonstrate variant-specific functions. Thus, we propose that biochemical heterogeneity gives rise to functionally specific properties of individual SWI/SNF complex variants, which provides greater regulatory control over transcriptional networks. Finally, the identification of SWI/SNF inhibitors that fine-tune inflammatory responses or provide gene-specific regulation of different functional aspects of the immune response is likely to have therapeutic utility in immune-related diseases.

EPIGENETIC REGULATION AND ADAPTATION IN HOST DEFENSE AND INFLAMMATION

Steven Z Josefowicz

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The lab applies chromatin biochemistry and molecular immunology approaches to understand epigenetic regulation and adaptation in host defense to infection and inflammation. Recent advances have established broad relevance of innate immune memory and the influence of inflammation on hematopoietic development. Molecular and cellular features of these phenotypes are poorly described. We have revealed durable alterations of innate immune and hematopoietic progenitor cells post-COVID-19. Enabled by new approaches, and using combined snRNA/ATACseq, we show that rare circulating HSPC reflect the diversity, proportions, and epigenetic phenotypes of their bone marrow counterparts and demonstrate that this approach can be used to study concepts of innate immune memory and epigenetic memory of infection and inflammation within HSPC populations. We reveal epigenetic reprogramming in progenitors that persists and is conveyed, for months to a year, to short-lived progeny monocytes. We find that epigenetic changes in HSPC are associated with increased myeloid and granulocyte differentiation and persisting migratory/inflammatory programs, with implications for altered immune status post-COVID and potential links to “long COVID”. We use in vitro and animal models to understand function and mechanisms of characteristics changes we observe in human post-COVID hematopoiesis and immune status. One example involves the exploration of AP-1 and IRF transcription factors and their chromatin binding activity as potential mediators of epigenetic memory of inflammation. In another example, we found that dynamic regulation of the histone variant H3.3 is a prominent feature of post-COVID HSPC and monocytes. The dynamic and regulatory histone variant H3.3 has been the subject of the lab’s mechanistic studies for years; we have shown that signals of pathogen sensing and inflammation are transduced directly to H3.3 in chromatin, resulting in H3.3 phosphorylation at target genes, which is critical for augmented transcription of these genes. Thus, we pursue a mechanistic understanding of H3.3 activity in the response to infection, including its positive and negative regulation or dosage, and its specific residues and their modifications. Our studies provide insights into how pathogens and inflammation shape host epigenetic and stem cell phenotypes and the function of these immune adaptations in defense and disease.

REGULATING THE GENOTOXIC POTENTIAL OF SOMATIC HYPERMUTATION

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Somatic hypermutation (SHM) produces point mutations in immunoglobulin (Ig) genes in B cells when uracils created by the activation induced deaminase are processed in a mutagenic manner by enzymes of the base excision repair (BER) and mismatch repair (MMR) pathways. Such uracil processing creates DNA strand breaks and is susceptible to the generation of deleterious deletions. We have found that the DNA repair factor HMCES strongly suppresses deletions without significantly affecting other parameters of SHM in mouse and human B cells, thereby facilitating the production of antigen-specific antibodies. The deletion-prone repair pathway suppressed by HMCES operates downstream of the uracil glycosylase UNG and is mediated by the combined action of BER factor APE2 and MMR factors MSH2, MSH6, and EXO1. HMCES's ability to shield against deletions during SHM requires its capacity to form covalent crosslinks with abasic sites, in sharp contrast to its DNA end joining role in class switch recombination but analogous to its genome stabilizing role during DNA replication. HMCES acts predominantly during the G1 phase of the cell cycle, and deletions generated in the absence of HMCES are strongly dependent on the DNA damage response protein 53BP1. Our findings lead to a novel model for the protection of Ig gene integrity during SHM in which abasic site crosslinking by HMCES intercedes at a critical juncture during processing of vulnerable gapped DNA intermediates by BER and MMR enzymes.

A MULTI-ENHANCER HUB AT THE ETS1 LOCUS ENDOWS COMPETENCE FOR T CELL DIFFERENTIATION

Aditi Chandra, Golnaz Vahedi

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Multi-enhancer hubs are spatial clusters of enhancers which have been recently characterized across numerous developmental programs. Yet, the functional relevance of these three-dimensional (3D) structures is poorly understood. Here we show that the multiplicity of enhancers interacting with the transcription factor Ets1 is essential to control the precise level of this protein in response to cellular cues and the failure to do so can lead to allergic diseases. Focusing on T cells as a model, we identified a highly connected multi-enhancer hub at the Ets1 locus, comprising a noncoding regulatory element that is a hotspot for sequence variation associated with allergic diseases. We deleted this hotspot and found that the multi-enhancer connectivity is dispensable for T cell development but required for CD4+ T helper (Th)1 differentiation in response to changes in the cytokine milieu. Mice lacking this hotpot are thus protected from Th1-mediated colitis but demonstrate an overt allergic response to house dust mite, a T cell-mediated response which is dampened by Th1 cells. Mechanistically, the multi-enhancer hub controls the expression level of Ets1 which is dispensable for the active enhancer landscape but required for the Th1-specific genome folding through recruitment of CTCF protein. Together, we establish a paradigm for the functional and mechanistic relevance of multi-enhancer hubs controlling cellular competence to specifically respond to an inductive cue.

STRATEGIC Igκ LOCUS MODIFICATIONS PROVIDE MECHANISTIC INSIGHTS INTO LONG-RANGE IgH VERSUS Igκ V(D)J RECOMBINATION

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Exons that encode IgH and IgL chain variable regions are assembled in developing B lymphocytes from V, D, and J segments. V(D)J recombination is initiated by RAG endonuclease, which generates DNA breaks between gene segments and flanking recombination signal sequence (RSSs). The mouse IgH locus (*Igh*) contains nearly 100 V_Hs in a 2.4 megabase (Mb) distal portion followed by 8 Ds and 4 J_Hs. In progenitor B cells RAG binds a J_H-based recombination center (RC) and then joins upstream Ds to J_Hs to form a DJ_H-RC. Our lab previously showed that joining of J_Hs to Ds occurs by a linear RAG chromatin scanning mechanism in which D-containing chromatin is presented to the J_H-RC bound RAG by cohesin-mediated loop extrusion (V(D)J recombination video). Cohesin mediates loop formation genome-wide by extruding chromatin between convergently-oriented CTCF looping factor binding elements (CBEs). For RAG-scanning, the RC, which lacks CBEs, serves as a downstream anchor. Remarkably, we have recently shown RAG scanning from a new DJ_H-RC in pro-B cells is extended through the upstream 2.4 Mb V_H domain to bring V_Hs into proximity for joining to DJ_H. Long-range scanning is achieved by neutralization of abundant V_H locus CBEs through down-modulation of the WAPL cohesin-complex unloading factor. By inverting the 2.4 Mb V_H locus, we found that, "deletional" orientation of V_Hs and their RSSs relative to DJ_H RC-RSS is required for recognition during RAG scanning. In the Igκ light chain locus, Vκs join to Jκs, which are the hub of an Igκ-RC, in the next precursor B cell developmental stage. However, Vκs and their RSSs are oriented in both inversional and deletional oriented blocks over the several Mb Vκ locus and, correspondingly are used for robust inversional or deletional V(D)J recombination. While robust inversional Vκ joining is not consistent with direct RAG scanning, such scanning can occur over downstream regions of an Igκ locus in which the Cer/Sis CBE-based scanning impediments between Vκs and Jκs is inactivated. Remarkably, the inverted 3 Mb Vκ locus still supports robust inversional and deletional joining of Vκs that are now in opposite orientation. Also, WAPL levels that impede V_H locus scanning allow normal Vκ rearrangement. We conclude that the Igκ and *Igh* use distinct mechanisms to incorporate their Vs across these loci into their RCs. We will describe new experiments that provide insights in the remarkable mechanism by which the Igκ locus employs cohesin-mediated loop extrusion to present both deletionally- and inversionally-oriented Vκs to the Jκ-RC. These studies implicate a novel Jκ-proximal element that promotes Vκ segments to access a RAG-bound Jκ based RC via short-range diffusional motion, somewhat analogous to how the IgH RC based DQ52-segment accesses the J_H-based RC.

DEEP LEARNING FOR HI-C IDENTIFIES DYNAMIC ENHANCER STATES, NOT GENE EXPRESSION, AS MAJOR DRIVERS OF 3D GENOME REORGANISATION IN T CELL DIFFERENTIATION

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Genomes are intricately organised in 3D nuclear space[1-3], but the extent to which genome form and function are interconnected remains subject to intense debate: 3D topology may be intimately linked to cell type-specific gene expression[4-6], or largely independent of gene regulation[1,7,8]. Here we generate developmental stage-specific maps of enhancer state, gene expression, and chromosome conformation capture (Hi-C) during CD4 T cell differentiation in the thymus to address the relationship between genome form and function. While progression from the DP to the CD4 SP stage of thymocyte differentiation is accompanied by activation and repression of hundreds of genes and enhancers, Hi-C maps of DP and CD4 SP thymocytes are visually highly similar, and conventional Hi-C analysis does not unambiguously link gene expression or enhancer activity to changes in compartments, domains, or loops. To address this conundrum we develop Twins, an AI approach that learns to distinguish technical from biological variation in Hi-C data. Twins uses Siamese neural networks to selectively identify 3D features that differ between biological conditions. At the Mb scale, regions with significant 3D change are enriched for developmentally regulated genes and enhancers. Interestingly, developmental stage-specific enhancers, not promoters, emerge as the principle drivers of 3D change at high resolution. This indicates that genome reorganisation during CD4 lineage choice and differentiation is linked to changes in enhancer state, rather gene transcription. We discuss the implications of these findings for the control of gene expression by distal regulatory elements.

1 Rao et al., 2017, Cell 171:305–20.e24
2 Schwarzer et al., 2017, Nature 551:51–6
3 Nora et al., 2017, Cell 169: 930-44.e22
4 Lupiáñez et al., 2015, Cell 161: 1012-25
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PHOSPHORYLATION OF RUNX PROTEINS CONTROLS THYMOCYTE FATE

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MHC-I and MHC-II selected $CD4^+CD8^+$ precursor thymocytes differentiate into $CD4^-CD8^+$ cytotoxic- and $CD4^+CD8^-$ helper-lineage T cells via induction of lineage-determining transcription factor Runx3 and Thpok, respectively. Runx proteins plays a crucial role in this lineage choice through suppression of *Cd4* and *Thpok* gene in cytotoxic-lineage cells via binding to the silencer in those genes. However, it remains unclear how TCR signals engaged by distinct MHC are linked with regulation of Runx function as well as silencer activity to establish the cytotoxic-lineage specific *Cd4/Thpok* silencing.
Runx proteins possess evolutionarily conserved WRPY sequences at the C-terminal end for interacting with TLE corepressor family proteins. Our current genetic approaches showed that amino acid replacement of the terminal Y to W or F in Runx1 or Runx3 converts Runx protein for constitutive transcriptional repressor. For instance, transgenic expression of $Runx^{WRPW}$ form in precursor thymocytes results in re-direction of MHC-II restricted thymocytes into $CD4^-CD8^-$ or $CD4^-CD8^+$ T cells in a silencer and TLE dependent manner. Mass Spectrometry analyses in Jurkat T cell line detected the phosphorylation of the terminal Y residue in Runx1 and Runx1 associates with Src family kinase proteins. Importantly, Runx-TLE interaction was abolished by phosphatase treatment. Our proximity ligation assay revealed that Lck or Zap70 interacts with Runx1 in the cytoplasm. Lck-Runx1 interaction and phosphorylation at the terminal Y residue in Runx1 were observed more frequently in MHC-I signaled $CD4^-CD8^+$ thymocytes than $CD4^+CD8^-$ thymocytes. Collectively, TCR signaling by distinct MHC is converted to distinct phosphorylation status at the terminal Y residue of Runx to link MHC restriction with the CD4/CD8 lineage choice via controlling a lineage-specificity of the silencers.

TRANSCRIPTIONAL CONTROL OF cDC SUBSETS: IMPACT ON IMMUNE RESPONSES TO PATHOGENS

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Conventional dendritic cells (cDCs) can influence the nature of the adaptive immune response through their influence on T cell differentiation. While in some settings, the terminal differentiation of adaptive responses is influenced by input at sites of infection by other innate immune cells, dendritic cell priming of T cells within lymphoid tissues can also influence the choice of the predominant effector module emerging against a particular pathogen.

Documenting the impact of cDC subsets on the choice of effector module has benefited from the development of various *in vivo* models wherein certain cDC subsets are ablated developmentally, or that allow for gene inactivation in these subsets. Such studies have confirmed a non-redundant role for cDC1 for *in vivo* cross-presentation in response to many viruses and tumors responses, and also uncovered an unknown role in the innate defense against T. gondii. However, while some studies support a role for the cDC2 subsets in promoting T_H2 and T_H17 modules, there has yet to be an effective model system that achieves cDC2 lineage ablation.

Previously, we found that *Nfil3* acted genetically upstream of *Id2*, *Batf3* and *Zeb2* in cDC1 development. Existing NFIL3 reporter mice over-reported expression. Therefore we generated a new NFIL3 reporter, knocking in an NFIL3-GFP fusion protein into the *Nfil3* locus. This reporter revealed that NFIL3 is expressed in a transient pulse in some CDPs, leading to cDC1 specification. CUT&RUN and ChIP-seq identified three NFIL3 binding in the already reported –165 kb *Zeb2* enhancer. These sites also bind C/EBPα and C/EBPβ *in vivo*. By mutating these sites in vivo, we found that these NFIL3–C/EBP sites are functionally redundant, and that C/EBPs support *Zeb2* expression, while NFIL3 inhibits. A mouse line with mutations at all three NFIL3–C/EBP sites was found to totally ablate *Zeb2* expression in myeloid progenitors, but not in lymphoid progenitors, and to have a complete loss of pre-cDC2 specification and mature cDC2 development. Thus, CDP divergence into cDC1 or cDC2 lineages is controlled by competition between NFIL3 and C/EBPs at the –165 kb *Zeb2* enhancer. So far, our functional analysis shows that tumor rejection of immunogenic fibrosarcomas remains intact in these mice, and that antibody affinity maturation continues to take place. However, we confirm our previous results that cDC2 are required for T_H2 responses to the helminth *Heligmosomoides polygyrus*. This system should allow for a deeper dissection of this requirement.

EARLY LIFE DETERMINANTS OF IMMUNE TOLERANCE

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The immune system must balance immunity to pathogens with tolerance to self and innocuous foreign antigens in order to avoid destructive autoimmune and inflammatory diseases. Within the thymus, medullary thymic epithelial cells (mTECs) expressing the AutoImmune Regulator gene, *Aire*, play a critical role in self-tolerance through deletion of autoreactive T cells and promotion of thymic regulatory T (Treg) cell development. Within weeks of birth, a second wave of Treg cell differentiation occurs in the intestine, in response to colonization by the gut microbiota, yet the cell types responsible for the generation of peripheral Treg (pTreg) cells are not known. Here we identified a new lineage of tolerogenic RORγt$^+$ antigen-presenting cells (APC) with a hybrid dendritic cell (DC)-mTEC phenotype, dubbed Thetis cells (TCs), comprising 4 major sub-groups (TC I-IV). We uncovered a developmental wave of TCs within intestinal lymph nodes during a critical early life window, coincident with the wave of pTreg cell differentiation. While Aire$^+$ TC I and III bore remarkable homology with Aire$^+$ mTECs, including expression of tissue restricted self-antigens, TC IV lacked Aire expression and were enriched for molecules required for pTreg generation, including the TGF-β activating integrin αvβ8. Loss of either MHCII or Itgb8 expression by TCs led to a profound impairment in intestinal pTreg differentiation, with onset of intestinal inflammation. In contrast, MHCII expression by RORγt$^+$ group 3 innate lymphoid cells (ILC3) and classical DCs was neither sufficient nor required for pTreg generation, further implicating TCs as the critical tolerogenic RORγt$^+$ APC. Our studies reveal parallel pathways for establishment of tolerance to self and foreign antigen within the thymus and periphery, marked by involvement of shared cellular and transcriptional programs.

THE TRANSCRIPTION FACTORS ZFP148 AND ZFP281 COOPERATE WITH GATA3 TO SUPPORT CD4$^+$ T CELL DEVELOPMENT AND FUNCTIONS.

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The transcription factor Gata3 is essential both for CD4$^+$ T cell development in the thymus and for the differentiation of Th2 cells, which are essential for responses against extracellular parasites and contribute to asthma and allergies. Because enforced expression of Gata3 is not sufficient to promote CD4$^+$-lineage differentiation, we speculated that additional transcription factors would assist its functions. Mining the transcriptome of differentiating thymocytes, we identified the related transcription factors Zfp148 and Zfp281 as potential candidates for this function. Genetic and biochemical analyses found that Zfp281, in part redundantly with Zfp148, promotes CD4$^+$ T cell development and matching of CD4$^+$-lineage differentiation to MHC-II specificity. Because Zfp281 binds a cis-regulatory element previously shown to recruit Gata3, and because Gata3 and Zfp281 associate in vitro, we considered the possibility that Zfp281 would also cooperate with Gata3 to promote Th2 differentiation. Accordingly, we found that Zfp281 and Zfp148 promote Th2 cytokine production both in vitro and in vivo and that Zfp281 binds to the type 2 cytokine chromosomal locus. In a mouse model of airway inflammation, Zfp148/281-deficient CD4$^+$ T cells failed to express Th2 cytokines, despite normal activation and expression of Gata3. Assessing Th2 cells by single-cell 'multiomics', which jointly measures gene expression and chromatin accessibility in the same cell, we found that Zfp148 and Zfp281 promote both the gene expression of *Il4*, *Il5*, and *Il13* and chromatin accessibility around these cytokine loci. However, despite this effect on chromatin accessibility in Th2 cells, Zfp148 and 281 are not required for Gata3 binding to cytokine loci. Altogether, these findings demonstrate that the redundant activity of Zfp148 and Zfp281 is essential for both CD4$^+$ T cell development and Th2 function and thereby identify these proteins as novel cofactors for Gata3 functions in the T lineage.

TOOTH AUTOIMMUNITY: FROM BROKEN CENTRAL TOLERANCE TO BROKEN TEETH

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Ameloblasts are specialized epithelial cells in the jaw, which facilitate the formation of the hardest body tissue – the enamel layer. This process involves activity of multiple ameloblast-derived proteins, whose loss of function results in a rare congenital disorder – amelogenesis imperfecta. Defects in enamel formation are also found in patients suffering from the Autoimmune polyglandular syndrome type-1 (APS-1), caused by AIRE deficiency with still unknown underlying mechanisms. Here we show that AIRE induces ectopic expression of a battery of ameloblast specific proteins in the thymus and that both APS-1 patients and Aire-deficient rodents develop autoantibodies against the enamel matrix, which consequently interfere with enamel formation. Therefore, these findings help uncover a new type of autoimmune disorder - Autoimmune Amelogenesis Imperfecta (AAI) – which is characterized by the autoimmune destruction of the permanent teeth. Moreover our findings also suggest that AAI may be more common as it can accompany other autoimmune diseases.

CONTROL OF THE T CELL AND INNATE LYMPHOID CELL DECISION IN THE THYMUS BY A TIMED EPIGENETIC SWITCH

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Stem and progenitor cells delay lineage commitment decisions when faced with differentiation signals, sometimes for extended periods lasting many cell generations. These timing delays in decision making could be important for regulating the lineage output of stem cells as they differentiate. Here, we investigate these ideas in the differentiation of immune progenitors in the thymus. Despite continual exposure to instructive signals, early thymic progenitors maintain a prolonged state of multipotency – lasting for 5-10 days – in which they can differentiate into either T cells or innate lymphoid cells (ILCs). This commitment delay arises due to a cis-epigenetic switch that generates a stochastic, multi-day delay in the activation of Bcl11b (Ng et al. eLife. 2018; Pease et al. Cell Rep. 2021), a transcription factor that is essential for T cell lineage commitment, but also facilitates development of Type 2 innate lymphoid cells (ILC2s). To investigate the role of this Bcl11b activation delay in differentiation control, we studied progenitors harboring a deletion of a distal Bcl11b cis-regulatory element. Deletion of this 'timing enhancer' prolonged the delay in activation of the Bcl11b epigenetic switch, but did not affect Bcl11b expression levels or T cell function after activation. This activation delay reduced thymic T cell output but enhanced the generation of thymic ILCs. We further found that delaying Bcl11b activation promoted ILC diversion by enabling the emergence of an early ILC transcriptional program in multipotent progenitors, marked by heightened expression of the ILC transcription factor PLZF. Despite the delay in Bcl11b expression, ILC-primed progenitors were still capable of later activating Bcl11b to permit the generation of Bcl11b-dependent ILC2 cells. These results highlight the importance of timing and order within developmental gene regulatory networks and their control of population sizes and proportions of differentiated cell types.

OCA-T1 AND OCA-T2 ARE COACTIVATORS OF POU2F3 IN THE TUFT CELL LINEAGE

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Tuft cells are a rare chemosensory lineage that coordinates immune and neural responses to foreign pathogens in mucosal tissues. Recent studies have also revealed tuft-cell-like human tumours, particularly as a variant of small-cell lung cancer. Both normal and neoplastic tuft cells share a genetic requirement for the transcription factor POU2F3, although the transcriptional mechanisms that generate this cell type are poorly understood. Here we show that the binding of POU2F3 to the uncharacterized proteins C11orf53 and COLCA2 (renamed here OCA-T1/POU2AF2 and OCA-T2/POU2AF3, respectively) is critical in the tuft cell lineage. OCA-T1 and OCA-T2 are paralogues of the B-cell-specific coactivator OCA-B; all three proteins are encoded in a gene cluster and contain a conserved peptide that binds to class II POU transcription factors and a DNA octamer motif in a bivalent manner. We demonstrate that binding between POU2F3 and OCA-T1 or OCA-T2 is essential in tuft-cell-like small-cell lung cancer. Moreover, we generated OCA-T1-deficient mice, which are viable but lack tuft cells in several mucosal tissues. Furthermore, the OCA-T1 knockout mice are defected in type II immune response upon IL-25 treatment. These findings reveal that the POU2F3–OCA-T complex is the master regulator of tuft cell identity and a molecular vulnerability of tuft-cell-like small-cell lung cancer. Additionally, the heterogeneous phenotype of OCA-T1 deficient mouse in tuft cell deficiency suggested that tuft cells from different tissues are regulated differently transcriptionally, which might be linked to the distinct functions of tuft cells in different tissues.

FUELLING T CELLS

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The immune activation of T cells requires the precise control of protein synthesis to allow the implementation of T cell transcriptional programs. Protein synthesis is critically dependent on the availability of amino acids. These can be obtained from the environment by the selective transport of amino acids across the plasma membrane but one other key pathway of amino acid supply is via autophagy, a recycling process that degrades intracellular proteins to supply amino acids to cells. Autophagy controls CD8 T cell survival and memory T cell differentiation but autophagy triggers and how autophagy shapes CD8 T cell proteomes is not understood. We have identified three critical regulators of CD8 T cell autophagy amino acid transporter levels, extracellular amino acid concentration and the activity of the class III PI3K VPS34. Naïve T cells have high VPS 34 controlled autophagic flux; these cells switch off autophagy when antigen receptor engagement induces amino acid transporter expression and increases intracellular amino acid delivery . Effector cytotoxic T cells with high levels of amino acid transporters have low autophagic flux but rapidly reinduce VPS34 controlled autophagy when amino acid supply is restricted. Mass spectrometry that maps the impact of amino acid deprivation and VPS34 inhibition on CTL proteomes identify substrates for VPS34 dependent degradation and informs how amino acid generated by autophagy are repurposed. The study reveals how VPs34 directed degradation of effector molecules and metabolic and cell cycle machinery supports the production of molecules that coordinate T cell migration and quiescence.

CONTROL OF POLARIZED SIGNALING IN CD8+ CYTOTOXIC T LYMPHOCYTES

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Cytotoxic T lymphocytes (CTLs) of the immune system are remarkably polarized cells that kill virally infected and cancer cells with remarkable specificity. In order to do so, they use their centrosome to direct secretion to destroy their targets, without destroying healthy neighbouring cells. Killing is controlled by the strength of receptor signaling after recognition with the strength of signal co-ordinating multiple events leading to centrosome docking and successful secretion. The effectiveness of CTL-mediated killing is enhanced by the ability of individual CTLs to serially kill multiple target cells, requiring CTLs to both engage and then disengage with targets. In order to understand these events we have used electron microscopy tomography with APEX-tagged T cell receptors (TCRs) and actin to follow the localization of TCRs from the point when killer cells first engage to the point when they disengage. Our results reveal a novel mechanism for self-limiting signaling that maintains cell polarity and can facilitate serial killing.

SINGLE MOLECULE ANTIGEN SENSITIVITY IN T CELLS IS ENABLED BY A PROTEIN CONDENSATION PHASE TRANSITION OF LAT

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LAT assembly into a two-dimensional protein condensate is a prominent feature of antigen discrimination by T cells. The high multivalency of LAT phosphotyrosine sites, along with several crosslinking interactions among various adaptor proteins, enables extended clustering into an elaborate signaling complex. While this basic behavior has been known for many years, more recent studies of LAT, Grb2, and SOS reconstituted on supported membranes have revealed that LAT undergoes a particular type of two-dimensional protein condensation phase transition, which offers some distinctly different behavior than a strongly driven clustering process. Formation of the LAT condensate has also recently been discovered to facilitate release of autoinhibition in SOS, thus enabling Ras activation, and possibly providing a signal gating function in T cells[1]. Here, we use single-molecule imaging techniques to resolve the spatial position and temporal duration of each pMHC:TCR molecular binding event while simultaneously monitoring LAT condensation at the membrane. An individual binding event is sufficient to trigger a LAT condensate, which is self-limiting, and neither its size nor lifetime is correlated with the duration of the originating pMHC:TCR binding event. Only the probability of the LAT condensate forming is related to the pMHC:TCR binding dwell time. LAT condenses abruptly, but after an extended delay from the originating binding event. A LAT mutation that facilitates phosphorylation at the PLC-γ1 recruitment site shortens the delay time to LAT condensation and alters T cell antigen specificity. These results identify a role for the LAT protein condensation phase transition in setting antigen discrimination thresholds in T cells.More broadly, this system represents an example of how qualitatively different behavior can be obtained from a specific type of protein condensation phase transition as compared with more general clustering processes--and I will discuss these differences.

1.*Science* 2019, **36**3: 1098-1103: “A molecular assembly phase transition and kinetic proof reading modulate Ras activation by SOS”, William Y. C. Huang, Steven Alvarez, Yasushi Kondo, Young Kwang Lee, Jean K. Chung, Hiu Yue Monatrice Lam, Kabir H. Biswas, John Kuriyan, Jay T. Groves*.

UNVEILING THE MOLECULAR BASIS OF T CELL MALFUNCTIONS AND DISORDERS USING MULTI-OMICS APPROACHES

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T cells play a central role in adaptive immunity. Although the T cell antigen receptor (TCR) primarily controls T cell physiology, it does not work in isolation and the signals it triggers are tuned by a multitude of other surface receptors that deliver positive (costimulators) and negative (coinhibitors) informations about the state of activation of antigen-presenting cells (primarily dendritic cells). Therapeutic antibodies (immune-checkpoint inhibitors) blocking coinhibitors have become standard treatment for several malignant conditions, leading to a revival in the study of T cell coinhibition and costimulation. However, we lack a satisfying comprehension of the way T cells integrate inputs from multiple signalling pathways and use inter-pathway crosstalk to make informed decisions. To make sense of the formidable complexity of the signal transduction networks involved in T cell activation and the role played by the different types of dendritic cells in T cell activation, we combined "omic" and mouse genetics. It allowed us to decipher in a time-resolved and quantitative manner the dynamics of the protein signaling complexes (signalosomes) that assemble in primary T cells following physiologic TCR engagement. To further illustrate the interest of multi-omics approaches, I will present recent data demonstrating how corrupted LAT signalosomes lead to an inflammatory and autoimmune disease recapitulating human IgG4-related disease. Data will be also presented revealing that, although humans and mice have evolved independently for over 90 million years, their TCR-signaling network has an unexpected high degree of qualitative and quantitative conservation.

STRUCTURE OF A JANUS KINASE CYTOKINE RECEPTOR COMPLEX REVEALS THE BASIS FOR DIMERIC ACTIVATION

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Cytokines signal through cell surface receptor dimers to initiate activation of intracellular Janus Kinases (JAKs). JAKs are large, multidomain proteins consisting of a receptor binding FERM-SH2 domain, a regulatory pseudokinase (PK) domain, and a catalytically active kinase domain. Despite the importance of this signaling pathway, how receptor dimerization leads to JAK activation has remained poorly understood. We determined the 3.6Å resolution cryo-EM structure of full-length JAK1 complexed with a cytokine receptor intracellular Box1/Box2 domain, captured as an activated homodimer bearing the Val→Phe (VF) mutation prevalent in myeloproliferative neoplasms. The seven domains of JAK1 form an extended structural unit whose dimerization is mediated by close-packed pseudokinase (PK) domains. The oncogenic VF mutation lies within the core of the JAK1 PK dimer interface, enhancing packing complementarity to facilitate ligand-independent activation. Mapping of constitutively active JAK mutants supports a two-step allosteric activation mechanism and reveals new opportunities for selective therapeutic targeting of oncogenic JAK signaling.

THE PHOSPHATIDYLINOSITOL-TRANSFER PROTEIN NIR3 MODULATES T CELL DEVELOPMENT AND FUNCTION

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Before emerging as functional T lymphocytes, thymocytes transit through multiple selection stages during which T cell antigen receptor (TCR) signaling controls the survival and subsequent maturation. Hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) by phospholipase C-γ (PLCγ1) represents a critical step in T cell receptor (TCR) signaling which leads to calcium increases as well as PKC and Ras activation, events that contribute to T cell activation. PIP2 in the plasma membrane (PM) is depleted rapidly upon TCR stimulation and the replenishment of PIP2 levels is dependent on delivery of its precursor phosphatidylinositol (PI) from the endoplasmic reticulum (ER) to the PM. It was not clear how the delivery of PI from ER is regulated in thymocytes and T cells or how the process impacts T cell development and function. Here, we show that a membrane-associated PI transfer protein, Nir3 (Pitpnm2), promotes PIP2 replenishment following TCR stimulation and is important for T cell development. The high expression level of the Nir3 gene in thymocytes suggests its role in thymocyte selection. In Nir3 deficient thymocytes, the replenishment of PIP2 following TCR stimulation is significantly slower. Moreover, Nir3 deficiency attenuates calcium mobilization in DP thymocytes in response to weak TCR stimulation. The impaired TCR signaling led to impaired thymocyte development at the beta-selection and positive selection stages in Nir3 deficient mice. These findings reveal the role of Nir3 in TCR signaling and thymocyte development. This study highlights the importance of PIP2 replenishment and the role of Nir3 in PI transfer through regulated ER-PM interactions during TCR signaling.

A CRISPR SCREEN TARGETING PI3K EFFECTORS IDENTIFIES RASA3 AS A NEGATIVE REGULATOR OF LFA-1-MEDIATED ADHESION IN T CELLS

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The integrin lymphocyte function–associated antigen 1 (LFA-1) helps to coordinate the migration, adhesion, and activation of T cells through interactions with intercellular adhesion molecule 1 (ICAM-1) and ICAM-2. LFA-1 is activated during the engagement of chemokine receptors and the T cell receptor (TCR) through inside-out signaling, a process that is partially mediated by phosphoinositide 3-kinase (PI3K) and its product phosphatidylinositol(3,4,5)P3 (PIP3). However, the PI3K effectors that contribute to inside-out signaling remain poorly defined. To evaluate potential roles of PI3K in LFA-1 activation, we designed a library of CRISPR/single guide RNAs targeting known and potential PIP3-binding proteins and screened for effects on the ability of primary mouse T cells to bind ICAM-1. We identified multiple proteins that regulated the binding of LFA-1 to ICAM-1, including the Rap1 and Ras GTPase-activating protein RASA3. We found that RASA3 suppressed LFA-1 activation in T cells and that its expression was rapidly decreased upon T cell activation, suggesting RASA3 plays a major role in controlling LFA-1 activation in naïve T cells. Notably, despite PI3K and pleckstrin homology domain-dependent recruitment to the plasma membrane, RASA3 activity was inhibited by PI3K. Loss of RASA3 in T cells led to increased Rap1 activation, defective lymph node entry and egress, and impaired responses to T-dependent immunization in mice. Our results reveal a critical role for RASA3 in T cell migration, homeostasis, and function.
This work was supported in part by the Intramural Research Programs of NIAID and NHGRI, NIH. Animal studies were carried out under approved protocol G98.3 from NHGRI, NIH.

SENSING DNA AS A DANGER SIGNAL THROUGH THE cGAS-STING PATHWAY

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The life of any organism depends on the ability of cells to detect and to respond to pathogens. In order to detect the immense variety of pathogenic entities, the innate immune system of mammals has evolved a range of distinct sensing strategies. One major mechanism is based on the recognition of microbial DNA - an invariant and highly immunogenic pathogen-associated molecular pattern. Host cells, however, contain abundant sources of self-DNA. In the context of cellular damage or metabolic derangement, “out-of-the-context” self-DNA can elicit potentially damaging inflammatory responses. Our research focuses on the so-called cGAS-STING system - an evolutionary highly conserved innate DNA sensing system. On DNA binding, cGAS is activated to produce a second messenger cyclic dinucleotide (cyclic GMP-AMP), which stimulates the adaptor protein STING to induce innate immune responses. While this process was originally discovered as a crucial component of the immune defense against pathogens, recent work has elucidated a pathogenic role for innate DNA sensing in a variety of sterile inflammatory diseases. In this talk I will discuss recent findings on cellular mechanisms that regulate STING activity and present novel work on the role of cGAS-STING to the development of ageing-associated neurodegeneration.

STRESS GRANULES AND ANTIVIRAL SIGNALING

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Self and non-self nucleic acid discrimination is a fundamental basis for pathogen detection in all organisms. Unlike the bacterial CRISPR system that detects pathogen-derived nucleic acid sequence, the vertebrate innate immune system does not directly read out DNA/RNA sequence, raising the question of how it distinguishes self from non-self. Our work on the RIG-I-like receptors (RLRs) in the vertebrate innate immune system led to the discovery of receptor polymerization and clustering as key mechanisms for foreign RNA detection and immune activation. Our group elucidated how RLR filament formation enables detection of various types of viral and host RNA signatures, such as secondary structure and modification, and integration of such disparate information for RNA discrimination. In this talk, I will discuss our recent findings on the role of molecular condensates, namely stress granules, in regulating the RLR signaling pathway.

NEW NANOMACHINES FOR CYTOSOLIC IMMUNITY TO INFECTION

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All living organisms deploy cell-autonomous defenses to combat infection. In plants and animals, these activities often use large supramolecular complexes to assemble immune proteins for protection. Here, we describe the native structure of a massive antimicrobial complex generated by polymerization of 30,000 human guanylate-binding proteins (GBPs) over the entire surface of virulent bacteria. Construction of this giant nanomachine takes ~1-3 minutes, remains stable for hours, and acts as a nucleating platform for cytokine and cell death signaling directly atop the coated bacterium. Cryo-ET of this “coatomer” revealed thousands of human GBP1 molecules undergo ~260 Å elongation to insert into the bacterial outer membrane, triggering lipopolysaccharide release that activates co-assembled Caspase-4. Together, our results provide an unprecedented view of how the GBP coatomer mobilizes cytosolic immunity to combat infection in humans.

THE PORE-FORMING PROTEIN GASDERMIN D IS A CELLULAR REDOX SENSOR.

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Reactive oxygen species (ROS) affect cellular processes in a multitude of ways, from the covalent modification of proteins and nucleic acids to activities as signaling entities and the induction of cell death. In innate immunity, ROS are known to play a role in the inflammatory response by promoting priming and activation of inflammasomes, the signaling hubs which induce an inflammatory form of cell death known as pyroptosis. However, whether ROS also regulate additional steps in the pyroptotic pathway remains less understood. We recently provided evidence that cells deficient for components of the Ragulator-Rag-mTORC1 axis exhibit defects in oligomerization of the pore forming protein gasdermin D (GSDMD) and downstream pyroptotic lysis. The defect in GSDMD oligomerization and pore formation correlated with lower levels of cellular ROS. Herein, we show that ROS produced in response to various stimuli, including common inflammasome priming agents or environmental toxins, promote GSDMD oligomerization and pyroptosis. ROS from diverse sources could act in parallel to Ragulator-Rag control of basal ROS, and thus induced ROS could functionally compensate for genetic loss of Ragulator-Rag. Furthermore, we show that ROS enhances GSDMD pore formation through oxidative modification of a specific cysteine residue (C192) in the N-terminal fragment of GSDMD. Cysteine oxidation within GSDMD depends on genetic sufficiency for RagA or treatment with ROS inducing agents that promote oligomerization and pore formation. These findings suggest that GSDMD acts as a redox sensor in response to cellular ROS levels in a cysteine-dependent manner to regulate its activity. Overall, our findings uncover novel aspects in the regulation of pyroptotic cell death by bolstering the relationship between ROS and GSDMD pore formation and suggest GSDMD oxidation as a biomarker of productive inflammasome activation.

AN ARCHITECTURAL ROLE FOR CELLULAR RNA AT THE MAVS SIGNALOSOME

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Immune signaling is tightly regulated to promote pathogen clearance, while preventing tissue damage. An antiviral innate immune response is activated by the detection of viral RNA motifs by RIG-I-like receptors (RLRs) which induce the production of interferons and antiviral genes. Signal transduction downstream of RLRs proceeds when several effectors coalesce at a multi-protein complex organized around the adaptor protein MAVS, termed the MAVS signalosome. Many protein-protein and protein-membrane interactions as well as post-translational modifications are required for the proper formation and function of the MAVS signalosome. While RNA molecules have been shown to modulate protein complex function by serving as molecular decoys, guides or scaffolds, whether RNA plays an architectural role at the MAVS signalosome remains unknown. We have found that MAVS directly interacts with cellular RNA through its conserved central intrinsically disordered domain. Ribonuclease (RNase) treatment disrupts the migration of MAVS and MAVS signalosome proteins through a sucrose gradient, indicating that RNA promotes MAVS signalosome formation. RNase treatment also inhibits in vitro phosphorylation of the transcription factor IRF3 by the MAVS signalosome during immune activation by viral RNA and non-RNA RIG-I agonists. Together, these findings support the hypothesis that cellular RNA molecules promote key protein-protein interactions at the MAVS signalosome for efficient immune activation. Therefore, this work has uncovered new RNA-centric mechanisms by which antiviral immune signaling is regulated.

MAMMALIAN STING FIBRIL FORMATION IS UNDER INTERNAL AND EXTERNAL REGULATION FOR MAINTAINING SIGNALING HOMEOSTASIS

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STING oligomer fibril formation leads to inflammatory cytokine production, autophagy, and cell death. We hypothesized that the formation and resolution of STING oligomers is critical to signaling homeostasis. Using a genome-wide CRISPR screen for regulators of STING activity, we found that loss of DNAJC13 led to accumulation of high-order STING oligomers, translocation of STING to Golgi/endosomes and increased STING-IRF3 signaling and autophagy. Upon STING activation, we observed enhanced physical association of STING with DNAJC13, and DNAJC13-dependent enhanced binding with HSP70, a critical factor for oligomer disaggregation. To test whether oligomerization is sufficient to mimic STING-agonist induced cellular phenotypes, we forced oligomer formation using an FKBP12-STING fusion protein and recapitulated these phenotypes. We further discovered that loss of the conserved STING Ser272-Arg284 interaction led to spontaneous activation by mimicking WT STING oligomerization. Thus, extrinsic and intrinsic regulation of STING oligomerization is critical in controlling the activity of the STING pathway.

INNATE IMMUNE CELL FATE DETERMINATION BY A NETWORK OF NUCLEATED POLYMERS

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Innate immune determination of cell fates such as programmed cell death coincides with the formation of large protein complexes called signalosomes. Signalosomes consist of a sensor, adaptor, and effector component, each containing at least one member of the Death Domain, TIR domain, and RHIM protein interaction modules. Previously, we found that a nucleation barrier to signalosome assembly gives rise to binary immune proinflammatory activation. Here, we aimed to uncover the broader role of nucleation barriers in innate immune cell fate determination. First, we applied Distributed Amphifluoric FRET (DAmFRET) to detect nucleation frequency as a function of intracellular protein concentration for 128 human protein modules. We found that 46 modules assemble in a nucleation-limited manner, 11 lack a nucleation barrier to assembly, and 68 do not assemble. Notably, 4 out of 5 signalosome adaptor proteins exhibit a nucleation barrier, suggesting that these central nodes in immunity signaling pathways contribute to the switch-like nature of innate immune activation. We then designed and carried out a massive protein-specific nucleating interaction screen to determine the critical components of signalosomes. The results of the screen accurately recapitulated known signalosomes such as the inflammasome. However, we also discovered inter-signalosome nucleating interactions linking pyroptosis and apoptosis cell death pathways. We are currently exploring whether FADD and ASC adaptor proteins engage in bidirectional activation of cell death programs using optogenetic tools to selectively nucleate specific proteins. Altogether, our results reveal that sequence-encoded nucleation barriers govern cell fates by acting as irreversible switches. Collectively, this information should expand our understanding of the root causes of age-associated inflammation.

GPR35 PROMOTES MYELOID CELL RECRUITMENT IN RESPONSE TO PLATELET- AND MAST CELL-DERIVED 5-HIAA

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Rapid recruitment of myeloid cells is crucial for innate immune responses. In recent work, we revealed that the G-protein-coupled receptor GPR35 is upregulated in activated neutrophils, and it promotes their migration. GPR35-deficient neutrophils are less recruited from blood vessels into inflamed tissue, and the mice are less efficient in clearing peritoneal bacteria. Using a bioassay, we found that serum and activated platelet supernatant stimulated GPR35, and we identified the platelet-derived serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) as a GPR35 ligand. GPR35 function in neutrophil recruitment was strongly dependent on platelets, with the receptor promoting transmigration across platelet-coated endothelium. Activated mast cells also attracted GPR35+ cells via 5-HIAA. Loss of GPR35 from neutrophils, pharmacological inhibition of serotonin conversion to 5-HIAA, or genetic deficiency in 5-HIAA production by platelets and mast cells was associated with reduced neutrophil recruitment to inflamed tissue. These findings identified 5-HIAA as a GPR35 ligand and neutrophil chemoattractant and established a role for platelet- and mast cell-produced 5-HIAA in cell recruitment to sites of inflammation and bacterial clearance. In ongoing work, we have identified a role for GPR35, 5-HIAA, platelets and mast cells in recruitment of myeloid cells to the pathogen-infected lung. Our most recent advances on this project will be presented.

USING IMAGING IN VITRO AND IN VIVO TO STUDY IMMUNE CELL SIGNALING, COMMUNICATION, AND FUNCTION

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Deciphering signaling events occurring at the single cell level, between pairs of cells, and in complex multicellular environments requires visualization of the biochemical processes involved in signaling across time as well as the communication among cells at various scales of tissue organization. We have developed a variety of imaging methods to enable such analyses, ranging from real-time in vitro methods to intravital 2-photon imaging in live animals, highly multiplex static imaging in thin sections, and multiplex analysis of 3D volumes. For tracking signals downstream of surface receptors in real time at the single cell level, we have developed an integrated imaging and computational approach called FILMSTAR and applied it with Jurkat T cells expressing a defined TCR and live reporters for p65 NF-κB, ERK, JNK, p38, and NFAT. These cells were stimulated with a panel of pMHC ligands to probe the events associated with PD-1 engagement by PD-L1. PD-1 : PD-L1 interaction suppressed the TCR signals in a hierarchical manner (NF-κB > NFAT > ERK) influenced by pMHC ligand quality and amount. The cis interaction of CD80 with PD-L1 results in synergistic enhancement of both T cell and CD28 signaling. These data provide strong support for prior studies suggesting that PD-1 acts primarily on TCR signaling. In a tumor environment with diverse pMHC ligands for distinct TCRs, checkpoint therapy will lead to non-linear effects, with T cells experiencing not simply more or less input, but rather changing ratios of the signaling events whose balance dictates response.
To study cellular organization in tissues, we have developed a highly multiplex iterative imaging method called IBEX. Because of the vast amount of data generated from such multiplex staining of thin sections, we have created a machine learning-based software suite called RAPID that enables pixel-level or object-based analysis, automating the definition of cell subsets and providing input for another software tool called SPACE that identifies unknown patterns in cell organization across scales of complexity. To expand these analyses to larger volumes of tissue, we have developed a new clearing method (Ce3D) and a pipeline for iterative staining based on IBEX (Ce3D-IBEX) that enables >25 parameters to be assessed in tissue hundreds of microns thick. Examples of how these tools can provide insight into the processes involved in immune response regulation, tumor immunity, and infectious diseases will be presented.
This work was supported by the Intramural Research Programs of NIAID and NCI, NIH, by NIH grant 633 5R01AI103867, HHMI, the Parker Foundation for Cancer Immunotherapy, and a 634 Bio-X seed grant.

REMEMBERING IMMUNITY: NEURONAL REPRESENTATION OF IMMUNE INFORMATION

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Thoughts and emotions can impact physiology. This connection is evident in the emergence of disease following stress, psychosomatic disorders, or recovery in response to placebo treatment. Nevertheless, this fundamental aspect of physiology remains largely unexplored. In this talk, I will focus on the brain's involvement in regulating the peripheral immune response and explore the question of how the brain evaluates and represents the state of the immune system it regulates.

UNIVERSAL RECORDING OF CELL–CELL CONTACTS IN VIVO FOR INTERACTION-BASED TRANSCRIPTOMICS

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Physical interactions between cells drive much of immune activity as well as communication between immune and non-immune populations. We previously developed LIPSTIC (Labeling of Immune Partnerships by SorTagging Intercellular Contacts), an enzymatic system capable of labeling, in vivo, interactions between T cells and antigen presenting cells mediated by the CD40L-CD40 pathway. Here we report on a universal version of this system (uLIPSTIC) that allows recording of interactions between cells of any type, regardless of the pathways involved. We combine this approach with single-cell transcriptomics to reveal the molecular logic of interactions between various cell types.

SECONDARY CUES SELECTIVELY REWIRE POTENT, BROAD-ACTING ANTIVIRAL DEFENSES TRIGGERED BY INTERFERONS

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Metabolic reprogramming plays a critical role in the activation of immune cells and regulation of host defense during infection. However, the crosstalk between metabolism and antiviral responses in non-immune cells - the site of early infection - is less resolved. Antiviral responses are triggered by cytokines like interferon-α (IFN-α) and interferon-γ (IFN-γ) in immune and non-immune cells. Both IFNs lead to the induction of a class of factors termed IFN-stimulated genes (ISGs). Many ISG-encoded proteins have antiviral functions. There is a growing appreciation that the collection of ISGs expressed is cell-type specific and that this bias impacts infection outcomes. To model the impact of metabolism on host defenses in non-immune cells, we infected A549 lung epithelium cells, a common model for the study of antiviral responses, with two biomedically relevant viruses: vaccinia virus (VACV), the prototypical poxvirus, and herpes simplex virus-1 (HSV-1). Unexpectedly, we find that cells favoring glycolysis, but not OXPHOS, catalyze potent IFN-γ restriction of VACV and HSV-1 as evidenced by inhibition of viral gene expression, viral protein expression, and a two-log reduction in infectious virus production. This antiviral response is specific as modulating IFN-α signaling by enhancing either glycolysis or OXPHOS cues has little to no effect on VACV and HSV-1 replication. This potent restriction is independent of differences in canonical IFN-γ signal transduction, occurs at low levels of IFN-γ, and induced by physiological levels of glucose. While RNA-seq analysis reveals changes in expression of factors linked to glucose homeostasis in cells primed with IFN-γ/glucose, we observe no marked changes in the “usual suspects” (e.g. interferon-stimulated genes). Instead, we find protein levels of key ISGs are differentially regulated in a manner dependent on metabolic secondary cues that promote either glycolysis or OXPHOS. These findings suggest robust antiviral defenses catalyzed by interferons can be unlocked by secondary cues, which shape cellular metabolism, within the same cell-type.

A CORONIN 1-DEPENDENT KIN-TO-KIN DENSITY-SENSING PATHWAY DEFINES T CELL POPULATION SIZE

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The maintenance of appropriate cell population size is fundamental to the proper functioning of multicellular organisms, yet the underlying mechanisms remain largely undefined. For T cells, the factors required for sustained survival in the peripheral lymphoid tissues are well described, yet how the homeostatic population size is defined remains unknown. We used a combination of techniques including cell biology, biochemistry and molecular biology on mouse and human models to study the regulation of T cell population size. We found a cell-intrinsic kin-to-kin density sensing pathway by which T cells define their appropriate population size. We show that the transcriptional regulation of the expression of the evolutionarily conserved protein, coronin 1, tuned cell population density and coordinated pro-survival signalling with inhibition of cell death until the cell population reached threshold densities. At or above threshold densities, coronin 1 expression levelled off allowing for the initiation of apoptosis through kin-to-kin adhesin-mediated signalling to return the cell population to homeostatic size. Mice and humans that lack coronin 1 have up to 90% reduction of mature T cells in circulation, with consequential deregulation of immunity. Our data suggest the existence of a coronin 1-dependent homeostatic mechanism by which T cells are informed of and coordinate their population size.

NON-CODING IL-2RA VARIATION IMBALANCES T CELL FEEDBACK CIRCUITRY TO SHAPE AUTOIMMUNE DISEASE RISK

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Genetic variants associated with human autoimmune diseases commonly map to non-coding control regions such as enhancers that function selectively in immune cells and fine-tune gene expression within a relatively narrow range of values. How such modest, cell-type-selective changes can meaningfully shape organismal disease risk remains unclear. To explore this issue, we experimentally manipulated species-conserved enhancers within the disease-associated IL2RA locus and studied accompanying changes in the progression of autoimmunity. Perturbing distinct enhancers with restricted activity in conventional T cells (Tconvs) or regulatory T cells (Tregs) — two antagonistic T cell subsets — caused only modest, cell-type-selective decreases in IL2Ra expression parameters. However, these same perturbations had dramatic yet opposing effects in vivo, completely preventing or severely accelerating the development of autoimmune diabetes. Quantitative tissue imaging and computational modelling revealed that each enhancer manipulation impinged on distinct IL-2-dependent feedback circuits. These imbalances altered the intra- and intercellular dynamics of activated Tconvs and Tregs to produce sharp, opposing transitions in each cell type's local density, thereby amplifying or constraining ongoing autoimmune responses. These findings demonstrate how subtle changes in gene regulation stemming from non-coding variation can propagate across biological scales due to inherent non-linearities within feedback circuitry, ultimately shaping disease risk at the organismal level.

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IMMUNOLOGY OF LONG COVID

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SARS-CoV-2 infection can result in the development of a constellation of persistent sequelae following acute disease or Long COVID. .Individuals diagnosed with Long COVID frequently report unremitting fatigue, post-exertional malaise, and a variety of cognitive and autonomic dysfunctions. However, the basic biological mechanisms responsible for these debilitating symptoms are unclear. I will discuss our ongoing work on immune phenotyping in long COVID vs. those who recovered from COVID, and highlight key differences that suggest possible underlying disease mechanisms.

MICROBIOME AND METABOLITES AND REGULATION OF IMMUNE RESPONSIVENESS

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The gut microbiota plays a key role in regulating host immunity throughout life. While there is continual communication between intestinal microbes and the immune system throughout life, these interactions are likely to be especially important in early life. While live microbes are very efficiently contained to mucosal sites, exposure to microbial products or metabolites is ubiquitous with exposure to maternal microbiota-derived metabolites even found to occur in utero. We utilize gnotobiotic mouse models to gain greater insight into the multiple mechanisms by which the intestinal microbiota determines the function of innate and adaptive immune cells and promotes homeostasis. We have been studying how the intestinal microbiota regulates immune development, sets immune thresholds, regulates innate and adaptive immune cells, and how microbes and microbial metabolites can regulate the efficacy of immunotherapy.

INFLAMMASOME-MEDIATED CROSSTALK BETWEEN MYELOID CELLS AND THE ALVEOLAR EPITHELIUM MEDIATES ANTI-BACTERIAL DEFENSE

Sunny Shin

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Alveolar macrophages are among the first immune cells that respond to inhaled pathogens, such as Legionella pneumophila, the causative agent of Legionnaires' disease. Upon entry into the lung, Legionella infects and replicates within alveolar macrophages. Legionella-infected macrophages initiate an IL-1-dependent inflammatory cytokine response by recruited monocytes and other cells that allows for successful control of infection in immunocompetent hosts. How IL-1 directs myeloid cells to produce inflammatory cytokines is unknown. We found that collaboration with the alveolar epithelium is critical, in that IL-1 induces the alveolar epithelium to produce granulocyte-macrophage colony-stimulating factor (GM-CSF). Intriguingly, GM-CSF signaling amplifies inflammatory cytokine production in recruited monocytes and bacterial control by enhancing TLR-induced glycolysis. Our findings reveal that alveolar macrophages use inflammasome-derived cytokines to amplify alveolar epithelial signals that metabolically reprogram monocytes for antibacterial inflammation.

EPIGENETIC CONTROL OF VIRUS SUSCEPTIBILITY AND SPECIES-SPECIFICITY

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The species- and cell type-specificity of viral tropism is a defining factor in establishing and patterning the global virome. The ability of viruses to broaden the range of permissive host organisms or cell types is frequently associated with the appearance of highly pathogenic new viruses.
We identified BET proteins as key restriction factors in defining the flaviviruses' cell and species tropism. We found that pharmacological inhibition of BET bromodomain binding to acetylated lysin residues increases the infection frequency of permissive human cells and allows infection of non-permissive murine cells of different types. A similar alteration in viral tropism and infectivity has also been observed in cells that have been rendered deficient for the three distinct BET proteins - BRD2, BRD3, and BRD4. Inactivation of individual BET genes suggested the critical function of BRD2 and BRD3, but not BRD4, in restricting virus infection. We also found that BET proteins control flavivirus entry into cells but do not significantly contribute to the rate of viral replication. Our findings suggest that BET proteins restrain flavivirus tropism and infectivity by suppressing the expression and/or function of factors that circumvent the need for virus- and cell type-specific entry factors. The nature of these BET-controlled entry factors will be discussed.

INFLAMMASOME ACTIVATION IN INFECTED MACROPHAGES DRIVES COVID-19 PATHOLOGY

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COVID-19, an infectious disease caused by SARS-CoV-2, in its severe form, presents with an uncontrolled, hyperactive immune response and severe immunological injury. By adapting recombinant adeno-associated virus (AAV)-driven gene therapy to deliver human ACE2 to the lungs of MISTRG6 mice, we created a comprehensively humanized mouse COVID-19 model that carries key features characteristic of chronic COVID-19: prolonged disease duration, chronic weight loss, persistent viral RNA and lung pathology with fibrosis. Overall, our mechanistic study of this model defined a cascade of events, which initiates with infection of lung macrophages with SARS-CoV-2, generating replicative intermediates and products including RdRp, dsRNA, sgRNA and expression of a virally encoded fluorescent reporter gene. These SARS-CoV-2 infected macrophages have a unique transcriptome associated with chemokines, cytokines, inflammatory molecules, and type I IFN response; they activate the NLPR3 inflammasome and undergo pyroptosis. Inhibitors of both caspase-1 and NLRP3 blocked the downstream aspects of inflammasome activation and the inflammatory cascade both in vivo and in vitro. More importantly, targeting inflammasome-mediated hyperinflammation or combined targeting of viral replication and the downstream interferon response in the chronic phase of the disease prevented immunopathology associated with chronic SARS-CoV-2 infection COVID-19, an infectious disease caused by SARS-CoV-2, in its severe form, presents with an uncontrolled, hyperactive immune response and severe immunological injury. By adapting recombinant adeno-associated virus (AAV)-driven gene therapy to deliver human ACE2 to the lungs of MISTRG6 mice, we created a comprehensively humanized mouse COVID-19 model that carries key features characteristic of chronic COVID-19: prolonged disease duration, chronic weight loss, persistent viral RNA and lung pathology with fibrosis. Overall, our mechanistic study of this model defined a cascade of events, which initiates with infection of lung macrophages with SARS-CoV-2, generating replicative intermediates and products including RdRp, dsRNA, sgRNA and expression of a virally encoded fluorescent reporter gene. These SARS-CoV-2 infected macrophages have a unique transcriptome associated with chemokines, cytokines, inflammatory molecules, and type I IFN response; they activate the NLPR3 inflammasome and undergo pyroptosis. Inhibitors of both caspase-1 and NLRP3 blocked the downstream aspects of inflammasome activation and the inflammatory cascade both in vivo and in vitro.. More importantly, targeting inflammasome-mediated hyperinflammation or combined targeting of viral replication and the downstream interferon response in the chronic phase of the disease prevented immunopathology associated with chronic SARS-CoV-2 infection in vivo.

THE BACTERIAL EFFECTOR GARD SHIELDS CHLAMYDIA TRACHOMATIS INCLUSIONS FROM RNF213-MEDIATED UBIQUITYLATION AND DESTRUCTION

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Chlamydia trachomatis (*C.t.*) is responsible for the most widespread sexually transmitted bacterial infection and represents a global threat to women's reproductive health. As an obligate intracellular pathogen, *C.t.* must overcome an offensive of Interferon-gamma (IFN-γ) induced innate immune pathways within the host cytoplasm, known as "cell-autonomous immunity". The bacteria evade these host immune responses by establishing specialized vacuoles, termed "inclusions", where the microbe is safe to replicate and survive. The exact mechanics by which *C.t.* armors itself against human cell-autonomous immunity is unknown. Utilizing a library of chemically-mutagenized *C.t.*, we performed a loss-of-function genetic screen and revealed CTL0390/GarD (gamma resistance determinant), a critical effector found within the inclusion membrane that protects against cell-autonomous immunity. In IFN-γ stimulated human cells, GarD-devoid *C.t.* become heavily decorated with antimicrobials, such as linear ubiquitin, and are destroyed. Through cellular genome-wide association studies, we identified the E3 ubiquitin ligase RNF213 as a candidate host protein with anti-Chlamydia activity. We found that ubiquitylation and destruction of GarD-devoid *C.t.* in IFN-γ stimulated cells was wholly dependent on RNF213. Lastly, we found that GarD acts in cis to defend *C.t.* inclusions from RNF213 translocation and ubiquitylation, thus establishing GarD as a "stealth-acting" virulence factor essential for its pathogenesis in environments exhibiting cell-autonomous immunity.

CLONAL ANALYSIS OF IMMUNODOMINANCE AND CROSS-REACTIVITY

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The importance of CD4 T helper cells is well appreciated in view of their essential role in the elicitation of antibody and cytotoxic T cell responses. However, the mechanisms that determine the selection of immunodominant epitopes within complex protein antigens remain elusive. Using ex vivo stimulation of memory T cells and screening of naive and memory T cell libraries, combined with T cell cloning and TCR sequencing, we found that naive CD4 T cells have a broad repertoire, being able to recognize naturally processed as well as cryptic peptides spanning the whole influenza hemagglutinin (HA) sequence. In contrast, memory Th cells were primarily directed against just a few immunodominant peptides that were readily detected by mass spectrometry-based MHC-II peptidomics. These findings reveal the presence of a broad repertoire of naive T cells specific for cryptic H1-HA peptides and demonstrate that antigen processing represents a major constraint determining immunodominance. We also characterized the CD4 response to SARS-CoV2 S protein in vaccinated and convalescent individuals and found that the receptor-binding domain (RBD) is highly immunogenic and that 33% of RBD-reactive clones and 94% of individuals recognized a conserved immunodominant S346-S365 region comprising nested HLA-DR- and HLA-DP-restricted epitopes. Furthermore, using pre- and post-COVID-19 samples and S proteins from endemic coronaviruses, we identified cross-reactive T cells targeting multiple S protein sites. The immunodominant and cross-reactive epitopes identified can inform vaccination strategies to counteract emerging SARS-CoV-2 variants.

I WILL SURVIVE: S1P SIGNALING IN NAIVE T CELL SURVIVAL

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T cells are often described as “primed” for apoptosis. Developing T cells with non-functional or self-reactive T cell receptors must be eliminated, and the bulk of effector T cells in an immune response must die to prevent excessive inflammation. However, naïve T cells must be long-lived to maintain a diverse repertoire to defend against diverse pathogens, and memory T cells must be long-lived to confer lasting protection. A T cell makes critical decisions about whether to live or die at each stage of its life, and the factors that the cell weighs remain incompletely understood.

We recently found that the signaling lipid sphingosine 1-phosphate (S1P), acting via S1P receptor 1 (S1PR1), plays an essential role in naïve T cell survival. S1P has been intensively studied in the context of T cell migration. It is well established that the abundant S1P in blood and lymph guides T cells out of the low-S1P environment of lymphoid organs into circulation, and that T cells follow this gradient primarily using S1PR1. Four drugs targeting S1PR1 are used clinically to treat multiple sclerosis and ulcerative colitis, and these drugs work in part by trapping pathogenic T cells in lymph nodes and hence preventing them from accessing sites of inflammation. The role of S1P in T cell survival was unexpected, and it remains unknown how S1P signaling limits cell death and whether the findings in genetically manipulated mice extend to patients treated with S1PR1 modulators.

Here we address how S1P promotes T cell survival. We find little evidence that S1P promotes survival by enabling T cell circulation among or within lymphoid organs; by acting as an essential metabolite or protein cofactor after S1PR1-mediated uptake into the cell; or by activating AKT or ERK signaling downstream of S1PR1. S1PR1 activation restricts JNK phosphorylation, thereby maintaining the appropriate balance of BCL2 family members within the T cell, and in turn limiting apoptosis. Interestingly, the same residues of the S1PR1 C-terminus that enable receptor internalization are required to prevent JNK over-activation. Our findings using genetic models were recapitulated in mice treated with the drug FTY720, which targets S1PR1, and in ulcerative colitis patients treated with the drug ozanimod, a similar S1PR1 modulator. Patients treated with drugs targeting S1PR1 respond poorly to COVID vaccines, with antibody titers correlating negatively with time on drug, and using a mouse model we find that this may reflect progressive loss of the naïve T cell repertoire.

PRIMING OF INNATE ANTIVIRAL IMMUNITY BY ACTIN CYTOSKELETON REMODELING

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Abstract: The RIG-I-like receptor (RLR) family of cytosolic RNA sensors is crucial for eliciting innate immune defenses against a variety of viruses including influenza, SARS-CoV-2 and Zika virus. RLR signaling in uninfected conditions is repressed by phosphorylation, whereas protein phosphatase-1 (PP1)-mediated dephosphorylation following virus infection is crucial for RLR activation. While PP1 has emerged as a key upstream activator of RLRs, how PP1 itself is activated upon viral infection and what determines its specificity towards RLRs is unknown. Here, we report that actin cytoskeleton remodeling induced by virus infection, virus-like particles, or commonly used reagents to intracellularly deliver RNA triggers the relocalization of F-actin residing PPP1R12C, which is one of >200 PP1-regulatory proteins, to cytoplasmic RLRs. This facilitates PP1 targeting to RLRs and dephosphorylation-mediated RLR priming by the PP1α/γ-PPP1R12C complex. Genetic ablation of PPP1R12C impairs antiviral responses and enhances viral replication in various cell types and in mice. We further demonstrate that two independent 'signals' – actin cytoskeleton disturbance and dsRNA agonist binding – are essential for the full activation of RLR signaling. Our work identifies PPP1R12C as a novel innate immune regulator and unveils that actin remodeling serves as a priming signal required for RLR-mediated antiviral responses.

Keywords: Innate immunity, antiviral response, RIG-I-like receptor (RLR), dephosphorylation, actin cytoskeleton rearrangement, RLR-priming, and interferons (IFNs) responses

MECHANISM OF TH2 FATE INSTRUCTION BY CD301B$^+$ MIGRATORY cDC2s

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Upon recognition of cognate peptide-MHCII complexes, naive CD4T cells undergo massive proliferation and simultaneously differentiate into various types of effector T helper (Th) cells. Many studies have shown that the type of dendritic cells (DCs) presenting the antigen to CD4T cells determines the type of Th cells during this process, but the mechanism underlying this fate instruction by DCs is incompletely understood. In particular, the differentiation into Th2 cells remains most enigmatic, as the critical cues provided by DCs onto CD4T cells for the Th2 fate instruction have not been clearly identified. For Th2 differentiation, some models imply mechanisms intrinsically wired to the T cell receptor (TCR) signaling (e.g., correlation of the Th1/Th2 balance with the TCR signal intensity), while others suggest the involvement of antigen-independent stimuli such as cytokines and co-stimulatory molecules. However, it remains unclear how each of these mechanisms is associated with specific DC subsets.
We previously showed that CD301b$^+$ DCs, a major subset of migratory cDC2s in peripheral organs, are specifically required for the differentiation of Th2 cells upon immunization with a protease allergen papain or infection with a helminth parasite Nippostrongylus brasiliensis. Here, we show that CD301b$^+$ DC-intrinsic MHCII expression is required for both effective priming of antigen-specific CD4T cell clones and their differentiation into Th2 cells, suggesting that CD301b$^+$ DCs instruct the Th2 fate through cognate interactions. Mechanistically, priming of CD4T cells in the absence of CD301b$^+$ DCs results in delayed activation and incomplete upregulation of CD25 in antigen-specific CD4T cells, the latter of which selectively impairs Th2 differentiation. Furthermore, expression of CD40 in CD301b$^+$ DCs is specifically required for CD25 upregulation and Th2 differentiation of antigen-specific CD4T cells. These results suggest that MHCII-dependent antigen presentation and CD40-dependent co-stimulation by CD301b$^+$ DCs cooperatively instruct the Th2 fate in antigen-specific CD4T cells.

THE TRANSCRIPTION FACTOR ELF4 TUNES THE INFLAMMATORY POTENTIAL OF $CD4^+$ T CELLS

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We recently discovered a monogenic autoinflammatory disease we termed Deficiency in ELF4, X-linked (DEX), which presents in male patients with recurrent fevers, oral ulcers, and mucosal autoinflammation. These patients harbor loss-of-function mutations in the X-linked gene encoding the ELF4 transcription factor (TF). ELF4 contains an evolutionarily conserved E26 transformation specific (ETS) DNA-binding domain and is highly expressed in hematopoietic cells, including $CD4^+$ T cells. ELF4 was previously found to restrain the differentiation of proliferating $CD4^+$ T cells into Th17 cells in mice, but broader roles for ELF4 in regulating inflammatory T cells remain to be defined. Surprisingly, we also found that in vitro differentiated ELF4-deficient Th1 cells from both mouse and humans produce more IFNγ compared to wild type (WT), indicating ELF4 fundamentally regulates cytokine production from $CD4^+$ T cell effectors. T cell-specific ELF4 deficiency caused hundreds of genes to be differentially expressed in resting naïve $CD4^+$ T cells, leading to the conclusion that ELF4 modulates the transcriptome landscape even before the initiation of differentiation. In contrast to what was previously reported in other cell types, subcellular fractionation in mouse and human $CD4^+$ T cells shows ELF4 is constitutively nuclear, supporting that its role in mediating these transcriptional changes is regulated by nuclear interactions rather than shuttling between the cytosol and nucleus. Additionally, CUT&RUN to define chromatin binding sites in WT naïve $CD4^+$ T cells revealed that ELF4 binds to over 2,500 gene transcription start sites, a rare feature for TFs. We also found ELF4-deficient naïve $CD4^+$ T cells have more accessible chromatin by ATAC-Seq compared to WT, suggesting ELF4 may facilitate repressive chromatin modifications. Work is ongoing to identify protein binding partners of ELF4 in $CD4^+$ T cells by immunoprecipitation and mass spectrometry, as well as to define the ability of ELF4 to interact with chromatin modifiers and mediate architectural changes. Building from our discovery in patients, this work advances our understanding of molecular and cellular pathways important in naïve T cell states that influence inflammatory T cell differentiation and offers new insights with translational relevance for inflammatory disorders.

A PROSTAGLANDIN E2-DEPENDENT SUBSET OF IL-1β+ MACROPHAGES FUELS PATHOGENIC INFLAMMATION AND IMMUNE ESCAPE IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by the co-existence of aberrant inflammatory responses that underlie loss of cell identity, angiogenesis, and fibrosis with factors that suppress cytotoxic immunity. To identify drivers of immune dysfunction in PDAC, we profiled the ecosystem of primary human and mouse pancreatic tumors with single-cell transcriptomics. We identified a conserved subset of macrophages – IL-1β+ TAMs – expressing high levels of inflammatory (*IL1B, IL6, CXCL2, CXCL3*) and immune regulatory (*IL10, PDL1, ARG1, VEGFA*) genes, but devoid of interferon (IFN) and cytotoxicity signatures. IL-1β+ TAM were undetectable in the healthy pancreas, but accumulated within hypoxic and angiogenic areas of the tumor. Notably, a gene signature of IL-1β+ TAMs negatively correlated with PDAC patient survival and blockade of IL-1β suppressed tumor growth in mice.
We identified the eicosanoid prostaglandin E2 (PGE2) as a key regulator of IL-1β+ TAM identity. In this context, PGE2 and TNFα – both of which were produced at high levels in PDAC – acted synergistically to trigger IL-1β expression in tumor-infiltrating monocytes and to promote IL-1β+ TAM development. Blockade of PGE2 synthesis by PDAC cells led TAM skewing in vivo – that is, reduced expression of IL-1β and upregulation of IFN response genes. Accordingly, PGE2-deficient tumors became infiltrated by cytotoxic lymphocytes and were efficiently controlled in vivo. While deletion of the IL-1 receptor in hematopoietic or stromal cells had no impact on disease progression, IL-1R1 ko PDAC showed profoundly defective growth in vivo – highlighting tumor cells as the main target of IL-1β. Mechanistically, IL-1β exposure triggered massive transcriptional reprogramming of PDAC and stimulated inflammatory cytokine production. Activated tumor cells also synthesized heightened levels of PGE2, which in turn cooperated with TNFα to drive IL-1β production by macrophages. Here, we have uncovered a feed-forward loop between tumor cells and a newly-described subset of macrophages that fuels pathogenic inflammation in PDAC. Together with previous findings, our data establish PGE2 as a key driver of aberrant TAM activity and immune dysfunction in pancreatic cancer. On the one hand, PGE2-driven suppression of IFN I responses hampers anti-tumor immunity and favors PDAC escape. On the other hand, PGE2-driven inflammation supports disease progression in an IL-1β-dependent manner. Rational targeting of PGE2 and/or IL-1β+ TAMs may represent a combinatorial immunotherapy approach for otherwise resistant pancreatic cancer.
Unpublished data.
Key ref: Cilenti, Immunity 2021; Caronni, Immunol Rev 2021; Natoli and Ostuni, Nat Immunol 2019.

PHYSIOLOGICAL ROLES OF TRANSCRIPTION FACTOR BCL11B IN T CELL FATE DETERMINATION

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The transcription factor Bcl11b plays a critical role in the development of multiple mammalian organ systems. While outright deficiency results in perinatal lethality in both mice and humans, Bcl11b hypofunctionality results in developmental defects in neurological, dental, adipose and other systems, and increased risk of some cancers. Of particular interest to us is T lymphocytes, a collection of lineages that are dependent upon Bcl11b expression to establish and maintain the T cell program.

Following its induction at the time of T lineage commitment, Bcl11b remains expressed in all subsequent T cell stages. However, there is conflicting attribution of T cell lineage commitment to the activity of Bcl11b or E proteins, with Bcl11b expression required to repress the E protein inhibitor Id2. We have performed developmental and transcriptomic analyses of *Bcl11b* and *Id2* double-deficient T cell precursors, dissecting the intrinsic role of Bcl11b in T lineage commitment and progression from those roles mediated by indirectly Bcl11b-dependent E protein activity.

Complete Bcl11b loss is incompatible with the normal function and underlying lineage integrity of mature thymus-derived lineages. As a result, homozygous Bcl11b deficient cells are often absent, or so altered as to have 'buried the evidence' of how Bcl11b maintained the lineage while it was still present. In the periphery, we have observed even modest reduction of Bcl11b protein levels to result in a dramatic phenotypic shift among the mature T cell compartment. These mutations derepress T cell proliferation in both activated and homeostatic contexts. A distinct impact of these mutations on T cell activation status is evident in even the earliest exported T cells in neonates, indicating a role for maximal Bcl11b expression in the thymus to program subsequent fate determination. A specific Bcl11b structural mutant that phenocopies the impact of reduced Bcl11b expression in the periphery but in not thymic DN cells implicates specific cofactor interactions in this dosage-dependent regulation of mature T cell fate determination.

Myriad modes of molecular function are available to Bcl11b, with a diverse array of cofactors capable of mediating both its interactions with chromatin and the specific outcomes thereof. This poses mechanistic questions as to its role in any one context versus another. Are its roles in establishing and then indefinitely maintaining T lineage identity related or not? How are the required Bcl11b functional partners appropriately chosen in each specific context? And how can the microcosm of Bcl11b activity that is T cell development inform our broader understanding of its role in the diverse body systems it supports?

Funded by NIH grant R01AI135200

INFLAMMATION AND HOMEOSTASIS

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Although usually considered as opposites, homeostasis and inflammation both operate based on the same principles and serve to achieve the same ultimate goal, albeit by different means. Inflammation supplements homeostatic capacity of the organism and is engaged when the latter is insufficient. Both homeostatic and inflammatory signal operate through the same control points, but the higher priority of inflammatory response established the hierarchy of regulation that creates vulnerability to stable inflammatory control that translates into chronic inflammation and associated diseases.

NEURO-IMMUNE CIRCUITS DRIVE THE INITIATION OF ALLERGIC IMMUNITY

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Allergic diseases are characterized by inappropriate Type-2 immune responses targeted against non-infectious environmental antigens and venoms. It is well established that the enzymatic, or functional, activity of allergens is required for the initiation of Type-2 immune responses, but the initial cellular sensors of allergens have been unclear. My laboratory recently established that cutaneous sensory neurons directly detect the functional activity of diverse protease allergens leading to the sensation of itch and the release of the neuropeptide Substance P. Substance P promotes the activation and migration of locally placed Th2-skewing dendritic cells through their expression of MRGPRA1, leading to the initiation of adaptive allergic immune responses. However, it remained to be determined how this neuro-immune pathway could become dysregulated in atopic disease, thereby permitting low-level allergen exposure to lead to pathophysiologic responses. Using a screen approach, we identified a novel cutaneous innate-like immune cell that acts to prime neuronal responses to allergens through the release of secreted factors. These factors, along with allergen-induced metabolic reprogramming events in sensory neurons, create a bidirectional neuro-immune circuit that both establishes the set-point for neuronal activation by allergens, but controls the initiation of downstream allergic immune responses.

SUBEPITHELIAL FIBROBLASTS RELAY FEEDING CUES TO INITIATE THE SMALL INTESTINAL ILC2- TUFT CELL CIRCUIT

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The small intestine represents a critical interface for absorption of nutrients. Group 2 innate lymphoid cells (ILC2s) are prevalent in small intestinal lamina propria and activated in allergies and helminth infections, but the mechanisms engaging type 2 immunity during basal intestinal physiology remain unclear. Thymic stromal lymphopoietin (TSLP) is a key driver of type 2 inflammation and an alarmin cytokine that activates ILC2s, although its cell sources and functions in the small intestine are less well understood. We generated a novel TSLP reporter mouse strain and identified populations of subepithelial fibroblasts designated villus telocytes and peri-cryptal trophocytes as prominent sources of small intestinal TSLP. Food feeding increased TSLP in the small intestine and activated type 2 cytokine production by lamina propria ILC2s. Telocytes and trophocytes express receptors for glucagon-like peptide-2 (Glp-2), a gut peptide hormone secreted by enteroendocrine L cells in response to diverse nutrients. Glp-2 receptor agonist activated ILC2s by a TSLP-dependent mechanism that initiated increased tuft cell prevalence in intestinal epithelia, which was attenuated by deletion of TSLP in stromal cells or in TSLP receptor-deficient mice. These data suggest that subepithelial telocytes produce TSLP to relay luminal nutrient signaling from epithelial L cells to activate ILC2s and initiate increased numbers of epithelial tuft cells. We propose a model whereby telocyte TSLP couples epithelial peptide hormones induced by feeding with inside-out amplification of the ILC2-tuft cell chemosensory circuit to enhance surveillance of constituents in luminal cargo.

REGULATORY T CELLS RESTRAIN SKIN INFLAMMATION BY MODULATING NOCICEPTION

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The skin is one of the largest interfaces between the body and the environment, integrating signals including temperature and mechanical stimuli, tissue damage, and pathogenic and commensal microbes. Both the nervous and immune system sense potentially damaging stresses and perturbations and orchestrate appropriate adaptive responses. Nociceptors, sensory neurons that respond to damaging stimuli, are sensitized by inflammatory mediators and also produce effector molecules that influence the function of innate and adaptive immune cells. Therefore, sensory neuron activation must be tightly regulated to prevent excessive inflammation and nociception, perpetuated by these feed-forward circuits. Regulatory T (Treg) cells are a key component of the inhibitory arm of the immune system which promote tolerance to self, commensal bacterial, and environmental antigens, as well as limiting responses to acute and chronic infections. In addition, Treg cells in non-lymphoid tissues have been shown to promote tissue function and repair by both immune-dependent and independent mechanisms. We find that Treg cells localize in close proximity to axons innervating the skin and that short-term ablation of Treg cells increases neuronal activation to noxious stimuli. In an established mouse model of psoriatic-like disease using TLR7 agonist Imiquimod (IMQ), we find that short term ablation of Treg cells increases neuronal activation in the dorsal root ganglia (DRG), as well as the production of IL-23 in the skin, the latter in a manner dependent on sensory innervation. We find that a population of skin Treg cells is highly enriched for expression of Penk, the gene encoding the neuropeptide precursor proenkephalin, as well as the corresponding proteases for its cleavage and maturation into enkephalins, endogenous opioids that act as analgesics. Acute ablation of Penk in Treg cells in mice treated with IMQ led to increased neuronal activation in the DRG and pro-inflammatory cytokine production, and eventual disease worsening. These results indicate that, during inflammatory challenge, a population of Treg cells prevents exacerbated activation of sensory neurons through the production of an endogenous opioid that reduces nociceptive signaling and associated inflammation.

THYMIC EPITHELIAL CELLS CO-OPT LINEAGE-DEFINING TRANSCRIPTION FACTORS TO ENFORCE T-CELL CENTRAL TOLERANCE

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Medullary thymic epithelial cells (mTECs) express thousands of peripheral-tissue antigens (PTAs) ectopically within the thymus, driving deletion of self-reactive thymocytes or their diversion to the regulatory T cell (Treg) lineage. Failure of PTA expression results in multiorgan autoimmunity, as occurs in APECED, the autoimmune syndrome caused by Aire deficiency. By assaying chromatin accessibility in individual mTECs, we found a strong and specific effect of Aire in enhancing chromatin accessibility at its binding sites and target genes. Surprisingly, however, we also uncovered signatures of lineage-defining transcription factors (TFs) for skin, lung, liver and intestinal cells—including Grhl, FoxA, FoxJ1, Hnf4, SpiB and Sox8—in distinct mTEC subtypes. Bulk and single-cell transcriptomics showed that these subtypes (and others that were uncovered), which we collectively term mimetic cells, expressed PTAs in a biologically logical fashion, mimicking extra-thymic cell types while maintaining mTEC identity. Lineage-defining TFs bound specifically to the open chromatin regions of mimetic cells, driving their differentiation and function. Accordingly, thymus-intrinsic deletion of two lineage-defining TFs, SpiB and Sox8, ablated their corresponding mimetic cell, the thymic microfold cell, and impaired microfold-associated PTA expression within the thymic epithelium. Mimetic cells accumulated downstream of Aire expression and dominantly expressed many Aire-induced PTAs, but deletion of Aire only partially and variably impaired mimetic-cell accumulation and did not directly influence mimetic-cell gene expression. Expression of a model antigen in three distinct subsets of mimetic cells sufficed to induce cognate T-cell tolerance in a polyclonal repertoire, negatively selecting antigen-specific conventional T cells while preserving antigen-specific Tregs. Thus, mTECs co-opt lineage-defining TFs to generate thymic mimetic cells, which drive PTA expression and self-tolerance. As with Aire and APECED, defects in the thymic action of lineage-defining TFs may help explain syndromic manifestations of autoimmunity.

A SELF-SUSTAINING LAYER OF EARLY LIFE ORIGIN B CELLS DRIVES STEADY STATE IGA RESPONSES IN THE ADULT GUT

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The adult immune system consists of cells that emerged at various times during ontogeny. We aimed to define the relationship between developmental origin and composition of the adult B cell pool during unperturbed hematopoiesis. Lineage tracing stratified murine adult B cells based on timing of output, revealing that a substantial portion originated within a restricted neonatal window. In addition to B-1a cells, early-life time-stamped B cells included clonally interrelated IgA plasma cells in the gut and bone marrow. These were actively maintained within gut chronic germinal centers and contained commensal microbiota reactivity. Neonatal Rotavirus infection recruited recurrent IgA clones that were distinct from those arising by infection with the same antigen in adults. Finally, gut IgA plasma cells arose from the same hematopoietic progenitors as B-1a cells during ontogeny as revealed by cellular barcoding. Thus, a complex layer of neonatally imprinted B cells confer unique antibody responses later in life.

NEW APPROACHES TO STUDY THE FUNCTION & DISTRIBUTION OF HISTONE MODIFICATIONS IN MURINE IMMUNE CELLS

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Complex organisms face daunting "epigenetic challenges" in organizing and regulating their genomes. These challenges are solved through integrating signal transduction and genome organization and are highly dependent on histones, proteins that package and regulate the genome, and the post-translational modifications (PTMs) that occur on their N-terminal tails. However, our understanding of histone PTMs, and their effects on gene expression, are largely based on studies done in simple model organisms. Moreover, since it's widely assumed that the increased cellular and genome complexity in mammals requires additional epigenetic mechanisms, an epigenetic paradox has arisen whereby functional data that indicate how specific histone PTMs control mammalian cell fate decisions and rapid responses to stimuli are starkly lacking. Furthermore, this paradox has been largely unaddressed, as the high copy number of mammalian histone genes has made them intractable for genetic studies. Notably, however, two of the fifteen histone H3 genes in mouse (and human) code for the ancestral H3.3 variant, which is evolutionarily conserved and enriched at dynamically regulated chromatin. Thus, we hypothesized that H3.3 has an outsized epigenetic regulatory role and, when combined with the fact that it has only two gene copies, we chose to utilize H3.3 as a model histone to study histone PTM function. To do so, we generated H3.3 conditional double knockout mice (H3.3 cDKO) and designed a novel lentiviral based "histone knockout and replacement" model to perform first-of-their-kind functional histone genetic experiments in mouse. Importantly, we used our H3.3 cDKO mice to first show that H3.3 is required for the differentiation and proliferation of both bone marrow derived macrophages (BMDMs) and hematopoietic stem cells (HSCs). We next demonstrated that H3.3 is required for stimulation induced transcription as H3.3 cDKO BMDMs were unable to upregulate inflammatory genes upon LPS stimulation. As a validation of our "knockout and replacement" system, we then transduced both H3.3 cDKO BMDMs and HSCs with a H3.3 lentiviral transgene, which functionally "rescued" the differentiation, proliferation, and stimulation-induced transcription of both cell types. We have now started to greatly expand this approach by introducing a library of lentiviral vectors with H3.3 point mutant transgenes (~200) in order to assess the explicit function of individual histone PTMs in both stimulation induced transcription and immune cell differentiation. Furthermore, by utilizing cutting-edge chromatin mapping techniques, we have initiated complementary epigenomics-based studies to comprehensively profile the genomic distribution of a vast array of histone PTMs in both resting and stimulated immune cells. It is through this paired approach that we seek to establish a "functional roadmap" of critical H3.3 PTMs that regulate proper immune cell function, differentiation, and transcriptional identity.

LARGE-SCALE SINGLE-CELL RNA SEQUENCING OF HEALTHY AND INFLAMED TERMINAL ILEAL SAMPLES YIELDS INSIGHTS INTO CROHN'S DISEASE BIOLOGY

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Inflammatory bowel disease (IBD) is characterised by chronic inflammation of the digestive tract and has two common subtypes, Crohn's disease (CD) and ulcerative colitis (UC). With the aim of better understanding IBD, we generated single cell RNA-sequencing data from terminal ileal biopsies taken from 49 CD patients with active ileal inflammation and 72 non-IBD controls, split into a discovery (25 CD; 26 non-IBD) and replication (24 CD; 46 non-IBD) cohort.

With a one-pot digest we were able to cluster 49 cell types across immune, enterocyte, secretory and mesenchymal populations. We computed cell type specific markers, differentially expressed genes (DEGs) in health versus disease, and learned expression programmes with non-negative matrix factorisation, in both of our cohorts. We identified a population of monocytes and macrophages which were both expanded in inflamed biopsies and expressed inflammatory cytokines. Our DEGs in epithelial, stem, B plasma, and B cells showed encouraging evidence of replication (Pearson's $R > 0.5$), whilst the mesenchymal, myeloid, and T cell DEGs showed little evidence of replication (Pearson's $R < 0.15$). Amongst the replicating DEGs were IBD biomarkers (LCN2, B2M, REG1A/1B/3A) as well as genes with IBD genetic evidence (CYBA, SDF2L1, STAT1/3). We also observe upregulation of expression and expression programmes for interferon-related genes, and both class I and class II MHC genes, with enrichment for antigen cross-presentation and interferon pathways.

We integrated the markers, and factors with CD and UC genome-wide association study (GWAS) statistics to identify cell types and processes with a genetic basis for involvement in disease. We observed a significant enrichment of CD and UC GWAS variants (FWER < 5%) in the markers and programmes for T cells, including regulatory T cells, cytotoxic T cells, helper T cells and gamma-delta T cells. We also observed genetic enrichment for CD for myeloid cells, including the previously mentioned monocyte and macrophage populations.

Here we present the largest single-cell RNA-sequencing study of the gut to date which we use to characterise the genes, processes and cell types involved in Crohn's disease. Our study design allowed us to examine the replication rate of our analyses and highlighted a cause of concern in DGE replication that may have wider consequences beyond IBD.

BINARY PROMOTER SWITCHES IN TRANSCRIPTION FACTORS: A MODEL FOR THE DIGITAL PROGRAMMING OF CELL FATE DETERMINATION

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Previous studies of the murine *Ly49* and human *KIR* gene clusters have revealed a role for bidirectional promoters in the control of variegated gene expression. Whether or not competing promoters control other instances of cell fate determination remains an outstanding question. Although divergent transcripts within 300 bp are found in ~6% of human genes, an analysis of human transcription factor (TF) genes (~1700) revealed that 20% possess stable divergent transcripts with a transcription start site (TSS) less than 300 bp upstream, with many separated by less than 30 bp, indicating an enrichment for potential binary switches in TFs.

We have performed a detailed examination of putative bidirectional promoter switches in three lineage-determining TF genes: *AHR*, *GATA3*, and *RORC*. These genes also contain additional intronic antisense promoters that would prevent simultaneous transcription of sense and antisense, and thus may represent simple on/off switches rather than probabilistic switches.

RT-PCR of sense/antisense transcripts in a human tissue panel revealed tissue-specific differences in the relative sense/antisense activity of the switch elements, and distinct tissue specificities of the probabilistic versus the on/off type of switches. Luciferase-reporter assays of isolated elements confirmed the tissue-specificity of switch behavior. In situ RNA hybridization of human tissues revealed mutually exclusive expression of sense versus antisense transcription, indicating switching between stable sense or antisense transcriptional states. Single-cell RNAseq with 5'-end capture was used to confirm the separate sense/antisense states, and to determine the identity of cells with active switch elements. Differential gene expression analysis of cells with antisense switch transcripts revealed an enrichment for genes found in immature/stem cells and a lack of genes associated with terminally differentiated cells.

In conclusion, these data indicate that there is a digital component to the differentiation program mediated by binary promoter switches in lineage-determining transcription factors.

HIC1 INTERACTS WITH FOXP3 MULTI-PROTEIN COMPLEX: A NOVEL MECHANISM TO REGULATE HUMAN REGULATORY T CELL DIFFERENTIATION AND FUNCTION

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Transcriptional repressor, hypermethylated in cancer 1 (HIC1) participates in several biological processes including tumor repression, immune suppression, embryonic development and epigenetic gene regulation. Earlier, we demonstrated that HIC1 is an important contributor to regulatory T (Treg) cell development and function. However, a detailed mechanism by which it regulates Treg cell development is poorly understood. To address this question, we systematically characterized the HIC-1 interactome by affinity-purification mass spectrometry in human regulatory T cells. We identified 62 high-confidant interactors, which belonged to protein transport, mRNA processing, non-coding (ncRNA) transcription and RNA metabolism processes. Importantly, we report for the first time that HIC1 is a part of a FOXP3-RUNX1-CBFβ protein complex that regulates Treg signature genes and is indispensable for the suppressive function of FOXP3+ regulatory T cells. In addition, IKZF3, a key protein involved in Treg cell development, was identified as an interactor of HIC-1. Altogether, the identified interactome of HIC1 highlights its essential role in the development and suppressive function of regulatory T cells.

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SOLID PHASE SYNTHESIS OF MATURE PEPTIDOGLYCAN FRAGMENTS

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The gut microbiome is host to trillions of commensal and pathogenic bacteria. These bacteria are recognized by peptidoglycan (PG) fragments that are shed as bacteria repair and maintain their cell wall. The synthesis of PG fragments is long and laborious. By utilizing solid phase synthesis (SPS), we aim to streamline the process and expand our library to include fragments that mimic mature PG. With these large fragments in hand, we can uncover how minute variations observed across bacterial species can trigger large immunological differences and help reveal mechanisms by which bacteria interact with our innate immune system.

ASSESSING THE ROLE OF GUT MICROBIAL METABOLITES IN IMMUNE MODULATION OF MULTIPLE SCLEROSIS

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The gut microbiota and microbial metabolites play a pivotal role in modulating extra-intestinal autoimmune diseases like Multiple Sclerosis (MS). Changes in the gut microbiota composition and gut mucosal immunity, i.e., high frequency of Th17 cells, were detected both in pre-clinical model (EAE) and relapsing remitting (RR)MS patients and correlate with disease activity. Emerging evidence identify microbial bile acid metabolites (BAMs) as key regulators of intestinal homeostasis and gut mucosal immunity. Alteration of BAMs was found in IBD and extra-intestinal autoimmune disease like type 1 diabetes and MS. We analyzed the microbiota-related metabolic profile in RRMS patients and evaluated whether BAMs could counteract brain autoimmunity by modulating gut immunity and mucosal homeostasis.

We collected serum, fecal, urine and duodenal tissue samples from RRMS patients and controls to perform untargeted (1H-NMR) and BAMs-targeted (HPLC-MS) metabolomics and correlate with disease activity and metagenomic profile (WGS). Additionally, we tested the beneficial effect of BAMs in a model of EAE.

We found an altered microbial profile in our cohort of RRMS patients, with a decreased abundance of beneficial bacteria including the BAMs' producers. Oral administration of BAMs in pre-clinical model significantly prevented the severity of EAE. Immunological analysis revealed their ability in shaping gut mucosal immunity decreasing the frequency of Th17 cells and increasing the relative percentages of both $FOXP3^+$ T reg cells and IL-10 secreting Tr1 cells. Such immune modulation extended in the draining lymph nodes and reflected at brain level, where a decreased frequency of $IL\text{-}17^+$ T cells expressing the gut homing marker $\alpha4\beta7^+$ was observed, thus reinforcing the role of the gut-related immune modulation in affecting brain autoimmunity.

Our results suggest that BAMs play an important role in shaping adaptive immune response and, if a defective release of BAMs in the intestine of RRMS patients will be documented, our study could pave the way to new therapeutic interventions in MS.

TAP DYSFUNCTION IN DENDRITIC CELLS ENABLES NONCANONICAL CROSS-PRESENTATION FOR T CELL PRIMING

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Specialized antigen-presenting cells are able to present exogenous antigen on MHC-I molecules through the highly regulated process of cross-presentation. Delivery of proteasome-generated peptides to MHC-I molecules for presentation to CD8 T cells is impaired upon dysfunction of the transporter associated with antigen processing (TAP) in diseases such as viral infection, cancer and TAP-deficiency syndrome. Here, we unveiled a non-canonical trafficking of MHC-I molecules that cell-autonomously counters TAP dysfunction within dendritic cells and allows cross-presentation. Blocking TAP depletes the MHC-I endosomal recycling stores thereby negating their Toll-like receptor-mediated availability for cross-presentation during infection. MHC-I instead accumulate in endoplasmic reticulum (ER)-Golgi intermediate compartments sequestered away from Toll-like receptor control, and coopt ER-SNARE Sec22b-mediated vesicular traffic to nevertheless reach phagosomes and prime CD8 T cells. TAP deficiency in hematopoietic cells, therefore, does not prevent the generation of a protective CD8 T cell response during viral infection. These findings highlight a novel MHC-I antigen presentation pathway with major implications to disease pathogenesis and therapeutic exploitation.

ROLE OF CLEAVAGE AND POLYADENYLATION FACTOR (CFIM25) IN MACROPHAGE DIFFERENTIATION.

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Monocytes and macrophages play a crucial role in our body's defense against pathogens and diseases. Monocytes migrate from the blood into infected or wounded tissue to differentiate into macrophages and control inflammation via phagocytosis or cytokine secretion. Recent evidence suggests that CFIm25, a global regulator of alternative mRNA polyadenylation, impacts gene expressions and promote the synthesis of mRNAs that modulate cellular proliferation and differentiation. We have observed a striking increase in the level of CFIm25 during the differentiation of monocytes to macrophages, suggesting that it may promote this process.
To study the possible function of CF1m25 in macrophage differentiation, we used 3 nM Phorbol Myristate Acetate (PMA) to differentiate monocytic cells (THP and HL-60) into naïve macrophages (M0). The differentiation process was characterized at various time points (0 hr, 1 hr, 6 hr, 15 hr, 18 hr, and 24 hr) after PMA treatment by the number of cells that became attached to the surface of the culture plates, by exit from the cell cycle as measured by a decrease of cells in S phase, and by assessment using flow cytometry of the increase in CD38, a naïve macrophage marker, and the concommitant decrease in CD14, a monocytic marker. Depletion of CFIm25 using siRNAs delayed macrophage marker expression, cell cycle exit, and attachment. Conversely, CFIm25 overexpression caused the appearance of differentiated phenotypes with a significant increase in attachment, macrophage marker expression, and much more efficient exit from the cell cycle. The maximum change in the differentiation potential upon overexpression was observed between the 6 hr and 15 hr time points. The overexpression and knockdown of CFIm25 were validated by western blotting analysis. In summary, overexpression of CFIm25 expedited the PMA-induced differentiation of monocytic THP and HL60 cell lines into naïve macrophages (M0), while knockdown impeded the differentiation process, indicating an important role of this factor in promoting efficient differentiation.

DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* SUPPRESSES HOST IMMUNE RESPONSES THROUGH TYPE I INTERFERON SIGNALING

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Mycobacterium tuberculosis (*Mtb*) causes nearly 10 million active cases of tuberculosis (TB) annually. The rise of multidrug-resistant (MDR) *Mtb* infections worldwide presents a significant obstacle to curbing TB globally. While human studies report dysregulated immune responses in MDR TB patients, we lack a clear understanding of the host-pathogen interactions following MDR *Mtb* infection. We previously showed that *Mtb* carrying a rifampicin drug resistance (RDR)-conferring single nucleotide polymorphism (SNP) in the RNA polymerase-β gene (*Mtb rpoB*-H445Y) modulates host macrophage metabolic reprogramming and drives the production of type I interferons (IFNs). Here, using a mouse model, we have characterized the host immune response *in vivo* following RDR *Mtb* infection. We show that, despite no differences in the bacterial burden in the lungs of mice infected with RDR or drug-susceptible *Mtb*, lung myeloid and lymphoid immune responses to RDR *Mtb* are suppressed through a type I IFN-dependent mechanism, which in turn promotes the survival of RDR *Mtb* at chronic stages of infection. These findings coincide with impaired responses in the bone marrow hematopoietic stem and progenitor cells (HSPCs) following RDR *Mtb* infection. Overall, our studies have implications for the pathogenesis of RDR TB disease with specific SNPs and the rational design of host-directed therapeutic approaches to treat DR TB specifically.

SETD3-REGULATED B CELL ACTIVATION AND DIFFERENTIATION

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During adaptive immunity, B cells play a vital role as they differentiate into antibody producing cells upon activation and contribute to various effector functions mediated by antibodies. Antigen specific B cell activation is achieved via B cell receptor (BCR) binding to an antigen (Ag). For efficient BCR activation, Ag affinity and valency must be sufficient to reach a certain signalling threshold, such that the BCRs are able to cluster, internalize with antigen and present the processed Ag to T follicular helper cells (Tfh). Our data show that Setd3, a histone lysine methyltransferase (HKMT) in muscle cells, was mainly expressed in the cytoplasm of B cells and was upregulated during B cell activation. Setd3 was not required for B cell development and maturation, but it was important for proper germinal centre (GC) responses upon low avidity Ag stimulation, such as sheep red blood cells (SRBCs). However, during immunization with high avidity Ag, Setd3-deficient B cells could respond and form GC similarly as that of wild-type cells. As Setd3 is shown to methylate histidine 73 on actin and thereby stabilizes F-actin, Setd3 may control BCR signalling strength by regulating cortical actin dynamics via actin methylation or other cytosolic substrates. The underlying molecular mechanisms will be further investigated.

IMMUNE CELL EXPRESSION OF THE IFNAR1 RECEPTOR IS REGULATED BY AN INTRONIC TRANSPOSON

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Transposons are known to be co-opted by their host genomes to serve a number of regulatory functions, especially in the immune system. When Long Interspersed Nuclear Elements (LINEs) occur within genes, they are silenced by the Human Silencing Hub (HUSH) complex, and have been assumed to serve no active regulatory function. However, here we describe an intronic LINE-1 element within the Interferon Alpha/Beta Reporter subunit 1 (IFNAR1) gene with apparent regulatory activity in human immune cell lines. This intronic LINE-1, nicknamed IFNAR1.L1M2a is marked by epigenetic modifications associated with both silencing and transcriptional activation. It shows transcriptional and epigenetic signs of activity as a cis acting enhancer of gene expression. We removed the predicted enhancer region within the IFNAR1.L1M2a element using CRISPR knockout, and observed altered expression of the IFNAR1 gene by both quantitative PCR and RNA sequencing. Further, we observed changes in the downstream transcriptomic response of the cells to interferon signaling. These observations suggest that, although the intronic IFNAR1.L1M2a element appears to be epigenetically silenced by the HUSH complex, as expected, it also retains the potential for regulatory activity. This opens up a whole new population of potentially regulatory transposons to be examined which were previously assumed to be inactive.

EPIGENETIC REGULATION OF iNKT CELL DEVELOPMENT

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Invariant natural killer T (iNKT) cells carry a semi-invariant αβ T cell receptor (TCR) specific to lipids presented on cluster of differentiation 1d (CD1d) molecules. Characterization of the functional subtypes of iNKT cells and the key transcription factors regulating their development helped bring more insights into the specific functions of each of their subtypes during antimicrobial defense and tumor surveillance. However, there is still limited understanding of the epigenetic regulation of their development. Here, we investigate the role of the H3K27 histone demethylase UTX in regulating thymopoiesis, with a special focus on iNKT cell differentiation using a mouse model with conditional inactivation of *Utx* in the hematopoietic system. We compared by scRNAseq the transcriptomic profile of (1) all thymocytes and (2) sorted iNKT cells, in control and UTX-KO mice. We observed that double-positive (DP) thymocytes exhibited significant downregulation of genes related to TCR signaling as compared to cells in other stages of thymopoiesis. This may prevent proper intracellular TCR signaling for iNKT to differentiate from DP thymocytes. To confirm this hypothesis, we are analyzing the gene expression changes occurring in thymic iNKT cells in the absence of UTX. Taken together, our results suggest that epigenetic regulators such as a histone demethylase may play hand in hand with TCR signaling to determine T cell lineages differentiation. This study thus provides new hypotheses to further explore the regulation of gene expression during T cell development.

A MULTI-ENHANCER HUB AT THE ETS1 LOCUS IS REQUIRED FOR T CELL FUNCTION

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Enhancer regions have been known to cluster together in the three-dimensional space of the nucleus in a cell-type specific manner. Previous studies from our lab have identified such hyperconnected enhancer hubs in T cells, of which the Ets1-Fli1 locus has been a top candidate. To understand the functionality of the multiple genomic interactions at this locus, we focused on a regulatory node of connectivity at the Ets1 locus that is also conserved in humans and overlaps with polymorphisms associated with allergic diseases. We deleted this regulatory element in mice using CRISPR-Cas9, and observed that this region was dispensable for T cell development but important for T-helper (Th)-1 differentiation in mouse. Mice lacking this region were unable to develop Th1-mediated colitis, but showed a severe Th2-mediated allergic response to house-dust mite exposure, indicating at a loss of balance between Th1 versus Th2 – mediated immune-response in these mice. To understand the mechanism behind this phenotype, we performed single-cell oligopaint DNA FISH studies and observed chromatin reorganization at this locus after the deletion. This led to reduced Ets1 expression, which impaired Th1-differentiation possibly through defective CTCF recruitment. Our study thus demonstrates the importance of understanding the functionality of the hyperconnected enhancer regions, due to their crucial role in normal cellular process as well as their potential to predispose to disease responses.

TYPHOID TOXIN SORTING AND EXOCYTIC TRANSPORT FROM SALMONELLA TYPHI-INFECTED CELLS

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Typhoid toxin is an essential virulence factor for Salmonella Typhi, the cause of typhoid fever in humans. This toxin has an unusual biology in that it is produced by Salmonella Typhi only when located within host cells. Once synthesized, the toxin is secreted to the lumen of the Salmonella-containing vacuole from where it is transported to the extracellular space by vesicle carrier intermediates. Here, we report the identification of the typhoid toxin sorting receptor and components of the cellular machinery that packages the toxin into vesicle carriers, and exports it to the extracellular space. We found that the cation-independent mannose-6-phosphate receptor serves as typhoid toxin sorting receptor and that the coat protein COPII and the GTPase Sar1 mediate its packaging into vesicle carriers. Formation of the typhoid toxin carriers requires the specific environment of the Salmonella Typhi-containing vacuole, which is determined by the activities of specific effectors of its type III protein secretion systems. We also found that Rab11B and its interacting protein Rip11 control the intracellular transport of the typhoid toxin carriers, and the SNARE proteins VAMP7, SNAP23, and Syntaxin 4 their fusion to the plasma membrane. Typhoid toxin's cooption of specific cellular machinery for its transport to the extracellular space illustrates the remarkable adaptation of an exotoxin to exert its function in the context of an intracellular pathogen.

REGULATION OF HETEROGENEOUS GENE EXPRESSION IN MEDULLARY THYMIC EPITHELIAL CELLS

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Negative selection in the thymus is a central tolerance process by which strongly auto-reactive T-cells are eliminated in order to prevent autoimmunity. Thymic epithelial cells (TECs), specifically medullary TECs (mTECs), mediate negative selection by expressing a molecular mirror of the body's tissue-restricted antigens (TRAs) and displaying them to developing thymocytes. This interaction with an antigen-presenting mTEC triggers the destruction of self-reactive thymocytes. At single-cell resolution, mTEC TRA expression is heterogenous, with individual mature mTECs expressing only 1–3% of TRAs at a given time. Prior studies have identified the transcription factors Aire and Fezf2, as well as chromatin remodelers as modulators of mTEC promiscuous gene expression, but the mechanisms regulating mTEC heterogeneity are poorly understood. Here, we use bulk and single-cell technologies to investigate different levels of mTEC heterogeneity and probe spatiotemporal patterns within mTEC gene expression and mRNA processing. We sorted live TECs from 40 equidistant, transversal mouse thymus slices, and sequenced these spatially mapped TECs in bulk and single cell. We found uniform spatial distributions of Aire and Fezf2, as well as of mTEC specific transcription factors and housekeeping genes. In contrast, we found significant spatial differential expression among the transcriptomic profiles of a wide range of TRAs. These results hint towards a spatial dependence of TEC expression heterogeneity. Future studies incorporating RNA processing and development-specific expression patterns will aid in obtaining a comprehensive profile of the mechanisms underlying the heterogeneity in promiscuous gene expression.

THE ROLE OF EMT TRANSCRIPTION FACTOR ZEB2 IN FETAL HEMATOPOIESIS

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Hematopoiesis is comprised of three waves: primitive, transient-definitive and definitive hematopoiesis. Transient-definitive and definitive hematopoiesis require the erythromyeloid progenitor (EMP) and the hematopoietic stem cell (HSC), respectively. The absence of EMPs results in midgestation lethality and EMP-derived monocytes are recognized as the main source of tissue-resident macrophages (TRMs) in the adult. During adult life, EMP-derived TRMs can be replace by monocytes derived from HSCs, at rates that vary among different tissues. However, much remains unknown regarding the developmental and functional differences between the monocytes and macrophages that are derived from the EMP vs. the HSC. A major challenge is that EMP-derived and HSC-derived monocytes/macrophages share many surface markers and transcription factors important for their development. Previous studies indicate that the transcriptional factor *Zeb2* is required for EMP formation and/or differentiation, and for the maintenance of adult TRMs. Our recent work has demonstrated that HSC-derived monocyte development requires the action of the -165kb enhancer in order to maintain *Zeb2* expression. The lack of the -165kb enhancer causes the complete abrogation of HSC-derived monocyte development but leaves the adult TRM populations intact. Here, we show that similar to HSC-derived monocytes, EMP-derived monocytes also require the -165kb enhancer for *Zeb2* expression. However, we find that *Zeb2* is unexpectedly not required for EMP-derived monocyte development. Additionally, our data show that *Zeb2* expression in fetal liver macrophages is not reduced in the absence of the -165kb enhancer. This suggests that other enhancers likely act to support *Zeb2* expression in EMP-derived macrophages. We have identified several candidate enhancers to consider for in vivo validation. Additionally, we will test the requirement of *Zeb2* for primitive and transient-definitive hematopoiesis.

EPIGENETIC MEMORY OF COVID-19 IN INNATE IMMUNE CELLS AND THEIR PROGENITORS

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The illness caused by infection SARS-CoV2, is characterized by a broad range of symptoms and severity and can result in a protracted course. Delayed adaptive immune response with robust innate immune cell activity features prominently in acute severe COVID-19. However, the long-term effects of COVID-19 on the immune system are unclear. Durable changes in the immune system following COVID-19 could influence subsequent immune responses to pathogens, vaccines, or even contribute to long-term clinical symptoms, i.e., post-acute sequelae of SARS-CoV-2 infection (PASC). Despite clinical observations of long-term sequelae, the nature of persistent molecular and cellular changes following COVID-19 is poorly understood.

Recent studies have established that innate immune cells and their progenitors can maintain durable epigenetic memory of previous infectious or inflammatory encounters, thereby altering innate immune equilibrium and responses to subsequent challenges. In addition, it has been revealed that HSPC can be epigenetically reprogrammed to persistently convey inflammatory memory to mature progeny cells with altered phenotypes. However, the complexities of studying human HSPC, especially in the context of cohort-level infectious disease studies, have limited our understanding of this type of HSPC cell-based memory.

We hypothesized that exposure of HSPC to inflammatory signaling events during severe COVID-19 requiring critical care unit admission could result in epigenetic memory and permanently changed phenotypes subsequent to COVID-19. By characterizing the molecular characteristics of the post-infection phase, we evaluated changes at the single-cell level in the epigenome and transcriptom of monocytes and HSPC.

To analyze HSPC at single-cell resolution, we created a new workflow to enrich and profile rare HSPC from peripheral blood, entitled Peripheral Blood Mononuclear Cell analysis with Progenitor Input Enrichment (PBMC-PIE), which allowed us to avoid bone marrow biopsies. A paired PBMC-PIE and single-nuclei RNA/ATAC-seq approach produced a high-resolution transcriptome and chromatin accessibility map of various HSPC subsets and PBMC in a cohort of convalescent severe COVID-19 patients ranging from months to a year.

We reveal the persistence of epigenetic and transcription programs in HSPC and monocytes following severe disease that are indicative of an altered innate immune responsiveness. These included durable epigenetic memory linked to inflammatory programs, myeloid lineage differentiation, and monocyte phenotypes. This highlights the potential for acute viral infection to drive a durable program of HSPC origin that is conveyed through to progeny monocytes to mediate an altered immune responsiveness, potentially misaligning immune homeostasis and influencing future immune challenges.

AN INTEGRATED TREG TRANSCRIPTION FACTOR NETWORK REVEALS CONTEXT-SPECIFIC ROLES FOR FOXP3

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Foxp3+CD4+ regulatory T cells (Tregs) are dominant controllers of immunological and organismal homeostasis. Reflecting their varied target cells and sites of residence, Tregs adopt remarkably heterogeneous functional states and molecular programs. While previous work has identified individual transcription factors (TFs) whose expression, along with that of FoxP3, enables differentiation into specialized sub-phenotypes, how the many TFs expressed in Tregs are systematically organized to determine Treg identity and diversity remains unclear. To address this question, we performed single-cell ATAC-seq (scATAC-seq) of splenic Tregs, relating the activity of open chromatin regions (OCRs), and the TFs that modulate them, to variation in Treg states. Unlike previous master TF models, we found that Treg states had overlapping accessibility at binding sites for several TF families, suggesting that each subset of Tregs was associated with combinatorial layering of multiple TFs. We parsed this combinatorial complexity in two ways. First, using probabilistic topic modeling, we computationally grouped co-varying OCRs into distinct Treg epigenomic programs ('topics'). Enrichment of TF motifs and binding sites within each topic nominated the combinatorial regulators of these state-specific programs. Second, we generated scATAC-seq profiles of Tregs from B6xCast F1 mice to identify the cell states and genomic loci in which TF motifs had causal effects by linking polymorphisms between B6 and Cast parental sequences to corresponding differences in allele-specific chromatin accessibility. Together, these two approaches resolved the organization of the Treg TF network, which we validated using TF binding data and Treg-specific TF knockouts (KOs). Finally, we investigated how FoxP3 impacted the Treg TF network. In scATAC-seq profiles of Tregs with or without functional FoxP3 from the same otherwise healthy heterozygous female mice, FoxP3-deficient Treg-like cells ('wannabe Tregs') displayed profound changes in chromatin accessibility and de-repressed cytokine production. FoxP3 had dual activating and repressive effects on chromatin accessibility, each component co-localizing with distinct TF cofactors. At the cellular level, wannabe Tregs had increased proportions of microbe-dependent Rorγ+ peripheral Treg-like cells in several tissues, but most pronounced in the colon and mesenteric lymph nodes, where wannabes had equal competitive fitness with their wild type Treg counterparts. Thus, dissecting the structure of the Treg TF network showed that Treg diversity arises not from the action of individual master TFs but rather graded multi-TF inputs with variable dependence on FoxP3.

A RETROTRANSPOSON-DERIVED IFNAR2 SPLICE VARIANT FUNCTIONS AS AN INTERFERON DECOY RECEPTOR

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Type I interferon (IFN) signaling mediates cellular innate immune responses to infection, and must be tightly regulated to avoid excessive inflammation or autoimmunity. Dysregulated responses to IFN underlie pathological responses to infection, but the molecular determinants of IFN sensitivity remain poorly understood. In the canonical model of human type I IFN signaling, it is assumed that the IFNAR2 receptor is encoded by a single full-length transcript (IFNAR2-L). However, the human IFNAR2 locus contains additional annotated splice variants, including a short isoform (IFNAR2-S) which uses an Alu element as an alternative terminal exon and generates a truncated protein. The IFNAR2-S isoform was first identified nearly thirty years ago but its potential role in IFN signaling has remained neglected over the past two decades.

Here, we re-analyzed short and long-read RNA-seq datasets and discovered that IFNAR2-S is consistently expressed at higher levels than the canonical IFNAR2-L receptor. The unexpectedly broad expression of IFNAR2-S implicates a functional role in human IFN signaling. To investigate this possibility, we used CRISPR, siRNA, and overexpression experiments in a panel of human cell lines to disentangle the functions of the short and long IFNAR2 isoforms. Deletion or depletion of IFNAR2-S potently increased the amplitude of IFN-stimulated gene activation in response to type I IFN stimulation, and increased sensitivity of human cells to IFN-mediated cytotoxic and antiviral activity in cells. Our evolutionary analysis indicates that IFNAR2-S evolved via a primate-specific gene conversion event that inserted an Alu element which provided an alternative splice acceptor site which is conserved in all simian primates. Notably, the Alu-derived terminal exon harbors two genetic variants linked to severe COVID-19 whereas the canonical terminal exon does not, suggesting that disease-associated variants at the IFNAR2 locus may act by altering IFNAR2 splicing or isoform-specific regulation.

Together, our findings uncover IFNAR2-S as a a novel decoy receptor that restrains human IFN signaling, and implicate IFNAR2 isoform-specific regulation as a previously unappreciated axis regulating type I IFN signaling. Our study highlights TE exonization as a process that fuels the evolution of species-specific isoforms and the emergence of new mechanisms of immune regulation.

CHARACTERIZATION OF THE FUNCTION OF TIF1γ IN THE CONTROL OF T REGULATORY CELLS

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TGF-β can regulate T cell differentiation and effector phenotypes. In particular, it can induce the differentiation and function of regulatory T cells (Tregs) in vitro and in vivo. TGF-β signaling depends on phosphorylation of Smad2 and Smad3, which interact with the common Smad: Smad4. This Smad2/3/4 complex was considered the main signal transduction element in the pathway. However, the transcriptional intermediary factor 1 γ (TIF1γ) was recently shown to interact with Smad2/3, leading to a distinctive gene expression profile and cell fate. The objective of this work is to elucidate the role of TIF1γ in the differentiation, cell function, and stability of Tregs. Using different conditional mouse models we demonstrated that under homeostatic conditions, TIF1γ is dispensable for Treg generation. However, it is required for the maintenance of suppressive functions and stability under inflammatory conditions, both in vivo and in vitro models. We also identified the molecular mechanism by which TIF1γ regulates Treg function, modulates the expression of different suppressive markers, and maintains Foxp3 expression under inflammatory conditions. In conclusion, TIF1γ is required for proper differentiation Treg lymphocytes and their acquisition of a suppressor phenotype.

IKK-α-DEPENDENT HISTONE PHOSPHORYLATION REGULATES IFNγ-INDUCIBLE TRANSCRIPTION

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A hallmark of innate immune cell activation is the rapid induction of inflammatory gene transcription. This process inherently requires efficient targeting of a small set of genes distributed throughout the three-billion base pair genome. Epigenetic genome cataloguing guide selective, high-level, and rapid transcriptional regulation following stimulation. Intrinsic to epigenetic mechanisms are core histone protein octamers that are wrapped by DNA to form nucleosomes. Histone proteins have unstructured tails that protrude from the nucleosomal core and are highly post-translationally modified, with specific modifications enriched at regions of the genome associated with distinct biological functions. Through unbiased mass spectrometry, we identified that the most dynamic histone post-translational modification after stimulation of bone marrow-derived macrophages (BMDM) with bacterial lipopolysaccharide (LPS) is phosphorylation of serine 31 (S31) on the amino-terminal tail of histone H3.3 (H3.3S31ph). This modification is catalyzed by IκB kinase alpha (IKK-α), a component of the NF-κB signaling cascade. Among 13 gene copies of histone H3, the variant H3.3 is the only one that contains this target S31 and is enriched in regulatory chromatin such as promotors, enhancers, and gene bodies. The contribution of histone H3.3 to regulating rapid immune responses remains poorly understood. We generated conditional H3.3 knockout (H3.3KO) mice, and found that H3.3KO BMDMs completely unable to initiate an inflammatory response to lipopolysaccharide (LPS). Additionally, H3.3KO mice are also unable to control infection with the intracellular bacteria Listeria monocytogenes. We found that signaling-to-chromatin is conserved among diverse immune cells. We thus speculated that the activity of IKKα may not be limited to NF-κB-dependent pathways. In accordance with this idea, we determined that H3.3KO BMDMs show deficient activation when stimulated with the cytokine interferon gamma (IFNγ). We utilized a lentiviral system to replace H3.3KO BMDMs with either WT H3.3, H3.3S31A, or H3.3S31E, stimulated these cells with IFNγ and found that both the WT H3.3 and the phospho-mimetic H3.3S31E restored IFNγ-induced activation, demonstrating that the deficiency in transcriptional responses to IFNγ is dependent on H3.3S31 phosphorylation, and ultimately indicating a conserved mechanism of signaling to chromatin across multiple unrelated pathways. To confirm utilization of IKKα in this pathway, we treated cells with an IKKα-specific inhibitor and found that H3.3S31ph, and subsequent transcriptional responses, were dependent on IKKα kinase activity in both NF-κB-dependent and -independent pathways. In the context of IFNγ stimulation, we also found that chromatin binding and activity of IKKα requires STAT1. We therefore propose a model wherein IKKα is targeted to specific genes by activated transcription factors, such as NF-κB or STAT1, where it phosphorylates H3.3S31 to promote rapid transcription, and that features of H3.3, including H3.3S31ph, provide preferential access to the transcription apparatus.

ROLE FOR LCK Y192 IN MODULATING AUTO-REGULATORY LCK-ZAP70 KINASE LOOP AND REGULATES TCR SIGNALING

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Role for Lck Y192 in modulating auto-regulatory Lck-Zap70 kinase loop and regulates TCR signaling.
Tightly regulated T cell receptor signaling is critical for proper T cell development and effector function. Lck, a Src family kinase, plays myriad roles in regulating TCR signaling by phosphorylating CD3 necessary for Zap70 recruitment and activation, and serving as a bridge between Zap70 and LAT. Phosphorylation of activating Y394 and inhibitory Y505 regulate Lck kinase activity. Recent reports show that phosphorylation of Lck Y192 inhibits the kinase activity by preventing docking of CD45 required for Lck activation via dephosphorylation of inhibitory Y505 (1) . Interestingly, in in vitro kinase assay the enzymatic activity of phospho-mimicking Lck Y192E mutant appeared to be intact and it remains unclear how Y192 modulates the Lck kinase function.
We recently showed that Zap70 expression inversely correlates with the Lck activity (2-3) . Since the two kinases act on each other, we took advantage of this system to evaluate role of Y192 in controlling Lck activity. Our results show that phospho-mimicking Y192E mutant inhibits Lck kinase activity in 293T and T cell lines, and the preliminary results suggest that it may act as a dominant negative. We also show that Lck auto-phosphorylates at Y192 and this is further augmented by Zap70. Together, these data suggest an important role for Lck Y192 in modulating auto-regulatory Lck-Zap70 kinase loop and as such may regulate TCR signaling. Ongoing experiments will investigate how Lck-Zap70 loop impacts TCR signaling during thymocyte development and effector T cell function.

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ROLE OF *IKZF3* AS A DRIVER MUTATION IN PRECURSOR B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Deletion of genes encoding the related E26-transformation-specific transcription factors PU.1 and Spi-B results in interleukin-7 (IL-7)-dependent B cell acute lymphoblastic leukemia (B-ALL) at 100% incidence in mice. Whole-exome sequencing of B-ALLs from this mouse model was used to identify recurrent mutations that may cooperate with PU.1/Spi-B deletion to drive B-ALL. We found recurrent mutations in *Ikzf3* encoding the zinc-finger transcription factor Aiolos. Two *Ikzf3* mutations were discovered, R137* and H195Y, and occur in the DNA-binding domain in zinc fingers 1 or 4, respectively. Mutations in Aiolos and the related protein Ikaros (Ikzf1) are frequent in human B-ALL, but the mechanism of how these mutations drive cancer is poorly understood. It was previously shown that Ikzf1 can interact with STAT5 binding sites to modulate the effects of cytokine signaling. We hypothesized that Ikzf3 R137* and H195Y mutants alter IL-7-dependent JAK/STAT signaling, resulting in pro-leukemic gain-of-function changes in PU.1/Spi-B deficient B cells. Ikzf3 R137* mutation resulted in a truncated protein, and therefore represents a loss-of-function mutation. Ikzf3 H195Y mutation resulted in altered DNA binding specificity as determined by electrophoretic mobility shift assays. To determine function, WT or H195Y Ikzf3 was ectopically expressed in 38B9 pre-B cells or preleukemic IL-7-dependent PU.1/Spi-B-deficient pre-B cells. Forced expression of Ikzf3 H195Y reduced proliferation of 38B9 cells and increased proliferation of pre-B cells. RNA sequencing analysis showed that IL-7-dependent gene expression was altered by expression of Ikzf3 H195Y. Chromatin immunoprecipitation analysis showed that Ikzf3 H195Y inhibited recruitment of STAT5 to the promoter of the gene *Cish*. We conclude that Ikzf3 H195Y mutation alters DNA binding specificity, resulting in altered STAT5 interaction with target genes following IL-7 signaling. Ultimately, this work is expected to provide insight into how Ikzf3 mutations may function as cancer drivers in pre-B-ALL.

CONDITIONAL DEPLETION OF BRD4 IN MICROGLIA LEADS TO A PARTIAL RESISTANT TO EAE PATHOLOGY

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Experimental Autoimmune Encephalomyelitis (EAE) is a widely studied animal model for multiple sclerosis (MS) a chronic autoimmune disease of central nervous system (CNS). EAE occurs with the infiltration of reactive lymphocytes and macrophages from the periphery. In EAE CD4 T cells get activated in the periphery and gain access to the CNS through blood brain barrier. In the CNS Infiltrated T cells are further activated by resident or infiltrating APC, which present MHCII associated peptides resulting in demyelination and neuronal damage. We show that BRD4 is critically required for T helper cell differentiation, particularly Th17 cells to express Il17a, Il17f, Il21, Rorc etc. Thus, mice with Brd4 deletion in CD4 T cells were resistant to EAE, with few reactive T cells infiltrating into the CNS. To elucidate the role of microglia in EAE, we asked whether BRD4 is required for microglia's APC function. To this end, we conditionally knocked out Brd4 in microglia (Brd4cKO). Surprisingly we observed that mice with Brd4 deletion had fewer microglia, fewer infiltrating T cells in the CNS showing no demyelination, which led to reduced paralysis. Further analyses showed that inflammatory response genes including Il1b, Ccl, Cxcl chemokines and MHCII, while upregulated in microglia from wild type mice, were markedly downregulated in Brd4cKO mice during EAE. Our results reveal that microglia plays a pivotal role in EAE pathology, which is driven by BRD4.

UNDERSTANDING THE ROLE OF ABERRANT EXPRESSION OF SP140 SPLICE VARIANT IN DISEASE

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Speckled Protein 140 (SP140) is a chromatin reader associated with maintaining immune cell identity, and SP140 splice variants are associated with immune diseases including Crohn's disease, multiple sclerosis, and chronic lymphocytic leukemia. SP140 expression is typically restricted to immune cells. However, by investigating splice junctions across ~50,000 publicly available RNA-seq datasets, we have discovered a novel splice variant driven by an intronic endogenous retrovirus (ERV) acting as an alternative promoter driving SP140 expression in a wide range of cancer cells. We hypothesize this novel disease-specific splice variant could contribute to immune dysregulation in cancer cells. Using CRISPR to generate SP140 knockouts/knockdowns in a panel of cancer cell lines, we have identified hundreds of differentially regulated genes. Ongoing work includes characterizing this splice variant biochemically and functionally via following up AP-1 transcription motifs found in the alternative promoter and following up with the differentially regulated gene networks which implicate cell motility and NFkB dysregulation. Our work suggests that this novel SP140 isoform contributes to immune dysregulation in cancer cells.

BCL6-DEPENDENT TCF-1^{+} PROGENITOR CELLS MAINTAIN EFFECTOR AND HELPER CD4^{+} T CELL RESPONSES TO PERSISTENT ANTIGEN

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Soon after activation, CD4^{+} T cells are segregated into BCL6^{+} follicular helper (Tfh) and BCL6^{-} effector (Teff) T cells. Here, we explored how these subsets are maintained during chronic antigen stimulation using the mouse chronic LCMV infection model. Using single cell-transcriptomic and epigenomic analyses, we identified a population of PD-1^{+} TCF-1^{+} CD4^{+} T cells with memory-like features. TCR clonal tracing and adoptive transfer experiments demonstrated that these cells have self-renewal capacity and continue to give rise to both Teff and Tfh cells, thus functioning as progenitor cells. Conditional deletion experiments showed Bcl6-dependent development of these progenitors, which were essential for sustaining antigen-specific CD4^{+} T cell responses to chronic infection. An analogous CD4^{+} T cell population developed in draining lymph nodes in response to tumors. Our study reveals the heterogeneity and plasticity of CD4^{+} T cells during persistent antigen exposure and highlights their population dynamics through a stable, bipotent intermediate state.

GENETIC DETERMINANTS OF ALTERNATIVE SPLICING IN STIMULATED IPSC-DERIVED MACROPHAGES ENHANCE THE UNDERSTANDING OF IMMUNE-MEDIATED DISEASE RISK.

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Macrophages represent the first line of defence against pathogens due to their ability to respond to environmental cues. Genetic variation can drive substantial alteration of this response, increasing susceptibility to immune-mediated diseases (IMD). Thus, understanding how macrophage cellular phenotypes such as gene expression are genetically controlled, particularly in response to environmental stimuli, can provide much needed insight into IMD biology and risk.

Since modelling environmental contexts in primary cells at scale is impractical, induced pluripotent stem cells (iPSC) offer an alternative in vitro system, which provides sufficient cells to study gene expression and its different layers of control. Alternative splicing (AS) is a major post-transcriptional layer of gene expression control, driving remarkable diversification of the transcriptome. Therefore, mapping splicing quantitative trait loci (sQTLs) improves our understanding of how genetic variation affects AS and predisposes to IMDs. However, the extent to which macrophage sQTLs affect IMD risk has not yet been systematically studied.

Here, we map sQTLs in iPSC-derived macrophages exposed to a panel of 10 different stimulants obtained from 209 individuals. Gene expression was measured 6 and 24 hours after stimulation in each state, resulting in 4,698 RNA-seq samples. We identify 5,734 genes with a sQTL in at least one state, with a median of 1,580 genes per state. We performed colocalization analysis between sQTLs or eQTLs (mapped from the same data), and results from 22 IMD genome-wide association studies. Over 50% of tested loci were likely to share a single causal variant with either an eQTL, sQTL or both ($PP4 \geq 0.75$). Almost half of these loci (25%) colocalized solely with an sQTL, clearly showing the important role that alternative splicing plays in IMD risk.

Interestingly, 53% of colocalized sQTL introns have a mean intron usage ratio (IUR) < 0.1 across samples. Moreover, 46% of these introns are not found in any annotated transcripts in GENCODE, compared with only 15.5% in introns with mean IUR ≥ 0.1. This suggests that many of the IMD-associated risk loci may result in the usage of rare splicing events that are underrepresented in annotated transcripts.

Our work highlights the added value of mapping context-aware sQTLs. We show that they provide independent high-confidence colocalizations with IMD risk loci, and highlight a particularly important role for rarely-used introns.

Z-DNA UNDERLIES THE TARGET SPECIFICITY OF AIRE BY PROMOTING POISING OF TRANSCRIPTIONAL PROMOTERS

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Aire is a transcriptional regulator that drives immunological tolerance by upregulating the expression in medullary thymic epithelial cells (mTECs) of thousands of genes encoding peripheral-tissue antigens. It does not bind to a specific DNA sequence motif, instead participating in large, chromatin-associated protein complexes. Aire has been shown to preferentially bind to and activate super-enhancers, to encourage promoter:enhancer looping, and to release RNA polymerase from poised promoters. However, its target choice remains a mystery. To investigate the *cis* elements distinguishing Aire target genes, we 1) built a convolutional neural network to study sequence features of the extended promoter regions of Aire-induced genes, and 2) analyzed the effects of natural genetic variations between the C57BL/6 and NOD/ShiLtJ mouse strains on allelic imbalances in chromatin accessibility and expression of Aire-induced genes. Results from these orthogonal approaches converged on Z-DNA as a putative positive regulator of the target specificity of Aire. By profiling DNA double-strand-break (DSB) formation genome-wide in mTECs, we found that Z-DNA motifs were positively associated with the inherent ability of a promoter to generate DSBs. We also noticed that promoters with strong DSB accumulation were more likely to be in a poised state with accessible chromatin and already-assembled transcriptional machinery. Genome-wide mapping studies revealed that Aire preferentially targeted genes with poised promoters enabled by Z-DNA formation. Enhancing either Z-DNA formation (via spermidine) or DSB generation (via topotecan) increased the expression of Aire-induced genes in mTECs from wild-type mice. Strikingly, promotion of Z-DNA formation in mTECs from Aire knock-out mice could substitute for Aire in upregulating the expression of a subset of Aire-induced genes. Thus, we propose a model whereby Z-DNA anchors the Aire-mediated transcriptional program by promoting DSB generation and promoter poising.

INVESTIGATING THE ROLE OF AN INTRAGENIC TANDEM AMPLIFICATION OF PAX5 IN B CELL DEVELOPMENT AND LEUKEMIA

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Alterations of the B cell lineage-defining transcription factor pair-box protein 5 (PAX5) are a central event in B cell acute lymphoblastic leukemia (B-ALL). The spectrum of B-ALL-associated *PAX5* lesions is incredibly diverse, comprising simple loss-of-function mutations, focal deletions, as well as chromosomal translocations that give rise to various Pax5 fusion proteins. Recently, a novel *PAX5* lesion, referred to as PAX5 intragenic tandem amplification (PAX5-ITA), has been described. This alteration is associated with 1% of all B-ALLs and is characterized by up to 5 in-frame intragenic amplifications of *PAX5* exon 2–5. As this segment includes the PAX5 DNA-binding domain, the amplification might impart PAX5-ITA with distinct molecular features and thus explain its B-ALL recurrence.
To study the molecular function of Pax5-ITA *in vivo*, we have generated a mouse model expressing Pax5-ITA from the endogenous Pax5 locus ($Pax5^{ITA}$). While heterozygous expression of Pax5-ITA had only a minor effect on B cell development, B cell lymphopoiesis was arrested at an early progenitor stage in homozygous $Pax5^{ITA/ITA}$ mice. Likewise, RNA-seq comparison of $Pax5^{ITA/ITA}$ and Pax5 deficient pro-B cells ($Pax5^{-/-}$) identified only few differentially expressed genes, suggesting that Pax5-ITA is unable to activate and repress canonical Pax5 target genes. Moreover, analysis of the genome-wide DNA-binding revealed that Pax5-ITA failed to efficiently bind the majority of canonical Pax5 target sites and thus provides one explanation for Pax5-ITA's inability to direct B cell development.
As heterozygous loss of Pax5 is a well-established B-ALL risk factor, inactivation of one Pax5 allele is one possible mechanism how the Pax5-ITA rearrangement contributes to B-ALL leukemogenesis.

TO EACH THEIR OWN: DISTINCT PATHWAYS TO TYPE 2 IMMUNITY FOR DISPARATE ALLERGEN CLASSES

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Unlike many pathogens that are detected directly by the innate immune system, protease allergens such as papain are initially detected by cutaneous sensory neurons enriched in *Trpv1* expression. TRPV1$^+$ neurons directly detect protease allergens and release substance P, which induce the migration of CD301b$^+$ dendritic cells (DCs) into the draining lymph node (dLN) to initiate T-helper 2 (Th2) cell differentiation. However, this mode of detection may not be universal as allergens are highly heterogeneous. We found that bee venom allergens such as phospholipase A2 (bvPLA2) activate neurons to specifically release calcitonin gene-related peptide (CGRP) instead of substance P. Since previous work from our laboratory showed that substance P, but not CGRP, promoted DC migration to allergens, this observation suggested that allergic sensitization to venoms may proceed via novel pathways. To elucidate the mechanisms of allergic immune initiation to venoms, we administered purified bvPLA2 intradermally in mice to study the DC and CD4$^+$ T cell responses in the dLNs. bvPLA2 induced the activation and migration of the Th2-skewing CD301b$^+$ dermal cDC2 subset similarly to that seen in response to the substance P-releasing allergen, papain. However, bvPLA2 immunization led to poor antigen uptake by the CD301b$^+$ DCs. This corresponded to decreased CD4$^+$ T cell activation and Th2 differentiation in comparison to papain immunization both *in vivo* and *in vitro*, despite equal numbers of activated CD301b$^+$ DCs in the dLN. To determine whether such defects in DC antigen uptake and CD4$^+$ T cell activation were mediated by allergen-induced neuronal signaling or neuropeptide release, we examined the response to bvPLA2 in mice depleted of TRPV1$^+$ neurons. Surprisingly, TRPV1$^+$ neuronal depletion rendered mice highly susceptible to death after a non-lethal dose of bvPLA2 injection. We conclude that (1) bvPLA2 immunization leads to robust DC activation and migration but defective antigen uptake; (2) said defect may be responsible for impaired antigen presentation and Th2-skewing of naïve CD4 T cells; and (3) TRPV1$^+$ neurons play a protective role against the toxicity of bvPLA2.

OTUD4 BALANCES THE PROTEASOMAL DEGRADATION AND NUCLEAR ACCUMULATION OF THE NF-κB TRANSCRIPTION FACTOR C-REL

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c-REL is a unique proto-oncogen of the NF-κB family of transcription factors that is frequently amplified in B-cell lymphoma and shows a direct link to antibody isotype switching and autoimmune disease. Multiple signals result in nuclear translocation and activation of NF-κB transcription factors leading to cell survival, inflammation, and immune-evasion in lymphomas and solid tumors. The ubiquitin proteasome system (UPS) critically regulates NF-κB activation via proteasomal degradation of the inhibitory IκB proteis. However, the direct ubiquitination of c-REL and its physiological consequences are not well established. Using a combination of proteomics, protein biochemistry experiments, mouse xenograft models and patient tissue analysis, we present a conserved molecular mechanism in Diffuse Large B cell lymphoma (DLBCL) and Pancreatic Ductal Adenocarcinoma (PDAC) to modulate the proteasomal degradation of c-REL. We identify *OTU domain-containing ubiquitin protease OTUD4* to stabilize c-REL in the cytoplasm and fine-tune nuclear translocation of c-REL. Importantly, OTUD4 regulates NF-κB transcription in DLBCL and is required for the expansion of DLBCL and PDAC tumor cell lines *in vitro* and *in vivo*. Finally, a direct correlation of OTUD4 and c-REL is observed in DLBCL Tissue Microarray. Our results reveal how a deubiquitinating enzyme regulates NF-κB activation beyond IκBs and provide a new strategy to target c-REL in disease

VIRUS-HOST CROSS-TRANSCRIPTIONAL REGULATION

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Viruses can rewire and leverage host gene regulatory networks for productive viral replication and to evade immune responses. Given the coexistence of viral genomes and host transcription factors (TFs) and host genomes and viral TF within infected cells, this provides the opportunity for cross-transcriptional regulation between virus and host. In the first type of cross-transcriptional regulation, cis-regulatory elements of DNA viruses and retroviruses bind host TFs to regulate viral gene expression in the appropriate cell types and states to promote viral replication or latency. In this regard, we have identified KLF2, KLF3, and PLAGL1 as novel regulators of HIV expression in CD4+ T cells, as well as upstream pathways that impinge on the HIV long-terminal repeat. In the second type of cross-transcriptional regulation, viral TFs bind to host cis-regulatory elements to modulate host gene expression and promote cell proliferation, metabolic changes, and immune suppression. We have recently generated a catalog of 419 viral TF belonging to 20 different virus families. Using this catalog and follow up experiments, we characterized shared and unique cellular genes, proteins, and pathways targeted by particular viral TFs and their role in human disease pathogenesis. Altogether, our studies are identifying mechanisms by which viruses control host gene regulatory networks to replicate and evade immune responses.

ESCRT-DEPENDENT STING DEGRADATION CURTAILS STEADY-STATE AND cGAMP-INDUCED SIGNALING

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STING is an intracellular sensor of cyclic di-nucleotides involved in response to pathogen- or self-derived DNA that induces protective immunity, or if dysregulated, autoimmunity. STING trafficking is tightly linked to its activity. We aimed to systematically characterize genes regulating STING trafficking and to define their impact on STING responses. Based on proximity-ligation proteomics and genetic screens, an ESCRT complex containing HGS, VPS37A and UBAP1 was found to be required for STING degradation and signaling shutdown. Analogous to phosphorylated STING creating a platform for IRF3 recruitment, oligomerization-driven STING ubiquitination by UBE2N formed a platform for ESCRT recruitment at the endosome, responsible for STING signaling shutdown. A UBAP1 mutant that underlies human spastic paraplegia and disrupts ESCRT function led to STING-dependent type I IFN responses at the steady-state, defining ESCRT as a homeostatic regulator of STING signaling.

TRANSCRIPTIONAL REGULATION OF THE THYMUS MASTER REGULATOR *FOXN1*

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FOXN1 is a transcription factor critical for the development of both thymic epithelial cell (TEC) and hair follicle cell (HFC) compartments. However, mechanisms controlling its expression remain poorly understood. To address this question, we performed thorough analyses of the conservation and chromatin status of the *Foxn1* locus in different tissues and states, and identified several putative cis-regulatory regions unique to TEC vs. HFC. Furthermore, experiments using genetically modified mice with specific deletions in the *Foxn1* locus and additional bioinformatic analyses helped us identify key regions and transcription factors involved in either positive or negative regulation of *Foxn1* in both TEC and HFC. Specifically, we identified SIX1 and FOXN1 itself, as key factors inducing *Foxn1* expression in embryonic and neonatal TECs. Together, our data provide important mechanistic insights into the transcriptional regulation of the *Foxn1* gene in TEC vs. HFC and highlight the role of FOXN1 in its autoregulation.
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INTRINSICALLY DISORDERED DOMAIN OF TRANSCRIPTION FACTOR TCF-1 IS REQUIRED FOR T CELL DEVELOPMENTAL FIDELITY.

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The transcription factor TCF-1 has long been demonstrated to be essential for normal T cell development, situated at the earliest stages of lineage specification downstream of Notch signaling in the thymus. Recent work has shown TCF-1 targets and is essential for the opening of chromatin in T cells to orchestrate the gene regulatory cascade that occurs during development. However, besides the high mobility group DNA binding domain, no portion of the TCF-1 protein has been characterized to determine its role in hematopoiesis. We fully characterized all regions of TCF-1 and assessed their importance in TCF-1 driven epigenetic and transcriptional regulation as well as in endowing developmental competency to the T cell lineage. Surprisingly, we identified an intrinsically disordered domain within the N terminus of TCF-1 (NTD) that is necessary for efficient transition from early thymic progenitors to DN2 T cells. The few T cells that develop from progenitor cells expressing mutant TCF-1 lacking the N terminal domain (ΔNTD) exhibit lineage infidelity that was distinct and unique from the lineage diversion TCF-1KO cells demonstrated. Transcriptional and epigenetic profiling indicated the NTD was required for efficient target gene transactivation which correlated with the de-repression of a set of GATA2 driven genes normally reserved to the mast cell and dendritic cell lineages. ΔNTD DN2 cells showed aberrant opening of previously silent chromatin at mast cell genes and GATA2 bound enhancers. These data suggest the integral role of TCF-1's intrinsically disordered N terminus in maintaining fidelity to the T cell lineage through a careful balance of activation of partner TF's that are required to repress alternative lineages.

COMPUTATIONAL MODELING OF STAT ACTIVATION FEATURES THAT PREDICT GENE EXPRESSION PROFILES

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Signal transducers and activators of transcription (STATs) integrate complex cytokine signals in the environment to mediate intricate cellular responses. In macrophages, cytokines IL-6 and IL-10 signal through common STATs (STAT1 and STAT3) to promote contrasting immune functions. This STAT functional specificity is encoded by variety of mechanisms, such as unique phosphorylation dynamics. While select regulators of STAT signaling have been shown to contribute to cytokine-specific phosphorylation and gene expression, we lack a comprehensive understanding of STAT signaling features that encode specific cytokine-induced gene profiles and mechanisms shaping STAT features. This knowledge gap limits our ability to effectively modulate cytokine-specific STAT-driven inflammatory responses. To this end, we have developed a novel computational workflow that uses an experimentally validated mechanistic model of IL-6 and IL-10 signaling dynamics and a machine learning model that predicts cytokine-induced gene expression patterns from STAT phosphorylation dynamics. The integration of these two computational models has identified early pSTAT1 features (<10 mins.) along with peak and late pSTAT3 time points as necessary for the prediction of select cytokine-induced gene clusters. Additionally, a sensitivity analysis of the mechanistic signaling model has identified JAK-related parameters that influence IL-6-driven STAT responses to a greater degree than the IL-10-driven responses, suggesting mechanisms that support cytokine-specific STAT phosphorylation dynamics. We are currently identifying the network parameters strongly associated with dynamic signaling features that are sufficient to predict gene clusters of interest. Overall, our novel approach will further our understanding STAT functional specificity and contribute to future efforts to target pathology-associated STAT-driven genes.

ROSEOMONAS MUCOSA ADHERES TO HUMAN KERATINOCYTES AND SUPPORTS HOMEOSTASIS VIA NOVEL INTERACTION WITH TLR5

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TLRs at barrier sites are exposed primarily to commensal microbes, and these interactions appear to play a role that is supportive of homeostasis of these sites, yet most studies of these receptors focus on interactions with pathogens. The commensal Gram-negative bacteria R. mucosa (Rm) has been developed as a therapy for atopic dermatitis (AD), a skin disease characterized by dysbiosis, impaired barrier function, immune dysregulation and increased susceptibility to infection. Initial mechanistic studies of Rm showed that its therapeutic actions, which include improved barrier function, reduced S. aureus burden, and significant reduction in disease severity, rely on TLR5 expression. However, as an α-proteobacteria, the flagellin of Rm lacks the consensus sequence for binding TLR5. Initial binding studies using co-immunoprecipitation confirmed that Rm flagellin does not bind TLR5, yet adhesion assays showed that blockade of TLR5 inhibits Rm adhesion. When applied topically to AD patients in a clinical trial, Rm had an anti-inflammatory effect, significantly reducing serum levels of TNFα, IL-8 and IL-13. Additionally, though it adheres to human keratinocytes in culture, it does not induce the expression of typical pro-inflammatory markers in these cells. To elucidate the mechanistic details of the interaction of Rm with TLR5 we are screening a library of transposon mutants for mutants deficient in adhesion and performing untargeted quantitative proteomics techniques to evaluate the responses of human keratinocytes co-cultured with Rm in vitro. These data will enrich our understanding of how commensal microbes interact with TLRs to affect immune regulation and other aspects of homeostasis at barrier sites. Furthering our knowledge of such interactions has therapeutic potential for allergic diseases and autoimmune disorders, preventing colonization by pathogens, and the improving overall support of human health.

SARS-COV-2 INFECTION AND mRNA VACCINE PRIME DISTINCT MEMORY CD4$^+$ T CELLS

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Adaptive immune responses are induced by vaccination and infection, yet little is known about whether T cell memory differs between these two contexts. For instance, it has been shown that spike-specific T cell responses to second and third doses of mRNA vaccines against SARS-CoV-2 differ in frequency and phenotype among individuals initially exposed to spike protein through infection versus vaccination. These differences are possibly due to distinct cues at the time of T cell memory development that influence subsequent responses to the antigen. Here, we explored SARS-CoV-2- specific CD4$^+$ T cell responses in the context of a third vaccine dose, referenced as the "booster" dose. We focused on the pre-booster and one month post-booster time points for ten individuals. We profiled spike-specific memory CD4$^+$ T cells using multimodal scRNA-seq following overnight Spike peptide pool stimulation in the activation-induced markers (AIM) assay. We identified a cluster of spike-specific CD4$^+$ T cells that differentially-expressed many genes associated with T cell activation, including *IFNG*, *IL2*, and *LTA*. To assess whether the mechanism of initial memory T cell priming affected subsequent responses to booster immunization, we compared spike-specific CD4$^+$ T cells between individuals whose first exposure was virus (SARS-CoV-2-Experienced) versus those whose first exposure was vaccine (SARS-CoV-2-Naive). At the pre-booster time point, we identified eight differentially-expressed genes among the spike-specific CD4+ T cells between these two cohorts. Gene set enrichment analysis (GSEA) revealed enrichment for Interferon Alpha Response and Interferon Gamma Response hallmark gene sets in spike-specific CD4$^+$ T cells from SARS-CoV-2-Experienced individuals, whereas there was enrichment for proliferative pathways by GSEA in spike-specific CD4$^+$ T cells from SARS-CoV-2-Naive individuals. The booster dose did not meaningfully alter the transcriptional profiles of spike-specific CD4$^+$ T cells from either SARS-CoV-2-Naive or SARS-CoV-2-Experienced individuals. Our data suggest that CD4$^+$ T cell memory is durably imprinted by the inflammatory context of SARS-CoV-2 infection.

TOOTH AUTOIMMUNITY: FROM BROKEN CENTRAL TOLERANCE TO BROKEN TEETH

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Ameloblasts are specialized epithelial cells in the jaw, which facilitate the formation of the hardest body tissue – the enamel layer. This process involves activity of multiple ameloblast-derived proteins, whose loss of function results in a rare congenital disorder – amelogenesis imperfecta. Defects in enamel formation are also found in patients suffering from the Autoimmune polyglandular syndrome type-1 (APS-1), caused by AIRE deficiency with still unknown underlying mechanisms. Here we show that AIRE induces ectopic expression of a battery of ameloblast specific proteins in the thymus and that both APS-1 patients and Aire-deficient rodents develop autoantibodies against the enamel matrix, which consequently interfere with enamel formation. Therefore, these findings help uncover a new type of autoimmune disorder - Autoimmune Amelogenesis Imperfecta (AAI) – which is characterized by the autoimmune destruction of the permanent teeth. Moreover our findings also suggest that AAI may be more common as it can accompany other autoimmune diseases.

DECIPHERING THE ROLE OF THE C-TERMINAL DOMAINS OF *PAX5 IN VIVO*

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The transcription factor paired box protein (Pax5) plays a unique role in B cell development by activating B cell-specific genes and simultaneously repressing genes required in other cell lineages. Multiple heterozygous Pax5 mutations have been identified in a large cohort of B cell acute lymphoblastic leukemia (B-ALL) patients. Notably, many of these variants consist of mutations or deletions of certain domains of Pax5, such as the highly conserved C-terminal transactivation domain (TAD) and inhibitory domain (ID), suggesting that these domains are crucial for the function of Pax5. Nevertheless, the individual roles of the TAD and ID and how they contribute to the activation and repression of Pax5 target genes *in vivo* still remains elusive.

To investigate how the C-terminal domains of Pax5 are involved in regulating the gene expression program in B cells *in vivo*, mutant mouse strains lacking the C-terminal domains of Pax5 have been generated. Upon deletion of the TAD or both the TAD and ID of Pax5, B cell development is blocked at the pro-B cell or pre-pro B cell stage, respectively. Transcriptomic analysis of the C-terminal Pax5 mutant pro-B cells also strongly indicates that Pax5-dependent gene regulation is highly dependent on the C-terminal sequences. Interestingly, both activation and repression of Pax5 target genes is affected upon deletion of the C-terminal domains of Pax5.

We previously showed that Pax5 regulates its target genes by binding to DNA through the N-terminal paired domain (PD) and subsequently recruiting the basal transcription machinery, histone modifiers and chromatin remodelers. Although the PD is present in the C-terminal Pax5 mutants, DNA-binding is altered at deregulated Pax5 target genes but not at genes, whose expression is not changed. In addition, Co-IP mass spectrometry analysis of the C-terminal mutant B cells revealed the loss of Pax5 interaction with many previously identified Pax5 interaction partners. In summary, we here show that the vast majority of Pax5 target genes are regulated through the C-terminal sequences of Pax5, which recruit the basal transcription machinery, histone modifiers and chromatin remodelers to Pax5 target genes. This identifies the C-terminal domains of Pax5 as a potent and essential structure in B cell lymphopoiesis *in vivo*.

INTERCELLULAR TRANSFER WITHIN THE TUMOR MICROENVIRONMENT

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Intercellular transfer of molecules is a phenomenon that has been described both within immunological and non-immunologic settings. T cells acquire molecules from other cells through various mechanisms including: TCR-dependent membrane acquisition (trogocytosis), extracellular vesicle uptake and through membrane nanotubes. The *in vivo* functional significance of these processes remains largely unexplored. Here, we generated a tumor mouse model, where tumor cells express a fluorescent protein ZsGreen (ZsG) and analyzed tumor infiltrating lymphocytes (TILs) by multicolor flow cytometry. We observed that in addition to phagocytic cells such as macrophages and monocytes that exhibited high levels of green fluorescence, both CD4 and CD8 T cells turned out to be ZsG-positive. Interestingly, half of the T cells had homogeneous cytoplasmic distribution of ZsG while the other half demonstrated a punctate pattern. Analysis by scRNAseq of sorted ZsG+ and ZsG- T cells revealed that the majority of ZsG+ CD4 T cells are Tregs with a highly suppressive ICOS+Tbet+ phenotype, while the majority of ZsG+ CD8 T cells have an exhausted or pre-exhausted phenotype. We found intercellular transfer increases with the differentiation status of T cells. Furthermore, gene set enrichment analysis (GSEA) revealed the positive correlation between intercellular transfer and metabolically active state of T cells. Finally, we showed that transfer is not dependent on TCR/MHC interaction or the presence of antigen. Thus, we discovered a new mechanism that modulates T cell function within tumor microenvironment.

THE PD-1/PD-L1 SIGNALING AXIS REGULATES STEM-LIKE PROGENITOR CD8+ T CELL DIFFERENTIATION

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Stem-like progenitor CD8+ T cells are a subset of activated cytotoxic CD8+ T cells that retain high proliferative capacity and self-renewal potential, enabling them to continuously give rise to terminally differentiated effector cells that promote clearance of infected and transformed cells. Importantly, stem-like CD8+ T cells – characterized by their high expression level of the transcription factor TCF-1 – have been identified as the major responding T cell subset during effective cancer treatment with checkpoint blockade and adoptive T cell immunotherapy. Thus, understanding how activated CD8+ T cells differentiate into TCF-1+ stem-like cells and retain their stemness is crucial towards designing therapeutics that generate optimal responses during cancer treatment as well as durable memory CD8+ T cell formation upon vaccination.

Using multiplexed 3D tissue imaging, we identified distinct niches in tumor draining lymph nodes occupied by antigen-specific TCF-1+ stem-like CD8+ T cells that co-express the inhibitory molecule PD-1 and are spatially associated with dense networks of antigen cross-presenting XCR1+ conventional dendritic cells (cDC1) late after initiation of tumor antigen-specific responses (Days 4-9). We present evidence of antigen-specific signaling among this subset, and *in vivo* depletion of cDC1 during this late antigen-presentation phase led to significantly reduced stem-like cell formation. Thus, we have uncovered a previously unappreciated antigen-presentation phase many days after response initiation involving TCF-1+ stem-like cell engagement with antigen-presenting cDC1 that is critical for driving the expansion of stem-like progenitor cells.

Intriguingly, PD-1 expression among polyclonal antigen-specific TCF-1+ stem-like cells is positively correlated with tetramer binding, suggesting that during effector differentiation, the PD-L1/cDC1 axis functions to promote selective enrichment and expansion of high affinity stem-like cells whose progeny would be especially effective in pathogen and tumor clearance. Disruption of PD-1 signaling *in vivo*, combined with statistical modeling, demonstrates the loss of high affinity stem-like clones after checkpoint blockade, along with a phenotypic shift towards terminally differentiated effector cells. Our findings thus suggest that the PD-1/PD-L1 axis is an integral signaling component involved in sustaining optimal stem-like progenitor formation. These results also raise questions about whether prolonged use of PD-1 checkpoint blockade therapy in cancer patients interferes with maintenance of key precursor population necessary for robust effector generation needed to achieve full tumor regression.

This work was supported by the Intramural Research program of NIAID, NIH.

THE DIVERGENCE OF PD-1^{+}CXCR5^{+}CD4^{+} T CELL DIFFERENTIATION AND STAGE-SPECIFIC REGULATION OF GC-TFH CELLS

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T follicular helper (Tfh) cells were discovered as CD4^{+} T cells in the B cell follicles that express CXCR5 and help germinal center (GC) B cell responses. However, it is not clear which PD-1^{+}CXCR5^{+}Bcl6^{+}CD4^{+} T cells will differentiate into PD-1^{hi}CXCR5^{hi}Bcl6^{hi} GC-Tfh cells while the others have a different fate, and how GC-Tfh cell differentiation is regulated. Here we report that the sustained Tigit expression in PD-1^{+}CXCR5^{+}CD4^{+} T cells marked the precursor Tfh (pre-Tfh) to GC-Tfh transition, whereas Tigit^{-}PD-1^{+}CXCR5^{+}CD4^{+} T cells upregulated IL-7Rα to become CXCR5^{+}CD4^{+} T memory precursor/memory cells—with or without a CCR7^{+} central memory phenotype. Our study further shows that pre-Tfh cells undergo substantial further differentiation to become GC-Tfh cells at transcriptome and chromatin accessibility levels, and we have identified novel factors that play stage-specific roles in GC-Tfh cell differentiation.

IDENTIFICATION OF TXP AS A MOLECULE INVOLVED IN ANTIGEN CROSS-PRESENTATION

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Presentation of exogenous antigens on major histocompatibility complex (MHC) class I by antigen-presenting cells (APCs) referred to as "antigen cross-presentation" is essential for priming naïve CD8+ T cells. In this pathway, exogenous antigens are taken up and transported into the endosome, followed by degraded and transported into the cytoplasm. At the cytoplasm, antigens are further degraded into peptides and transported into the endoplasmic reticulum (ER) through TAP1 on the ER membrane. Antigen peptides in ER bind to MHC class I molecules and peptide-MHC class I complexes are transported to the cell surface. However, it is reported that peptides are transported from the cytosol into the ER through the TAP1-independent mechanism.
Here, we screened a membrane transporter protein involved in antigen-processing. Through a combination of gene expression profile and functional analysis, we identified one candidate protein that we named “transporter associated with cross-presentation (TXP)”. TXP is a putative solute carrier (SLC) transporter which consists of 12 transmembrane domains with unclear function. We generated TXP-deficient murine embryonic fibroblast (MEF) by CRISPR/Cas9 genome editing system and evaluated antigen cross-presentation efficiency. We found that TXP-deficient MEFs exhibit a decrease in antigen cross-presentation capacity. Thus, TXP may play a critical role as a peptide transporter in antigen cross-presentation in MEFs.

EXAMINING TRANSCRIPTION FACTOR-COFACTOR COMPLEXES IN VARIOUS T CELL STATES

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Characterizing the gene regulatory mechanisms that define distinct T cell states is essential to understanding T cell biology in health and disease. Despite advances in identifying transcriptional and epigenetic differences across T cell states, the regulators of these changes remain poorly defined. Here, we describe a system-level proteomic and genomic study to identify the transcription factor (TF)–cofactor (COF) complexes that regulate cell state in resting, activated, and exhausted T cells. To rapidly and thoroughly profile TF-COF complexes present in cells, we introduce a high-throughput, protein-binding microarray (PBM)-based approach called CoRec (Cofactor Recruitment). We used CoRec to profile numerous COFs in resting and stimulated Jurkat T cells, as well as in acutely stimulated and exhausted CD4+ primary cells. We identify unique TF-COF recruitment networks for each of these various T cell states and examine the differences in their TF-COF interactions. We demonstrate that within the same cell state, COFs are recruited to distinct classes of TFs, and that this can happen between close paralogs, such as the histone acetyltransferases (HATs) P300 and CBP. We further highlight how some COFs are recruited specifically to TFs known to drive T cell state changes. For example, we have identified several COFs, including histone deacetylases (HDACs), that were only recruited to the T-box family (EOMES, TBX21, etc) during T cell exhaustion. This TF family has been implicated in the exhausted state and its exhaustion-specific recruitment of COFs may suggest a possible mechanism by which these TFs act to help establish the exhausted state. During establishment of exhaustion this TF family appears to recruit histone modifying cofactors that then place or remove epigenetic marks that alter expression of exhaustion-related genes and help create a functional change in the T cell state. Additionally, by integrating our TF-COF interaction data with mass spectrometry and ChIP-seq data, we further examine how these TF-COF networks relate to protein abundance levels, COF recruitment across the genome, and genome-wide chromatin marks. The CoRec method and its integration with other methods provides an exciting new approach for profiling cell-specific TF-COF complexes and allows us to better understand and identify the epigenetic regulators of T cell states. We anticipate that by identifying cell state-specific TF-COF complexes, our approach may also provide novel therapeutic targets that can alter T cell states and improve current T cell therapies.

GERMINAL CENTER EXPANSION BUT NOT PLASMABLAST DIFFERENTIATION IS PROPORTIONAL TO PEPTIDE-MHCII DENSITY VIA CD40-CD40L SIGNALING STRENGTH

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Selection of germinal center (GC) B cells with high affinity for a diversity of epitopes is essential for an efficient and cross-reactive immune protection against mutating pathogens. Although cognate peptide-MHCII (pMHCII) is critical for B cell selection in the germinal center (GC), it is unclear how cell intrinsic differences in peptide levels contribute to selection and cell fate decisions. Here, we applied the α-DEC205-OVA (dec) model system, to deliver different levels of OVA peptide to GC B cells in situ in order to interrogate how intermediate and high levels of pMHCII affect selection and cell fate on a per cell basis. We used *Ly75*$^{+/-}$ (DEC-het) B cells, which expressed ~50% of surface DEC205 protein compared to WT B cells, and presented proportional amount of OVA peptide after dec treatment. Using competitive co-transfers, we found that WT B cells expanded two-fold more than DEC-het B cells. This 2-fold difference in expansion was maintained at a wide range of dec administration. Lymph node intravital imaging did not discriminate differences in T-het and T-WT contact size or duration, suggesting dynamics were similar at this timepoint, despite differences in functional T cell help. To further study T cell recognition in situ, we developed a novel caged ovalbumin peptide, which is readily detected by OT-II T cells only upon photo-uncaging by intravital imaging, and triggered rapid T cell recognition and increased number of B-T serial contacts, suggesting integration of multiple T cell help. Differential CD40 signaling, was both necessary and sufficient to mediate 2-fold differences in GC B cell expansion and also promoted GC-like B cell morphology, suggesting CD40 signaling is a rheostat of pMHCII dose. Surprisingly, we found that while plasmablast numbers were increased upon dec stimulation, both WT and DEC-het GC B cells were equally capable of differentiating into plasma cells, suggesting that pMHCII density does not directly control the output or quality of plasma cells generated. Thus, these results delineate distinct roles pMHCII play in expansion vs. differentiation in the GC. Our findings explain how selection for high affinity clones can also accommodate intermediate affinity clones, which may provide breadth and evolution in the response.

SIGNALING AND ANTIGEN-PRESENTING BIFUNCTIONAL RECEPTORS FOR UNDERSTANDING AND ENGINEERING IMMUNITY

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The key to the effectiveness of T cells is their exquisite antigenic specificity, which can be harnessed to combat diseases. T cells use their surface T cell receptor (TCR) to recognize peptide epitopes on Major Histocompatibility Complex molecules (pMHC). While signaling through the TCR and its consequences have been studied extensively, pMHC is conventionally seen as merely a 'flag' on target cells for recognition by T cells. MHC molecules do not have canonical intracellular signaling domains, and therefore do not elicit any function into the cells presenting them, making TCR-pMHC interaction a 'one-way street' in terms of functional response. This presents a unique engineering opportunity: can TCR-pMHC interactions become 'two-way streets'? To address this, we developed chimeric receptors called Signaling and Antigen-presenting Bifunctional Receptors (SABRs). SABRs consist of extracellular full-length MHC complexes with genetically (and hence covalently) linked epitopes, fused with intracellular signaling domains. SABRs can present epitopes to T cells and elicit intracellular signaling upon successful recognition, thereby converting TCR-pMHC interactions into 'two-way streets. Here we showcase this platform in two ways: 1) SABR libraries for T cell antigen discovery, and 2) SABRs for combinatorial engineering of immune and non-immune cells to achieve desired immune outcomes. First, we demonstrated that SABRs are modular in design, allowing engineering of mouse and human, class I and class II MHC. Harnessing this powerful platform, we first showed that libraries of SABRs comprising of a large number of epitopes could be used for discovering the cognate epitopes of T cells. We showed successful identification cognate epitopes of human CD8+ T cells from a library of 12,055 epitopes as a proof-of-concept. Furthermore, using a 4,075 epitope SABR library, we de novo identified the epitope specificities of several islet-infiltrating CD4+ T cells from NOD mice. Second, we demonstrated that SABRs engineered to present specific antigens could be used to engineer immune cells to respond to their cognate TCRs. Specifically, we demonstrated that SABR-engineered primary mouse CD8+ T cells were able to specifically kill pathogenic mouse CD4+ T cells. These studies offer glimpses into the powerful cell engineering platform provided by SABRs. We envision widespread utility of SABRs as cellular therapeutic modalities, immune monitoring and perturbation tools, as experimental model systems, and to uncover new immune phenomena.

EARLY RESPONDER MEMORY CD4$^+$ T CELLS MODULATE HETEROLOGOUS DISEASE OUTCOME

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In times of epidemic outbreaks, individual differences regarding disease susceptibility, course and severity become exceedingly apparent. Epidemiological data suggest that the infection history can alter an individual's susceptibility to subsequent unrelated diseases. Prior infections have a lasting effect on the host by promoting generation of memory cells, which have an accelerated and enhanced response when confronted with the same pathogen. However, how they can affect the disease course of unrelated challenges is less clear. We identified a subset of virus-specific CD4$^+$ T cells termed early responder memory T (T_{ERM}) cells that are protective in heterologous bacterial infection. This protection was mediated by rapid IFN-γ production due to TCR-independent re-activation. In contrast, antigen-independent re-activation of virus-specific CD4$^+$ T_{ERM} cells accelerated disease onset in an autoimmune model of multiple sclerosis. Our findings demonstrate that T helper cells can acquire additional TCR-independent functionality to mount innate-like responses that modulate susceptibility to heterologous challenges *in vivo*.

NC2 REPRESSES THE PRODUCTION OF IMMUNOSTIMULATORY RNAs

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To have a meaningful antiviral response that rests on the detection of the aberrant modification and location of nucleic acids, an organism must first be able to regulate those similar aberrations which it itself produces. Recent research into interferonopathies has demonstrated that this is achieved by (i) the metabolism of aberrant nucleic acids, and (ii) establishing a threshold of activation for the respective sensors that is higher than the homeostatic levels of immunostimulatory nucleic acids. But if the presence and detection of these immunostimulatory nucleic acids must be tightly regulated, what about their initial production? To understand the cellular machinery involved in limiting the production of immunostimulatory nucleic acids, we constructed a reporter cell line (from A549s) in which expression of Gaussia luciferase is driven from the native locus of MX1 (an interferon stimulated gene (ISG)). We combined these cells with an arrayed CRISPR library that targets ~1600 genes involved in transcriptional regulation. In the absence of exogenous stimuli, loss of several genes increased luciferase activity in a STAT1-dependent manner. Two of these proteins (DR1 and DRAP1) form the heterodimeric complex NC2 (negative cofactor 2) which inhibits the aberrant assembly of the RNA Pol II pre-initiation complex at cryptic sites throughout the genome, thus preventing "pervasive transcription." qPCR and RNA-seq analyses show that loss of NC2 results in the induction of ISGs and pervasive transcription. NC2-deficient cells secrete high levels of interferon β (IFN-β) and are potently protected from viral infection. We show that NC2 instigates IFN signaling through a RIG-I-dependent pathway, suggesting increased immunostimulatory dsRNA. We performed RNA-sequencing analyses on bulk cellular RNA and immunoprecipitated dsRNA to show an increased burden of dsRNA inside of NC2-deficient cells. Our data leads us to suggest a model wherein loss of NC2 increases pervasive transcription which leads to increased immunostimulatory dsRNA that is sensed by RIG-I, thus instigating a potent inflammatory state. Our future work will focus on understanding the link between pervasive transcription and inflammation, and whether a specific dsRNA is responsible for the activation of IFN signaling.

SPLICING OF ULTRACONSERVED POISON EXON ELEMENTS IN RNA BINDING PROTEINS REPROGRAMS THE T EFFECTOR IMMUNE RESPONSE

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Optimal T cell effector function is dependent on RNA binding proteins (RBPs) that carry out RNA splicing of transcripts.[1] Our prior work demonstrated that two families of RBPs regulating alternative splicing, serine- and arginine-rich (SR) RBPs and heterogeneous nuclear ribonucleoprotein (hnRNP) RBPs, are upregulated in response to costimulation of $CD8^+$ T cells.[1] SR and hnRNP transcripts contain ultraconserved poison exon (PE) elements that are alternatively spliced. When included in a transcript, PEs introduce premature stop codons that trigger nonsense-mediated decay.

Here, we demonstrate the necessity of SR and hnRNP family proteins for T cell clonal expansion and effector cytokine production *in vivo*. We show differential splicing of RBP PE regions within these SR and hnRNP factors upon $CD8^+$ T cell activation in both human and mouse. Further, we demonstrate that PEs are cell-state associated by using single-cell RNA sequencing analysis of splicing changes across mouse thymic T cell subsets and in $CD8^+$ T cells over the course of an acute *Listeria* infection. RNA-seq analysis in human samples reveals that specific SR and hnRNP PEs are differentially spliced in T cells from patients with T cell acute lymphoblastic leukemia, plaque infiltrating T cells from patients with atherosclerosis, and T cells from melanoma patients compared to healthy controls.

SR protein Tra2β contains a PE that is most consistently differentially spliced across mouse and human T cell subsets. Human T cell activation and effector function is suppressed by specifically promoting PE inclusion in TRA2β using an antisense oligonucleotide after stimulation with anti-CD3/CD28. Further, CRISPR-Cas9 mediated deletion of the PE element in Tra2β reprograms the T effector response during influenza infection, maintaining a prolonged effector-like state and changing tissue-resident memory T cell localization. In total, our work demonstrates a fundamental role for ultraconserved elements in defining T cell states, controlling T effector-mediated immunity, and altering T cell response in pathologic conditions such as lymphoblastic leukemia, atherosclerosis, and cancer.

[1] Karginov, T. et al. Optimal CD8+ T cell effector function requires costimulation-induced RNA-binding proteins that reprogram the transcript isoform landscape. Nat Commun 13, 3540 (2022)

IL-2 RESCUES IMPAIRED T CELL FUNCTION UPON LOSS OF ITK VIA METABOLIC REPROGRAMMING

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Adaptive immune responses depend on properly balanced activity of different T cell subtypes. The Tec family non-receptor tyrosine kinase, IL-2 inducible T cell kinase (Itk), is a critical component of T cell receptor (TCR) signaling required for activation of downstream transcription factors (NFAT, ERK, AP1, NF-KB) that regulate expression of effector molecules. Loss of Itk impairs T-cell activation by modulating the strength or duration of TCR signal. We and others have previously reported that *Itk*$^{-/-}$ mice have defects in T cell development and maturation with decreased proliferation and impaired differentiation to multiple CD4 T helper lineages. Most severe defects are seen in the differentiation of Th9 cells, which produce IL-9, an inflammatory cytokine. We find that *Itk*$^{-/-}$ cells initially appear similar to Wt cells under Th9 differentiation conditions but subsequently fail to maintain expression of activation markers and IL-9. Th9 differentiation could be rescued by inclusion of IL-2, a potent T cell growth factor, in the media. To understand how IL-2 rescues defects in *Itk*$^{-/-}$ cells, we examined phenotypes of *Itk*-deficient cells differentiated under Th9 conditions. We found that *Itk*$^{-/-}$ CD4 T cells show decreased phosphorylation of S6 and reduced expression of IRF4, c-Myc and multiple nutrient transporters—including the Glucose transporter 1 (GLUT1), transferrin receptor (CD71), and amino acid co-transporter (CD98), all of which were rescued by IL-2. As expression of many nutrient transporters was low in *Itk*$^{-/-}$ cells, we used SCENITH—a flow cytometry-based technique that monitors effects of metabolic pathways on protein translation. We found that WT Th9 cells were primarily glycolytic but *Itk*$^{-/-}$ T cells were more dependent on mitochondrial respiration and exhibited less protein synthesis thus, linking sub-optimal TCR signaling to compromised protein translation rate in CD4 T cells. *Itk*$^{-/-}$ Th9 cells also had exhibited less polarized mitochondria when compared to Wt cells. Inclusion of IL-2 in *Itk*$^{-/-}$ cultures rescued metabolism, protein synthesis and the percentage of polarized mitochondria so that metabolic profiles resembled those of WT cells. Our results suggest that metabolic fitness and flexibility are central to dictating the functional/effector fate of CD4 T cells and that this requires the Tec kinase Itk and IL-2-mediated pathways, providing novel insight into mechanisms by which IL-2 can rescue T cell functional defects.

ABLATION OF cDC1 DEVELOPMENT BY TUMOR-DERIVED IL-6 THROUGH C/EBPB INDUCTION IN DC PROGENITORS.

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The type 1 conventional dendritic cell (cDC1) is essential for driving effective CD8 T cell immunity for most viruses and against tumors. In many cases, various types of tumors are recognized as producing an immunosuppressive environment that impairs anti-tumor immune responses. One example is the production of myeloid-derived suppressor cells (MDSCs) induced by various cytokines, including interleukin (IL)-6. MDSCs are a heterogeneous group of cells, and the basis for their suppressive activity is still obscure. Another recognized action of tumor-derived IL-6 is the impairment of the development and function of cDC1. Here, we have identified the molecular mechanism by which tumor cell-derived IL-6 suppresses cDC1 development. Recently, we described the basis for cDC1 specification from the common dendritic cell progenitors (CDP), showing that CDP divergence occurs through transient induction of *Nfil3* expression. NFIL3 acts to repress the expression of *Zeb2* at sites in the *Zeb2* -165 kb enhancer, normally bound by C/EBPα and C/EBPβ to support *Zeb2* expression, and thereby repress cDC1 specification. We now demonstrate that IL-6, derived from tumors, can act on progenitors in the murine bone marrow (BM) to ablate cDC1 specification. This observation can be recapitulated in human umbilical cord blood *in vitro*. In agreement, we find that cDC-specific *Zbtb46*^{GFP} reporter mice bearing IL-6-producing EL4 lymphoblast have severely reduced cDC1 production. We show that the mechanism of these effects of IL-6 is through the induction of *Cebpb*, encoding C/EBPβ, in CDPs and MDPs. The increased level of C/EBPβ acts to support *Zeb2* expression and prevent cDC1 specification induced by NFIL3. To test this directly, we demonstrated that in mice harboring mutations of the three CEBP/NFIL3 binding sites within the *Zeb2* -165 kb enhancer, IL-6 no longer impairs cDC1 specification despite the induction of C/EBPβ in cDC progenitors. These results suggest that tumor-derived IL-6 ablates cDC1 development by upregulation of C/EBPβ which maintains *Zeb2* expression in cDC progenitors and prevents cDC1 specification.

ZBTB20 TRANSCRIPTION FACTOR DEFINES INNATE-LIKE TREGS ESSENTIAL FOR INTESTINAL HOMEOSTASIS

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Several of the 49 BTB-ZF (broad-complex, tramtrack, and bric-à-brac - zinc finger) genes have been shown to be essential, non-redundant transcription factors that control the development and function of lymphocytes. By generation of fluorescent reporter mice to track BTB-ZF transcription factor expression, we identified a distinct subset of Tregs that is defined by the expression of Zbtb20. These Tregs are genetically and phenotypically distinct from the larger conventional Treg population. For instance, analogous to natural killer T (NKT) cells they have an activated phenotype (CD62Llo, CD44hi), and constitutively express the Il10 message which correlates with their ability to rapidly secrete a large quantity of the cytokine after primary activation. The cells acquire these characteristics during thymic development soon after Zbtb20 is induced in DP thymocytes. Zbtb20-expressing Tregs selectively accumulate in the intestine and expand during DSS-induced colitis. The targeted deletion of Zbtb20 in T cells results in intestinal epithelial layer damage causing loss of the barrier function making mice highly vulnerable to severe inflammation and death from induced colitis. Importantly, adoptive transfer of the Zbtb20-expressing Tregs was sufficient to rescue mice from death, whereas the transfer of non-Zbtb20 Tregs did not. In the absence of the transcription factor in T cells, normal intestinal macrophage responses were disrupted, likely in part due to decreased production of IL-10 by the Tregs. Consistent with this, high levels of myeloid cell-associated cytokines were produced in conditional Zbtb20 knock-out mice. Furthermore, activation of macrophages with anti-CD40 treatment induced substantial weight loss in the knock-out mice. Therefore, by profiling BTB-ZF transcription factor expression, we have identified a distinct subset of T cells with potent immunosuppressive abilities that are essential for the health of the intestine. Numerous aspects of Zbtb20-expressing Tregs are parallel to NKT cells, indicating the existence of “naïve” Tregs that have innate-like effector functions acquired independently from the differentiation in the periphery.

EPITRANSCRIPTOMIC SHAPING OF SIGNAL TRANSDUCTION CONTROLS THE DEVELOPMENT, ACTIVATION AND SURVIVAL OF T CELLS

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Signals from T cell receptor (TCR) are critical for the development, activation and survival of T cells by involving multiple layers and numerous mechanisms of gene regulation. In the last decade post-transcriptional regulation of immune responses by RNA-binding proteins (RBPs) and miRNAs has been established. Recently, the new field of "epitranscriptomics" has been emerged and we now have to integrate into the current concepts of post-transcriptional gene regulation. N6-methyladenosine (m6A) is the most prevalent mRNA modification affecting splicing, translation and stability of transcripts.
In T cells m6A modification has recently been proposed to determine the intensity of cytokine signal transduction. We have now provided evidence for an involvement of m6A methylation in TCR signaling that ultimately causes T cell-driven pathology. We describe how the essential role of the Wtap protein in the m6A methyltransferase complex exerts crucial control over TCR and Ca2+ signaling. We found that m6A modifications were required for the differentiation of thymocytes, control of activation-induced death of T cells and prevention of colitis by enabling gut RORγt+ Treg differentiation and function. Young mice lacking Wtap in T cells spontaneously develop a colitis phenotype. While the m6A methyltransferase complex is dispensable for the persistence of naive CD4+ T cells, it becomes essential for the survival of activated T cells upon TCR stimulation.
To globally identify m6A targets in T cells, we utilized RNA-sequencing and crosslinking, immunoprecipitation (CLIP) using antibodies against m6A or Ythdf2, a prototypic m6A-binding protein. The analyses revealed that m6A modification destabilizes Orai1 and Ripk1 mRNAs. Lack of post-transcriptional repression of the encoded proteins correlated with increased store-operated calcium entry activity and diminished survival of T cells, accompanied by dysregulated signaling pathways that are related to the upregulation of Orai1 and Ripk1. These findings highlight a decisive role of m6A in the TCR response. Creating near-nucleotide resolution maps for m6A modifications as well as for the binding of Ythdf2 or other RBPs to the transcriptome of T cells and thymocyte provides a basis to form and test hypotheses how individual cis-elements in mRNAs employ different post-transcriptional RNA modifications and trans-acting factors to cooperate or antagonize. Ultimately, this will enable a molecular understanding how the different mechanisms of post-transcriptional gene regulation shape T cell responses in a context dependent manner.

LARGE SCALE NASCENT RNA ANALYSIS OF HUMAN PERIPHERAL LEUKOCYTE SHOWS THE DIVERGENCE OF IMMUNE AND STRESS-RESPONSIVE TRANSCRIPTIONAL PROGRAMS

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Nascent RNA sequencing (RNA-seq) is a powerful method to analyze transcription with high resolution, sensitivity, and directional information. It provides a robust measure of gene expression by quantifying transcribing RNA on gene bodies. It can also measure activities of transcriptional enhancers by capturing the signature of bidirectional enhancer RNAs (eRNA). Therefore, nascent RNA-seq is an ideal all-in-one solution for analyzing transcriptional regulation and gene expression in the immune system at once. However, broader application of nascent RNA-seq had been limited by sample requirements and extensive handling.

To address this, we developed a nascent RNA-seq method compatible with human whole blood samples from clinical sources requiring minimal to no pretreatment. This peripheral blood Chromatin Run-On (pbChRO) is combined with deep learning and hierarchical co-expression network analysis, and can discover enhancers, promoters, and transcription factor networks that define peripheral leukocyte-specific transcription programs.

Using pbChRO, we generated nascent transcription profiles of peripheral leukocytes from untreated raw whole blood in ~50 healthy individuals. Inflammatory transcription factors such as NFkB were pivotally associated with enhancer-gene networks in peripheral leukocytes. Intriguingly, transcription factors related to cell stress response, such as ATF-6 and CHOP, were also associated with inter-individual variation of leukocyte transcription. We further tested the divergent perturbation of transcription factor networks in Lipopolysaccharide and Thapsigargin stimulus, to model immune cell activation and Endoplasmic Reticulum stress response, respectively. We also probed the variations of these transcriptional programs depending on gender and race.

These results demonstrate that transcriptional programs are functionally divergent across individuals in immune cells, which can be associated with various disease susceptibilities. Overall, our integration of experimental and computational nascent RNA methods provided an efficient strategy to dissect transcriptional programs in peripheral blood immune cells with minimal sample requirements, and can further be a source of convenient genome-wide transcriptional data for personalized medicine.

IDENTIFYING NOVEL METHYLATION SUBSTRATES OF SETD3 METHYLTRANSFERASE IN IMMUNE CELLS

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Protein methylation is a form of post-translational modification that has long been appreciated to play a major role in epigenetic regulation via methylation of histone lysine residues. In recent years, increasing numbers of non-histone substrates has been identified for multiple histone lysine methyltransferases. Importantly, many of these non-histone methylation events have been linked to regulation of important cellular functions such as in protein stability, protein activity, or in major cell signalling pathways. However, majority of non-histone substrates still remain unidentified, and the functional significance of their methylation remains largely unknown in immune cells. Interestingly, our preliminary findings demonstrate that SETD3 methyltransferase, a known histone lysine and actin histidine methyltransferase, exhibited predominantly cytoplasmic localization in multiple immune cells, and exhibited increased expression upon mitogenic stimulation. Using in vitro methylation assay approaches, we further observed purified recombinant wild-type SETD3, but not catalytically inactive SETD3 mutants, was able to strongly methylate several non-histone substrates ranging from 25kDa to 50kDa, and to a lesser degree substrates ranging from 90-250kDa, in lysates derived from Setd3-deficient bone marrow-derived macrophages and dendritic cells. These findings strongly suggest that SETD3 may play functional roles in immune cells, via the methylation of multiple cytosolic non-histone substrates. Future work will be needed to determine the identity of these substrates and nature of these methylation modifications.

HOW FAITHFUL ARE T CELL MEMORIES?

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T cells develop memory after infection or vaccination. Once recalled during subsequent infection, these cells can convey rapid and effective protection. Such recall responses are conventionally assessed by flow cytometric examination of <10 marker proteins, usually secreted proteins called cytokines. However, systematic assessment of memory T cell recall has yet to be reported, leaving their dynamics and relationship to initial responses unresolved. Here, we tracked the heterogeneous and dynamic changes of memory $CD4^+$ T cells in the spleens of mice experiencing re-infection with malaria parasites. We studied both TCR transgenic (PbTII) and endogenous polyclonal $CD4^+$ T cells using droplet-based scRNA-seq+VDJ, computational modelling and spatial transcriptomics at near single-cell resolution via Slide-seqV2. We noted three major $CD4^+$ T cell states present prior to re-infection, termed Th1-memory, T central memory (Tcm), and germinal centre T follicular helper (GC-Tfh) cells. Upon re-infection, PbTII GC-Tfh cells remained surprisingly unaltered over three days, while PbTII Tcm cells initiated a slow conversion to a Tfh-like phenotype, but with no evidence of clonal re-expansion. In contrast, we traced a dramatic co-ordinated series of transcriptional change in PbTII Th1 memory cells. Deconvolution of Slide-seqV2 spatial data using RCTD or Cell2Location confirmed GC localisation for GC-Tfh transcriptomes, and localisation of Tcm and Th1-memory cells to T cell zones, close to antigen-presenting cells, cDC2. Using spatialDE, gene expression dynamics of Th1-memory cells were mapped, revealing an unexpected biological process that was linked, as inferred by pseudotemporal gene-gene correlation network analysis, to immune function initially, and later to cellular proliferation. Importantly, we found no evidence of genes expressed uniquely during recall. Instead recalled genes were solely a minor subset of those expressed during initial infection, revealing for the first time the faithful yet imperfect nature of Th1 memory recall relative to initial Th1 responses. Finally, we validated that the differential dynamic responses of splenic GC-Tfh, Tcm, and Th1 memory cells were also evident for polyclonal $CD4^+$ T cells that possess highly diverse antigen receptors. Thus, antigen-experienced $CD4^+$ T cells, located in specific areas of the spleen, exhibit vastly different recall responses to re-infection, which are faithful, though imperfect, recollections of initial responses.

CYTOKINE-DRIVEN INTEGRIN EXPRESSION CONTROLS CD8 T CELL HOMEOSTASIS

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Cytotoxic CD8 T cells play critical roles in anti-viral and anti-tumor immunity. CD8 T cells are generated in the thymus and then migrate into peripheral lymphoid organs where they are maintained as mature but antigen-inexperienced naïve T cells or differentiate into effector and memory CD8 T cells upon self- and/or foreign- antigen exposure. Thus, the peripheral CD8 T cell pool comprises both naïve and memory T cells, whereby their relative ratio remains remarkably consistent under steady-state condition. The prevailing view posits that utilization of distinct survival cytokines accounts for maintaining naïve and memory CD8 T cells at a set ratio, in that naïve T cells depend on IL-7 while memory CD8 T cells preferentially require IL-15. Consequently, naïve and memory CD8 T cells would occupy disparate survival niches, but it remains unexplained how the relative abundance of naïve versus memory CD8 T cells could be controlled in the steady state. Here, we report a new regulatory mechanism of CD8 T cell homeostasis where the integrin alpha E (CD103) acts as a rheostat to determine the relative ratio of naïve and memory CD8 T cells in peripheral tissues. So far, the only known ligand for CD103 is the cell adhesion molecule E-cadherin which is mostly expressed in barrier tissues but also found in a subset of dendritic cells in lymphoid organs. Because CD103 is highly expressed on all naïve CD8 T cells, E-cadherin-expressing dendritic cells (E-cad$^+$DCs) would tightly interact with naïve CD8 T cells and provide tonic TCR signaling and critical survival cues. Notably, we also found that CD103 is markedly downregulated upon memory cell differentiation in an IL-15-dependent manner. Thus, memory CD8 T cells lack CD103, which would disengage them from E-cad$^+$DCs. Since we found that E-cad$^+$DCs supported the survival and homeostasis of CD103-positive naïve CD8 T cells, the loss of CD103 dissociates memory CD8 T cells from potential competition with naïve CD8 T cells for engaging E-cad$^+$DCs. As a corollary, the forced expression of CD103 on memory CD8 T cells substantially promoted memory CD8 T cell survival and accumulation, while it impaired the maintenance of naïve CD8 T cells, as they could not sufficiently interact with E-cad$^+$DCs. Collectively, our results suggest that IL-15-mediated downregulation of CD103 in memory CD8 T cells is important to constrain the size of memory CD8 T cell pool and to preserve naïve CD8 T cell pool, revealing a previously unappreciated role of cytokine-driven integrin expression in controlling CD8 T cell homeostasis by orchestrating T cell-dendritic cell interactions.

CONTROL OF FOXP3 INDUCTION AND MAINTENANCE BY SEQUENTIAL HISTONE ACETYLATION AND DNA DEMETHYLATION

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Regulatory T (Treg) cells play crucial roles in suppressing deleterious immune responses. The determination of Treg cell fate is a process converting fluctuating induction cues to a committed cell fate. Here, we investigate how the stage-specific activities of individual epigenetic mechanisms controlling Treg cells induction in vitro (iTreg) and stabilization via transcriptional regulation of Treg lineage specifying factor Foxp3. We find that acetylation of histone tails at the *Foxp3* region is required in cis for inducing *Foxp3* transcription. Upon induction, histone acetylation signal via bromodomain-containing proteins, particularly targets of inhibitor JQ1, sustains Foxp3 transcription via a global or trans effect. Subsequently, Tet-mediated DNA demethylation of Foxp3 cis-regulatory elements mainly conserved non-coding sequence 2 (CNS2) increases chromatin accessibility and transcription factors binding, stabilizing *Foxp3* transcription and obviating the need for the histone acetylation signal. These processes transform stochastic iTreg induction into a stable cell fate, with the former sensitive and the latter resistant to genetic and environmental perturbations. Thus, sequential histone acetylation and DNA demethylation in *Foxp3* induction and maintenance reflect stepwise mechanical switches governing iTreg cell lineage specification.

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A HEPATOCYTE SPECIFIC INTERFERON-INDUCIBLE LONG NON-CODING RNA, HPN-AS1, CONTROLS THE INDUCTION OF TYPE I IFN

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Timely induction of type I interferon (IFN) is crucial fight against invading viruses and generally is present in all nucleated cells. However, depending on the actual expression levels of proteins involved in the induction of innate immunity, the intensity of the response may vary. We previously reported that Hepsin, predominantly presenting in hepatocytes, could inhibit the induction of type I IFN during virus infections by suppressing the STING-mediated type I IFN induction. This negative regulator of hepatic induction of type I IFN needs to be turned off when real infections occur in the hepatocytes. We identified that a long noncoding RNA (LncRNA), Hepsin-Antisense 1 (HPN-AS1), which is transcribed partially overlapped with Hepsin coding sequences, was induced during viral infections and/or IFNβ treatments, and the interferon-inducible expression of HPN-AS1 was only observed in the hepatocytes. Ectopic expression of HPN-AS1 may enhance antiviral innate immunity in non-hepatocytes, while knock-down of HPN-AS1 by shRNA hepatocytes are more permissive to virus infections, suggesting that HPN-AS1 is a positive regulator of type I IFN induction. The genomic DNA methylation levels of the HPN-AS1 upstream regions between hepatocytes and non-hepatocytes were comparable, and therefore we searched for hepatocyte-specific transcription factors which may promote HPN-AS1 expression. We identified that in addition to interferon responsive transcription factors, hepatocyte-specific transcription factors also contribute to the expression regulation of HPN-AS1. In conclusion, we have identified a hepatocyte-specific machinery to regulate type I IFN induction in liver cells to control viral infections.

SPATIAL MAPS OF T CELL RECEPTORS AND TRANSCRIPTOMES REVEAL DISTINCT IMMUNE NICHES AND INTERACTIONS IN THE ADAPTIVE IMMUNE RESPONSE

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T cells mediate antigen-specific immune responses to disease through the specificity and diversity of their clonotypic T cell receptors (TCRs). Determining the spatial distributions of T cell clonotypes in tissues is essential to understanding T cell behavior, but spatial sequencing methods remain unable to profile the TCR repertoire. We developed Slide-TCR-seq, a 10-μm-resolution method, to sequence whole transcriptomes and TCRs within intact tissues. We confirmed the ability of Slide-TCR-seq to map the characteristic locations of T cells and their receptors in mouse spleen. In human lymphoid germinal centers, we identified spatially distinct TCR repertoires. Profiling T cells in renal cell carcinoma and melanoma specimens revealed heterogeneous immune responses: T cell states and infiltration differed intra- and inter-clonally, and adjacent tumor and immune cells exhibited distinct gene expression. Altogether, our method yields insights into the spatial relationships between clonality, neighboring cell types, and gene expression that drive T cell responses. Our next directions include exploring thymic involution and the decline in TCR repertoire diversity in humans and mice, as well as building methods for incorporating B cell receptor sequence readouts while retaining spatial tissue information.

ABLATION OF cDC2 DEVELOPMENT BY TRIPLE MUTATIONS WITHIN THE *ZEB2* ENHANCER

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The divergence of the common dendritic cell progenitor (CDP) into the conventional type 1 and type 2 dendritic cell (cDC1 and cDC2,respectively) lineages is poorly understood. Some transcription factors act in the commitment of already specified progenitors—such as BATF3, which stabilizes *Irf8* autoactivation at the +32 kb *Irf8* enhancer—but the mechanisms controlling the initial divergence of CDPs remain unknown. Here we show the transcriptional basis of CDP divergence and describe the first requirements for pre-cDC2 specification. Genetic epistasis analysis suggested that *Nfil3* acts upstream of *Id2*, *Batf3* and *Zeb2* in cDC1 development but did not reveal its mechanism or targets. Analysis of newly generated NFIL3 reporter mice showed extremely transient NFIL3 expression during cDC1 specification. CUT&RUN and ChIP-seq identified NFIL3 binding in the –165 kb *Zeb2* enhancer at three sites that also bind C/EBPα and C/EBPβ. In vivo mutational analysis using CRISPR–Cas9 targeting showed that these NFIL3–C/EBP sites are functionally redundant, with C/EBPs supporting and NFIL3 repressing *Zeb2* expression at these sites. A triple mutation of all three NFIL3–C/EBP sites ablated *Zeb2* expression in myeloid, but not lymphoid progenitors, causing the complete loss of pre-cDC2 specification and mature cDC2 development in vivo. Thus, CDP divergence into cDC1 or cDC2 lineages is controlled by competition between NFIL3 and C/EBPs at the –165 kb *Zeb2* enhancer.

A SINGLE AMINO ACID SUBSTITUTION IN THE ADAPTOR LAT ACCELERATES TCR PROOFREADING KINETICS AND ALTERS T CELL SELECTION, MAINTENANCE, AND FUNCTION

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T cells balance their needs to mount efficient immune responses and maintain self-tolerance by exerting tight control over T cell receptor (TCR) ligand discrimination. A selective temporal delay in LAT Y136 phosphorylation ensures discrimination of TCR interactions with longer-binding to agonist peptide:MHC and briefer interactions with self-peptide:MHC. However, the functional significance of this time delay in LAT Y136 phosphorylation in vivo remains unclear. Here, we report that speeding LAT Y136 phosphorylation by altering a single neighboring amino acid profoundly affects kinetic proofreading and ligand discrimination in vivo. Knocking G135D LAT into a mouse line endowed the T cells with sustained increases in LAT Y136 phosphorylation speed and magnitude, specifically augmenting the activation of Y136-mediated PLCγ1 recruitment, enhancing calcium mobilization, and facilitating the nuclear translocation of transcription factor NFAT. This G135D substitution thereby markedly enhanced the sensitivity of T cells to ligands that are unable to activate wild-type T cells. The enhanced reactivity to self-peptides or weak ligands also instructed peripheral T cell fate decisions. CD4 or CD8 G135D T cells expressing either the OT-II or OT-I transgenic TCR also showed augmented reactivity toward self-peptides or weak OVA altered peptide ligands in vitro and in vivo. During infection using Listeria monocytogenes expressing OVA, G135D OT-I CD8 T cells become terminally differentiated effector T cells with diminished cytokine production ability compared with wild-type LAT-expressing cells. Interestingly, during Armstrong LCMV infection, G135D SMARTA CD4 T cells showed stronger expansion than wild-type LAT-expressing SMARTA CD4 T cells and promoted germinal center development. These biased cell fate decisions were also observed for G135D OT-II CD4 T cells after immunization with OVA emulsified in complete Freund's adjuvant, and in aged G135D mice, in which the generation of anti-dsDNA autoantibodies was substantially increased. Taken together, our data show that LAT Y136 phosphorylation is a bona fide regulator of signaling that establishes proper T cell sensitivity to guide appropriate ligand discrimination and cell fate decision-making.

DEFECTIVE TOLEROGENIC RESPONSE TO COMMENSAL LPS IN THE PREDIABETIC PHASE PROMOTES GUT INFLAMMATION AND LOSS OF GUT BARRIER INTEGRITY IN AUTOIMMUNE DIABETES.

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An increase in gut barrier (GB) permeability and abnormal crosstalk between commensal gut microbiota and immune cells underlies the pathogenesis of Type 1 Diabetes (T1D). In line with this view, we recently demonstrated that damage of the GB triggers intestinal activation of islet-reactive T cells and T1D in preclinical models. Several mechanisms contribute to maintenance of gut immune homeostasis and GB integrity. For example, LPS of commensal gut bacteria triggers the activation of a tolerogenic TLR4-mediated pathway on intestinal epithelial cells (IEC) mediated through activation of the TRAF3 signaling pathway promotes mucin expression and Treg cell differentiation thus maintaining immune tolerance and GB integrity. On the other hand, the activation of a pro-inflammatory pathway through TLR4 and TRAF6 signaling contribute to intestinal inflammation, damage of the GB and breakage of intestinal immune homeostasis. Here, we specifically tested whether loss of GB integrity and intestinal inflammation in preclinical models of T1D is related to a defective response of IEC to commensal LPS.

Our ex-vivo results demonstrate that NOD mice, the spontaneous preclinical model of T1D, carry an inherent functional defect with abnormal differentiation of Goblet cells and an overstimulation of the TRAF6 signaling pathway under basal homeostatic conditions at the expenses of the tolerogenic TRAF3-mediated signaling pathway. Importantly, IEC of NOD have an impaired response to the commensal LPS leading to defective FoxP3+Treg cell differentiation, impaired mucus barrier structure and intestinal inflammation compared to both non autoimmune strains of mice as well as diabetes-resistant NOD mice thus suggesting that the abnormal response to commensal LPS is directly linked to T1D pathogenesis. Importantly, we confirmed those findings in human T1D showing an overactivation of the TRAF6-mediated inflammatory signaling pathway at the expenses of the tolerogenic TRAF3-mediated pathway in the small intestine of T1D patients compared to healthy controls.

Here we demonstrate that preclinical models and humans affected by autoimmune T1D have a dysregulated response to commensal LPS that triggers a pro-inflammatory TLR4-signaling pathways leading to damage of GB integrity and reduced intestinal immune tolerance. Further studies are necessary to link the defective response to commensal LPS to activation of islet-reactive T cells and autoimmune pathogenesis of T1D in preclinical models and humans.

METHODS FOR SCREENING CANDIDATE CAUSAL REGULATORY VARIANTS IN HUMAN T CELLS BY CRISPR RIBONUCLEOPROTEIN

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Non-coding variants that are causal for human traits, such as those implicated by GWAS, often have small effects on gene expression. Current methods for determining the function of candidate causal variants sacrifice statistical power and genomic context for screening throughput, resulting in the loss of important biological information. To screen non-coding candidate sequences with power and versatility in situ, we have optimized a robust ribonucleoprotein method for CRISPR deletion in primary human T cells. We apply this method to determine candidate causal variants of type 1 diabetes at the *CTLA4* locus. Finally, we extend this method for direct capture of genomic edits to enable powerful, combinatorial single-cell screening of regulatory sequences.

MILD RESPIRATORY SARS-COV-2 INFECTION CAN CAUSE MULTI-LINEAGE CELLULAR DYSREGULATION AND MYELIN LOSS IN THE BRAIN

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Survivors of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection frequently experience lingering neurological symptoms, including impairment in attention, concentration, speed of information processing and memory. This long-hauler cognitive syndrome shares many features with the syndrome of cancer therapy-related cognitive impairment (CRCI). Neuroinflammation, particularly microglial reactivity and consequent dysregulation of hippocampal neurogenesis and oligodendrocyte lineage cells, is central to CRCI. We hypothesized that similar cellular mechanisms may contribute to the persistent neurological symptoms associated with even mild SARS-CoV-2 respiratory infection. Here, we explored neuroinflammation caused by mild pulmonary SARS-CoV-2 infection – without neuroinvasion - and effects on hippocampal neurogenesis and the oligodendroglial lineage. Using a mouse model of mild pulmonary SARS-CoV-2 infection induced by intranasal SARS-CoV-2 delivery in two distinct genetic background strains, we found white matter-selective microglial reactivity, a pattern observed in CRCI. Human brain tissue from 9 individuals with COVID-19 or mild SARS-CoV-2 infection exhibits the same pattern of prominent white matter-selective microglial reactivity. In mice, pro-inflammatory CSF cytokines/chemokines were elevated for at least 7-weeks post-infection; among the chemokines demonstrating persistent elevation is CCL11, which is known to impair neurogenesis. Humans experiencing long-haul syndrome with cognitive symptoms (48 subjects) similarly demonstrate elevated CCL11 levels compared to those with long-haul syndrome who lack cognitive symptoms (15 subjects). Impaired hippocampal neurogenesis, decreased oligodendrocytes and myelin loss in subcortical white matter were evident at 1 week, and persisted until at least 7 weeks, following mild respiratory SARS-CoV-2 infection in mice. Taken together, the findings presented here illustrate striking similarities between neuropathophysiology after cancer therapy and after SARS-CoV-2 infection, and elucidate cellular deficits that may contribute to lasting neurological symptoms following even mild SARS-CoV-2 infection.

DYNAMIC REGULATION OF TFH SELECTION DURING THE GERMINAL CENTRE REACTION

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The germinal centre is a dynamic microenvironment in which B cells that express high-affinity antibody variants produced by somatic hypermutation are selected for clonal expansion by limiting the numbers of T follicular helper cells1,2. Although much is known about the mechanisms that control the selection of B cells in the germinal centre, far less is understood about the clonal behaviour of the T follicular helper cells that help to regulate this process. Here we report on the dynamic behaviour of T follicular helper cell clones during the germinal centre reaction. We find that, similar to germinal centre B cells, T follicular helper cells undergo antigen-dependent selection throughout the germinal centre reaction that results in differential proliferative expansion and contraction. Increasing the amount of antigen presented in the germinal centre leads to increased division of T follicular helper cells. Competition between T follicular helper cell clones is mediated by the affinity of T cell receptors for peptide–major-histocompatibility-complex ligands. T cells that preferentially expand in the germinal centre show increased expression of genes downstream of the T cell receptor, such as those required for metabolic reprogramming, cell division and cytokine production. These dynamic changes lead to marked remodelling of the functional T follicular helper cell repertoire during the germinal centre reaction.

TRANSCRIPTOME DIVERSITY IN HUMAN MEDULLARY THYMIC EPITHELIAL CELLS

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The induction of central T-cell tolerance in the thymus depends on the presentation of peripheral self-epitopes by medullary thymic epithelial cells (mTECs), enabled by a process known as promiscuous gene expression (pGE). Transcriptome diversity generated during pGE has many contributors, including non-canonical transcription initiation, alternative splicing and expression of endogenous retroelements (EREs). However, their significance and regulation are poorly understood in the healthy human thymus. Here, we mapped the expression of genome-wide transcripts in immature and mature human mTECs using high-throughput 5'Cap and RNA sequencing. Overall, 96% of protein coding genes were represented across five human mTEC samples, with mature mTECs showing increased rates of global transcript mis-initiation. Both mTEC populations have increased splicing entropy, which appears to be driven by expression of peripheral splicing factors. Furthermore, EREs enriched in long terminal repeat retrotransposons are up-regulated during mTEC maturation and enriched in genomic proximity to differentially expressed genes. Our findings represent an important first step towards the generation of a comprehensive map of transcriptomic diversity in the healthy human thymus. Ultimately, a complete map of thymic expression diversity will allow for the identification of epitopes that contribute to the pathogenesis of auto-immunity and that drive immune recognition of tumor antigens.

PUSTULAR PSORIASIS AND THE POTENTIAL THERAPEUTIC USAGE OF AN IL-36 RECEPTOR MONOCLONAL ANTIBODY

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Pustular psoriasis is an uncommon subtype of psoriasis that dramatically affects the quality of life of affected patients. Pustules can emerge anywhere along the trunk, limbs, soles, palms, and fingers, which debilitates the functionality of these appendages. Currently, there are no approved treatments for pustular psoriasis in the US; off-label usage of psoriasis vulgaris medications is usually prescribed. These treatments are insufficient for patients with difficult to manage and/or severe forms of pustular psoriasis. Psoriasis vulgaris biologic medications mainly target the IL-17 and IL-23 axis. However, novel clinical findings have demonstrated that pustular psoriasis's central inflammatory axis depends on the dysregulation of the IL-36 family of cytokines. The lack of inhibition of IL-36α, IL-36β, and IL-36γ, as well as the absence or insufficiency of an IL-36 receptor antagonist, is involved in the upregulation of proinflammatory cytokines and neutrophil chemotaxis necessary for pustular lesion formation. An IL-36 receptor monoclonal antibody has the potential to disrupt the IL-36 inflammatory loop and inhibit the pathogenesis of pustular psoriasis. Spesolimab is an IL-36 receptor (IL-36R) monoclonal antibody undergoing clinical trials for the treatment of pustular psoriasis that has exhibited promising results.

EVALUATING THE IMPACT OF DIETARY FAT SOURCE ON GUT MICROBIOTA DIVERSITY AND GRAFT-VERSUS-HOST DISEASE OUTCOMES IN MICE

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It has been observed that a constriction of the gut microbiome occurs with a high fat diet. Likewise, decreased microbiome diversity is directly correlated with more severe graft-versus-host disease (GvHD) following allogeneic hematopoietic stem cell transplantation. Taken together it is plausible that not only fat content, but the source of dietary fat could influence gut microbiota and GvHD outcome. We sought to address this question and developed experimental rodent diets that have the same fat content (10% fat - similar fat content to normal chow) which differ only by fat source: animal- versus plant-derived fat and found that these diets drive alterations in gut microbiota diversity. Both diets yielded similar outcomes in an acute model of GvHD, however we found that mice fed the animal-derived fat diet exhibited worse survival and outcomes in a model of chronic GvHD, than mice fed the plant-derived fat diet. These findings correlated with alterations in intestinal immune cell populations following chronic GvHD induction. Collectively, our data show that diets that are equal in macronutrient content but different in the source of fat (only 10% of diet) can promote different gut microbiomes and in turn alter intestinal homeostasis and chronic GvHD outcome.

DEVELOPMENT OF A 96 TRANSWELL BARRIER FUNCTION SYSTEM USING PRIMARY HUMAN GUT ORGANOIDS

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Disruptions in the gut epithelial barrier can lead to the development of chronic indications such as inflammatory bowel disease (IBD). Historically, barrier function has been assessed in cancer cell lines, which do not contain all human intestinal cell types, leading to poor translatability. To bridge this gap, we adapted human primary gut organoids grown as monolayers to assess barrier function. In this work we describe a 96-well human gut organoid-derived monolayer transwell system that enables medium/high throughput quantitative assessment of candidate therapeutics. Normal human intestine differentiation patterns and barrier function were characterized and confirmed to recapitulate key aspects of in vivo biology. Next, cellular response to TNFα (a central driver of IBD) was determined using a diverse cadre of readouts. These outputs included kinase phosphorylation, target gene expression, cytokine/chemokine production, and barrier function. Additionally, we showed that several TNFα pathway antagonists rescued damage caused by TNFα in a dose-dependent manner, indicating that this system is suitable for quantitative assessment of barrier modulating factors. Taken together, we have established a robust primary cell-based 96-well system capable of interrogating questions around mucosal response. This system is well suited to provide pivotal functional data to support target and drug discovery efforts.

THE TRANSCRIPTIONAL LANDSCAPE OF AUTOIMMUNITY ASSOCIATED WITH X-LINKED CGD REVEALED BY SINGLE-CELL RNA-SEQ

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Precipitated by inactivating mutations in cytochrome b-245, beta chain (CYBB), X-linked chronic granulomatous disease is a rare primary immunodeficiency which affects about 1:200,000 live births. Patients suffer from specific bacterial and fungal infections, as well as a higher burden of autoimmune disease. Heterozygous female carriers typically present on a spectrum, with symptomatic severity being determined by skew in X-inactivation. Here, we performed single-cell sequencing on PBMCs and monocytes from 14 probands, and 10 carriers of X-CGD, as well as 15 age- and sex-matched controls, making it the largest sequenced cohort in X-CGD research. Differential expression analysis revealed the presence of a strong and shared signal across clusters driven by type I interferon signaling, previously implicated in immune dysregulation associated with autoimmune manifestations. Geneset enrichment analysis also revealed the enrichment of terms associated with antigen processing and presentation through the class I MHC pathway, which is crucial in maintaining self-tolerance as well as immunity from viruses and intracellular bacteria. Signatures defined from probands with X-CGD were conserved in female carriers, in a skew-independent manner, as validated by orthogonal dihydrorhodamine assay measurements. Proband-derived, cluster-specific disease signatures were able to confidently classify female carriers from sex-matched controls (average AUROC=0.79). Furthermore, genes associated with these disease signatures appeared to be strongly co-expressed in both reference human co-expression networks, as well as across multiple species using 1-1 orthologs, indicating that they likely constitute a novel, ancestrally conserved functional gene module. Disease phenotypes are composed of both cell intrinsic and cell extrinsic or systemic effects, and in X-CGD probands and carriers without any obvious active infectious foci, most transcriptomic dysregulation appears to be cell extrinsic in nature, as evidenced by its shared nature across cell types, and independence from orthogonal measures of cell intrinsic phenotypic penetrance.

SYSTEMATIC PREDICTION OF STAT-COOPERATING SIGNALING PATHWAYS THAT SUPPORT CYTOKINE-SPECIFIC GENE EXPRESSION

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Cells integrate extracellular cues through biochemical signaling pathways to induce the appropriate transcriptional programs. The signaling pathways that drive cytokine-induced gene expression are often depicted by a dozen or so landmark phosphorylation and transcriptional events, evoking the perplexion of how cytokines utilize common STAT transcription factors (TFs) to induce distinct functional programs, and to what extent cooperating pathways support response diversity. We hypothesized that in reality, thousands of dynamic post-translational modifications orchestrate the activation or repression of cytokine-specific genetic programs. Our mass spectrometry-based phosphoproteomic analysis of macrophage responses to IL-6 and IL-10, two opposing cytokines that are dependent on the overlapping usage of STAT1 and STAT3, identified 1,944 cytokine-specific phosphosites and 250 cytokine-induced phosphosites on TFs. To systematically identify STAT-cooperating TFs that support the expression of specific cytokine-regulated gene sets, we developed an interdisciplinary strategy that utilizes global, temporally-resolved phosphoproteomics with transcriptomic data, together with statistical modeling and motif analysis to identify TFs that potentially cooperate with the STATs. We are currently experimentally validating multiple candidate STAT-cooperating TFs, which have motifs that are enriched in unique gene sets, and which are dynamically phosphorylated in a cytokine-specific manner. Our ability to identify causal links between phosphorylation events and cytokine-driven functional specificity will improve our fundamental understanding of how extracellular cues are integrated, and is a step towards identifying and manipulating the biochemical events required for healthy versus pathological gene expression

STOCHASTIC IFN PRODUCTION AT THE SINGLE-CELL LEVEL SHAPES SPATIAL INFECTION DYNAMICS

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Upon viral infection, the production of type I interferon (IFN) by infected cells confers highly conserved negative feedback against viruses. The circuit operates in different parameter regimes, depending on the genetic backgrounds of the viruses and hosts, leading to different dynamics of IFN induction. A delayed and sustained production of IFN correlates with massive inflammation, tissue damage, and high mortality, as shown in lethal influenza and COVID-19 infection. Therefore, the timing and quantity of IFN production could shift the balance between its antiviral vs proinflammatory effects. The organismal-level correlation prompted us to ask at the single-cell level how an infected cell decides when and how much IFN to produce, and how the decision affects viral propagation dynamics in the neighborhood. To quantitatively answer these questions, we applied single-molecule fluorescence in situ hybridization on influenza-infected human lung epithelial cells. During primary infection, only 1-2% of infected cells can intrinsically produce IFN. The probability of production correlates with the viral load while the amplitude lacks significant correlation. The production of IFN induces a positive feedback loop that increases the probability of producing IFN in infected cells. Consequently, 10% of infected cells produce IFN. Both probability and amplitude of production strongly correlate with viral load. Despite the positive feedback, not all infected cells produce IFN. Therefore, we hypothesize that the stochastic IFN induction could lead to heterogeneity in the spatiotemporal dynamics of viral spreading. To correlate the timing/amplitude of IFN induction with the growth of infection foci, we performed quantitative analysis on hundreds of plaques. When we blocked IFN signaling for 24 hours post infection, the number of infected cells per plaque significantly increased, suggesting potent antiviral effect of IFN in early infection. Given that 2% of infected cells can produce IFN, we observed 10% of plaques positive for IFN with exactly 1 producer. Interestingly, IFN+ cells were significantly enriched at the border of the plaque. Bystander cells around the plaque responded strongly to IFN. However, infected cells showed varying degrees of response, suggesting the antiviral activity of IFN depends on the relative timing between infection and IFN exposure. Given the heterogeneity of IFN induction and responses in a single cell type, we are applying spatial transcriptomics to characterize the antiviral and proinflammatory states across different cell types within and surrounding *in vivo* plaques in infected lung tissues. By untangling the relationship between IFN signaling dynamics and cell state changes, we hope to make prediction about the fate of the plaques and infer the infection and immune response dynamics at the tissue level.

REDEFINING BLOOD CELL STATES AND THEIR FUNCTIONALITY ACROSS DISEASES

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Blood is the most commonly studied human specimen which informs scientific inquiry and directs clinical care. With the advent of single-cell genomics technologies, there is an opportunity to revisit our understanding of circulating immune cells. We postulate that there might be additional immune cell states and rare subsets that have yet to be discovered. We hypothesized that by analyzing blood from a wide range of autoimmune, inflammatory and infectious disease conditions we will be able generate a detailed map capturing all existing immune cell subsets and spectrum in human.

We collected peripheral blood mononuclear cells from patients with 6 immune-mediated diseases (at least 20 patients/disease), including auto-immune and infectious diseases and healthy controls. We implemented a unique experimental design to profile rare immune cell populations and concurrently measure gene expression and 282 surface protein levels at the single-cell resolution. Our growing cellular compendium already includes 3.5 million cells – we are in the process of generating data from 14 additional immune-mediated conditions. We identified immune cell populations which are shared across conditions and others which are unique to specific disease states, shedding light on disease dynamics. Studying gene programs across diseases and cell states allows an in-depth understanding of what defines a ‘cell state’, beyond the unique markers it expresses. Co-expression analysis of gene programs from different cell states gives a systems-immunology view of how different cells operate together. This circulating immune cell compendium will be the largest single-cell blood atlas described to date, both in magnitude of number of immune cells profiled and in the diversity of human disease analyzed in a single study.

ANTIVIRAL DEFENSE MECHANISM VIA VITAMIN K-MEDIATED CYTOPLASMIC PROTEIN CARBOXYLATION

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Protein carboxylation, mediated by gamma-glutamyl carboxylase (GGCX) in a vitamin K (VK)–dependent manner, has been considered to regulate only extracellular proteins. However, in this study, we discovered protein carboxylation activity in the cytoplasm using a reporter system. Moreover, we identified mitochondrial antiviral-signaling protein (MAVS), located at mitochondria and peroxisomes, is carboxylated by GGCX. This carboxylation selectively modulates the antiviral functions of MAVS, promoting interferon induction and suppressing apoptosis induction. Indeed, the susceptibility to vesicular stomatitis virus (VSV) infection in mouse brains was increased by GGCX ablation, VK–free diet, or anticoagulant warfarin treatment, which depletes VK, indicating a protective role of carboxylation and VK. This is the first example of functional cytoplasmic carboxylation of proteins. Moreover, these results highlight a possible beneficial effect of VK and an adverse effect of warfarin on antiviral therapies.

REGULATION OF IL-2 SIGNALING BY A TRUNCATED IL2Rβ PROTEIN IN RUMINANTS

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Introduction: Interleukin-2 (IL-2) is a master regulator of lymphocytic proliferation and differentiation, integral for initiating immune responses against pathogens or disease. It is required for the establishment of various cell groups, including those with opposing functions, i.e. T effector (Teff) and natural killer (NK) vs T regulatory (Treg) cells. IL-2 is a highly attractive therapeutic candidate for cancer and autoimmune disease, but progress has been challenged by inconsistent responses and negative side-effects. Understanding the complex regulation of IL-2 signaling can go a long way in the development of this cytokine for clinical applications. IL-2 is highly conserved across mammals but may be subject to different evolutionary adaptations, from which we can learn alternative regulation or function. In cow (Bos taurus), we have found that a truncated sequence of IL2Rβ, one part of the multimeric receptor for IL-2, is highly expressed in response to interferon. Interestingly, this transcript, referred to here as bIL2Rβ-short, arose from an incomplete gene duplication of canonical bovine IL2Rβ that co-opted a nearby MER41 transposable element (TE) as a promoter. bIL2Rβ-short lost the C-terminus signaling domain and transmembrane domain but retains an intact N-terminus IL2 binding domain. We predict the protein product of this transcript could have an important modulatory role in bovine IL-2 signaling.
Methods: We are characterizing bIL2Rβ-short at the protein level and aim to uncover any effects it may have on IL-2 signaling. We are over-expressing the construct in a panel of in vitro T and NK cell models and evaluating differential phenotypic responses and gene expression by growth assays and RNA-seq. Moreover, protein tags introduced into the over-expression constructs will be used to localize the protein and evaluate protein interactions.
Results/future directions: Over-expressing bIL2Rβ-short in the murine CTLL2 cell line, which is dependent on IL-2 for in vitro proliferation, results in an increased growth phenotype. Luminescence assays with HiBiT tagging of bIL2Rβ-short show that the protein is synthesized and secreted. In future directions, we will be using primary immune cells from cow to study bIL2Rβ-short expression endogenously.
Conclusions: Initial experiments show that over-expressing bIL2Rβ-short increases the growth rate of cytotoxic T cells cultured in vitro. Additionally, the evolution of this transcript, that may be important in modulating IL-2 signaling in cow, derived from a TE co-opted promoter. This shows that transposable elements can be exonized to generate novel gene products, introducing new function in immune responses.

THE EPIGENETIC AND TRANSCRIPTIONAL LANDSCAPE OF MONOCYTES RESPONDING TO ALLOGENEIC NON-SELF

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Introduction: Murine monocytes require A-type paired immunoglobulin-like receptors (PIR-As) to specifically recognize and acquire memory to major histocompatibility complex I antigens. Beyond the need for PIR-As, little is known about the mechanisms of specific innate immune memory. In this study, we aim to explore possible mechanisms of monocyte memory by investigating the epigenetic and transcriptional changes that specifically occur after allogeneic stimulation in splenic monocytes.
Methods: B6.RAG$^{-/-}\gamma c^{-/-}$ (BRG) mice were immunized by intraperitoneal injection of 20 million Balb/c irradiated splenocytes (allo). Splenic monocytes were FACS sorted at 0, 3, 7 and 28 days post-immunization. Simultaneous snRNAseq and snATACseq was then performed. To identify the changes that are specific to allo-stimulation and related to monocyte memory, monocytes were also sorted and sequenced from BRG injected with irradiated B6 splenocytes (syn) and allo-immunized BRG PIR-A$^{-/-}$ mice at day 7 after injection.
Results: Weighted nearest neighbor UMAP represented 4 visually distinct cell neighborhoods (N). N1 and N2 increased in abundance after allo-stimulation. Differential gene expression analysis revealed that N1 highly expressed genes encoding cell cycle proteins, Ly6C, and PIR-A. Monocytes in N2 from syn vs allo immunized groups were segregated. Clustering mainly resulted in the division of N2 into 3 clusters (C). C3 and N1 were more abundant in allo-immunized BRG and had a 3.5 and 2.4 fold reduction in allo-immunized BRG PIR-A$^{-/-}$ mice respectively. Flow cytometric analysis with EDU pulse-chase confirmed the patterns seen in the sequencing data. Real time trajectory inference using Waddington OT supported that N1 is the starting state. Real time and pseudotime trajectory inference suggested that the differentiation pathway connects N1 to N2 then splits to N3 and N4. N3 and N4 had a profile consistent with differentiation to macrophages and mo-DCs respectively. Gene expression and pathway analyses uncovered unique transcription factors, genes and gene networks associated with immunological memory in N1 and C3. While N1 and C3 shared accessible regions, N1 lacked the corresponding gene expression of those regions. This is in line with N1 being the source of change in the monocyte landscape after allo-stimulation.
Conclusion: Splenic monocytes are heterogenous and contain subsets that respond specifically to allo-stimulation with unique transcriptional and epigenetic changes that are consistent with a memory response.

A SYNTHETIC HIGH-AFFINITY *IRF8* +32 KB ENHANCER CONVERTS PRE-cDC2 TO cDC1 AND cDC1-LIKE CELLS

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The common dendritic cell progenitor generates pre-cDC1 and pre-cDC2, which are clonogenic progenitors restricted to cDC1 and cDC2, respectively. A complex of 3 transcription factors IRF8, BATF3 and JUNB binds the *Irf8* +32 kb enhancer, which induces IRF8 autoactivation and commits pre-cDC1 to cDC1. However, how pre-cDC2s prevent *Irf8* +32 kb enhancer activation and cDC1 differentiation is unclear. In the current study, we demonstrate that AICE motifs in the *Irf8* +32 kb enhancer are of low affinity for the IRF8/BATF3/JUNB complex, which suggests that a threshold amount of IRF8/BATF3/JUNB may be required to activate this enhancer. We generated a knock-in mouse strain that has multiple additional high-affinity AICE motifs in the *Irf8* +32 kb enhancer. In these mice, pre-cDC2 are no longer restricted to the cDC2 lineage and can generate cDC1, suggesting that the high-affinity enhancer can capture the low amount of IRF8/BATF3/JUNB complex in pre-cDC2 to drive IRF8 autoactivation. Unexpectedly, mice bearing the high-affinity enhancer develop a $Sirpa^{+}XCR1^{+}$ DC (DP DC) population that expresses both cDC1- and cDC2-specific genes. Similar to cDC1s, DP DCs could cross-prime $CD8^{+}$ T cells against cell-associated antigens in vitro. DP DCs are present in *Nfil3*$^{-/-}$ background which lacks cDC1 specification, suggesting that they can be derived from cDC2-committed cells *in vivo*. Collectively, these results demonstrate that a synthetic high-affinity *Irf8* +32 kb can redirect cDC2-committed cells to the cDC1 and cDC1-like cells, and suggests that the affinity of the natural *Irf8* +32 kb enhancer is tuned to a low affinity that enables cDC2 development.

POLYCOMB REPRESSIVE COMPLEX 2 EPIGENETICALLY REGULATES PRO-INFLAMMATORY RESPONSES IN LIPOPOLYSACCHARIDE-TOLERANT MACROPHAGES

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To avoid exaggerated inflammation and injury, host cells have been adapted to become hyporesponsive or “tolerance” in response to successive exposure to stimuli such as lipopolysaccharide (LPS). Such tolerized response is a part of innate immune memory which are mainly regulated via epigenetics changes and metabolic reprograming. Polycomb repressive complex 2 (PRC2) mediates the transcriptional repression by catalyzing H3K27me3 modification but little is known about the roles of PRC2 in tolerant macrophages. We examined the impact of lacking PRC2 components on LPS-induced tolerance in macrophages focusing on Eed or Ezh2. We observed not only the significant reduction in H3K27me3 but also the augmentation of H3K27Ac as the result of Eed or Ezh2 deficiency. LPS-tolerized Eed KO macrophages exhibited attenuated pro-inflammatory cytokines productions (TNF-α and IL-6). In addition, the tolerant *Eed* KO macrophages also exhibited lower glycolytic activity than the littermate control. RNA-Seq analyses revealed that most of differentially expressed genes were upregulated in Eed KO tolerant macrophages and most of which are involved with the cytokine-mediated signaling pathway. Loss of H3K27me3 in the regulatory regions of some of these genes were validated by ChIP-seq. These results indicated that PRC2 via Eed/Ezh2 epigenetically suppresses key inflammatory genes in response to LPS re-exposure and lacking PRC2 activity results in hyperresponsive to LPS re-stimulation. Therefore, we provide strong evidence that PRC2 via EED mediates LPS tolerance in macrophages by epigenetically suppressing proinflammatory responses with the link to dysregulated metabolic pathway.

A HIERARCHICAL GBP NETWORK PROMOTES NON-CANONICAL INFLAMMASOME ACTIVATION AND SEPSIS

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Lipopolysaccharide (LPS) from Gram-negative bacteria is one of the most bioactive substances known. Trace amounts trigger robust immunity to infection but also life-threatening sepsis causing millions of deaths each year. LPS contamination of the host cell cytosol elicits a caspase-dependent inflammasome pathway promoting cytokine release and a form of inflammatory cell death known as pyroptosis. Here, we report an immune GTPase family termed guanylate-binding proteins (GBPs) control multiple steps in this pathway by generating genome-engineering mice to lack tissue specific, chromosomal Gbp cluster and 7 different GBP family members. Gbp2-/- and Gbp3-/- mice had severe caspase-11-driven defects that protected them from septic shock. Mechanistically, Gbp2 interacted with caspase-11 to recruit it for upstream LPS recognition whereas Gbp3 trafficked the pyroptotic pore-forming protein, gasdermin D, to the plasma membrane after caspase-11 cleavage for initiating host cell death. Together, our results identify a new functional hierarchy wherein different GBPs choreograph sequential steps in the non-canonical inflammasome pathway to control Gram-negative sepsis.

DISPROPORTIONAL ENRICHMENT OF FOXP3^{+}CD4^{+} REGULATORY T CELLS SHAPES A SUPPRESSIVE TUMOR MICROENVIRONMENT IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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The objective response rate to PD-1 blockade remains low in human papilloma virus (HPV)-positive head and neck squamous cell carcinoma (HNSCC), highlighting the importance of investigating underlying resistance mechanism. Here, we explored immunologic characteristics of HNSCC tumor microenvironment to give insights involved in anti-PD-1 resistance and suggest effective treatment strategy. Distinct characteristics of HNSCC tumor microenvironment compared to other types of cancer was explored based on transcriptome profiles from The Cancer Genome Atlas. Features of peripheral blood, normal adjacent mucosa, and tumor-infiltrating lymphocytes from patients with HNSCC were analyzed by flow cytometry. Patients were classified based on HPV status to corroborate immunologic characteristics and survival outcomes by underlying etiology. Therapeutic relevance of inhibition of upstream regulators involved in immune suppression was tested *in vitro* with suggestion of direct evidence for robust antitumor efficacy with combination strategies. Although HNSCC is predominating with IFN-γ dominant subtype with highest lymphocytic infiltrate, FoxP3^{+}CD4^{+} regulatory T cells (Tregs) were disproportionally enriched to antagonize antitumor immunity. Tumor-infiltrating Tregs of HNSCC highly express immune checkpoint coinhibitory receptors, exerting suppressive capacity to inhibit effector T cell proliferation. Disproportional enrichment of highly suppressive TI Tregs was prominent in HPV-positive HNSCC with prognostic implications. Activation of IDO pathway was directly involved in inducing Tregs in the tumor microenvironment of HPV-positive HNSCC and IDO-1 inhibitor epacadostat reduced fate decision toward Treg during *in vitro* differentiation. Moreover, combined blockade of PD-1 and IDO exhibited robust treatment response in a patient with HPV-positive HNSCC. IDO pathway-mediated Treg accumulation shapes suppressive tumor microenvironment and drives anti-PD-1 resistance in HPV-positive HNSCC. Targeting Treg or IDO pathway would provide benefit in patients with HPV-positive HNSCC either alone or combined with PD-1 blockade.

NUCLEAR PARASPECKLES ARE DYNAMICALLY REMODELED BY PATHOGEN SENSING IN MACROPHAGES TO REGULATE INNATE IMMUNE GENE EXPRESSION

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When macrophages sense a pathogen, they rapidly upregulate hundreds of genes encoding cytokines, chemokines, and antimicrobial mediators that are essential components of the innate immune response to infection. The timing and magnitude of innate immune gene expression are tightly regulated to ensure that macrophages mount responses that are strong enough to fight off infection without risking hyperinflammation and autoimmunity. Thus, there is a need to identify and characterize the factors that fine tune this critical response. Despite the substantial impact RNA processing has on gene expression outcomes, little is known about how these pathways (e.g. pre-mRNA splicing, cleavage and polyadenylation, mRNA export) are differentially regulated in the context of the innate immune response. My lab studies how components of these RNA processing pathways, mainly RNA binding proteins, are functionalized in response to pathogen sensing to dictate specific innate immune outcomes. We recently became interested in identifying mechanisms through which the macrophage could simultaneously relocalize/sequester/refunctionalize many RNA binding proteins at once. This interest prompted us to study a nuclear subcompartment called the nuclear paraspeckle.

Paraspeckles are dynamic membraneless organelles that nucleate on the *Neat1* lncRNA and are thought to regulate a variety of nuclear RNA processing pathways by sequestering RNA binding proteins and mRNAs in response to stress. Using RNA-FISH and immunofluorescence microscopy, we found that paraspeckle structure is incredibly dynamic over the course of macrophage activation. Within minutes after lipopolysaccharide (LPS) treatment, the paraspeckle lncRNA *Neat1* accumulates in macrophages, leading to dramatic aggregation of paraspeckles. By 2h post-LPS treatment, we can no longer detect *Neat1* or paraspeckles by RNA-FISH. By 4h post-LPS, paraspeckles return to normal basal levels of 1-2 per nucleus. We predict that these tightly regulated dynamics signify an important role for the paraspeckle in controlling early macrophage activation. So far, we have implicated the nuclear exosome in regulating Neat1 stability in macrophages and are working to determine whether transcription of *Neat1*, possibly via NFκB/RelA, contributes to paraspeckle accumulation following TLR4 engagement.

In addition to characterizing paraspeckle dynamics, we are also working to determine precisely how paraspeckles control innate immune gene expression. Using *Neat1*(lacZ) knockout mice, we have identified a number of pro-inflammatory and anti-microbial genes that fail to be properly induced in macrophages lacking paraspeckles. Our data support a model whereby sequestration of repressive RNA binding proteins into paraspeckles plays a key role in activation of innate immune gene expression following engagement of pattern recognition receptors in macrophages.

ANALYSIS OF THE IMMUNOGLOBULIN μ ENHANCER PROTEOME

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The intronic immunoglobulin μ heavy chain (IgH) enhancer, termed Eμ, regulates the rearrangement and monoallelic expression of the μ gene in pro-B cells. Previous experiments aimed at identifying Eμ-binding proteins utilized short DNA fragments, which precluded the analysis of higher-order protein complexes. Here, we employed both reverse ChIP assays with the entire Eμ enhancer region and quantitative mass spectrometry to determine the Eμ enhancer proteome. We identified several novel Eμ interaction partners including the MSL/MOF complex, which is responsible for X gene dosage compensation in male fruit flies. By using mutated Eμ enhancer fragments, we found that the MSL/MOF complex is recruited to Eμ via the E1 box-binding protein YY1. Analysis of primary pro-B cell cultures, carrying a heterozygous mutation of the Mof gene or a CRISPR/Cas9-mediated mutation of the Msl2 gene showed an average two-fold reduction of μ gene expression both at the RNA and protein level. In addition, the induced degradation of the transcription factor YY1 or a CRISPR/Cas9-mediated genomic deletion of the E1 box resulted in a reduction of μ gene expression in pro-B cell cultures. Thus, the YY1-mediated recruitment of the MSL2-MOF complex to Eμ likely confers an additional layer of enhancer-promoter communication, which may be important in the context of monoallelic gene expression.

LET-7 microRNAs DEFINE CD8 T CELL FATE

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Effector and memory CD8 T cells are critical for an efficient immune response, but in chronic infection or cancer prolonged exposure to antigen leads to the stage of exhaustion, where T cells lose effector function and eventually die. Therefore, controlling differentiation of T cells into effector, memory, or exhausted cells has much therapeutic potential, but the mechanism behind these processes remains poorly understood. Using transgenic mouse models, we found that the inhibition of let-7 microRNA biogenesis in CD8 T cells promotes terminal differentiation including exhaustion, while increased expression of let-7 leads to differentiation into memory T cells. Comparative transcriptome analysis of let-7 transgenic and deficient CD8 T cells revealed dysregulation of early signaling events in T cells. Specifically, we found that let-7 inhibits mTOR-pathway that controls ROS production that is essential for differentiation of terminal effector T cells. Furthermore, we observed that such let-7-mediated regulation of CD8 T cell fate has a direct impact on anti-tumor immune responses. Therefore, we identified a novel molecular pathway that is involved in terminal differentiation of CD8 T cells and can be antagonized by let-7 which promotes memory formation.

DIFFERENTIAL REGULATION OF INNATE IMMUNE SIGNALING BY PEPTIDOGLYCAN DISACCHARIDES

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The human innate immune system is the body's first line of defense against invading pathogens, responding to and differentiating between pathogenic bacteria and the 39 trillion commensal bacteria that compose the human microbiome. Misrecognition of commensal bacteria has been linked to chronic inflammatory diseases such as Crohn's disease and rheumatoid arthritis. It has long been established that canonical peptidoglycan (PG) fragment muramyl dipeptide (MDP) activates a NOD2-dependent NF-kB immune response, but this minimal synthetic mimic fails to capture the vast peptidoglycan diversity sensed by the immune system. We have recently demonstrated that biologically-relevant disaccharide N-acetylglucosamine N-acetylmuramic acid tripeptide (GMTP) gives a markedly different gene transcriptional signature and more robust activation compared to MDP, validating that PG signaling is more complex than previously understood. Here, we have synthesized an expanded suite of disaccharide carbohydrate fragments with biologically-relevant modifications and chemical probes to be utilized for investigation of immunostimulatory properties. With these fragments, we have stimulated both macrophages and dendritic cells. Subsequently, RT-PCR and RNAseq experiments were performed to better dissect immune signaling mechanisms. These diverse fragments showed that small modifications in PG structure significantly impact downstream immune response pathways and PG receptors. These studies provide new insights into our understanding of differential PG innate immune signaling which is critical to develop novel therapeutics to control inflammation and adjuvants to bolster vaccine response.

RELATING T CELL RECEPTOR SIMILARITY TO ANTIGEN SPECIFICITY

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An integral requirement of cell-mediated immunity is the ability for T cells to recognize a diverse set of foreign antigens. This antigen recognition is mediated by the interaction between T cell receptors (TCRs) and peptides presented on major histocompatibility complexes (pMHCs). Despite the crucial role of TCR-pMHC binding in T cell mediated immune responses, predicting peptide specificity from TCR sequence remains an open problem. In this work, we develop a mechanistic model of TCR-pMHC binding, that quantifies the influence of sequence changes on TCR specificity. Specifically, we derive a distance metric that characterizes the influence of point substitutions in the TCR sequence on its specificity. Using publicly available databases of TCR sequences, we applied our distance metric to relate the inferred functional distance between pairs of TCR sequences to the physical and chemical properties of the substituted amino acids, as well as the position of the substitution.

A COMPREHENSIVE THYROID CELLULAR ATLAS REVEALS THYROCYTE-STROMAL-IMMUNE INTERACTIONS THAT DRIVE TISSUE INFILTRATIVE AUTOIMMUNITY

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Autoimmune thyroid disease (AITD) is found in 10% of Americans and is a valuable model to understand immune tolerance and tissue homeostasis in humans. In Hashimoto's thyroiditis, self-reactive T cells target hormone-producing thyroid epithelial cells (thyrocytes), leading to destruction of local tissue architecture. Intriguingly, 70% of Hashimoto's patients retain tissue function despite immune infiltration, suggesting additional tissue-specific mechanisms to constrain inflammation. To understand how cellular population dynamics drive loss of tissue function in AITD, we applied multimodal single-cell genomics approaches to 23 thyroid surgical specimens from patients with and without AITD. We generated an unprecedented cellular atlas of paired single-cell RNA sequencing with T cell receptor, B cell receptor and 204 surface proteins (CITE-seq) comprising 375,337 parenchymal, stromal, and immune cells.

Our results highlight the involvement of several cellular and transcriptional programs underlying the clinical spectrum of AITD. For example, our results highlight that thyrocytes from Hashimoto's patients display interferon-gamma responsive transcriptional programs, including up-regulation of the MHCI and MHCII antigen-presentation machinery. In addition, thyrocytes specifically upregulate expression of transcriptional programs known to modulate the immune system, including the checkpoint protein PD-L1. Strikingly, these inflammatory responses are dominated by a distinct thyrocyte cell subset that is enriched in Hashimoto's and strongly correlated with thyroid immune infiltration. Our data suggest that these "immunothyrocytes" respond to and subsequently constrain thyroid immune infiltration. We also identified an immunofibroblast population that is specifically enriched in Hashimoto's thyroiditis, strongly correlated with thyroid immune infiltration, and resembles fibroblasts previously shown to promote tertiary lymphoid structure formation in multiple autoimmune diseases. Analysis of tissue-infiltrating immune cells identified several disease-specific populations of NK, B, CD4+ T, and CD8+ T lymphocytes as well as myeloid cells. Our work may help decipher how immune-parenchymal-stromal interaction networks promote chronic tissue infiltration and alter tissue function in organ-specific autoimmunity more generally.

ABERRANT TGF-β SIGNALING DOWNSTREAM *IPO8* DEFICIENCY TRIGGERS INTESTINAL INFLAMMATION

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The TGF-β/BMP cytokine family orchestrates pleiotropic functions during embryonic development, tissue homeostasis and repair as well as within the immune system. Accordingly, aberrant TGF-β signaling underlies the pathogenesis of severe congenital disorders associating developmental defects with or without immune dysregulation. Thus, heterozygous loss-of-function (LOF) variants in *TGFBR1/R2*, *TGFB2* or *TGFB3*, *SMAD2* or *SMAD3* are notably the cause of the Loeys–Dietz syndrome (LDS), while the Shprintzen-Goldberg syndrome (SGS) results from heterozygous variants in *SKI*, a negative regulator of the TGF-β signaling, and the Marfan syndrome (MFS) from variants in *FBN-1*, encoding fibrillin-1, the main component of extracellular matrix microfibrils which scaffolds latent TGF-β. Patients with LDS, SGS and MFS present with connective tissue disorders characterized by arterial aneurysms and dissections, craniofacial and skeletal abnormalities, joint laxity. Strikingly, increased frequency of allergic manifestations and a 10-fold increased risk of inflammatory bowel diseases (IBD) have been reported in LDS patients. We have recently identified bi-allelic LOF variants in *IPO8* as cause of a connective tissue disorder largely overlapping with LDS. *IPO8* codes for importin 8, a member of the β-karyopherin family, the largest group of nuclear transport receptors implicated in cytoplasm-to-nucleus shuttling of a broad spectrum of cargos. We have shown that importin 8 plays a critical role during early stage of development in zebrafish by controlling phospho-Smad (p-SMAD) nuclear translocation downstream and TGF-β/BMP-dependent transcription. Thus, *ipo8*$^{-/-}$ zebrafish displayed severe cardiovascular and skeletal defects that mirror the human phenotype. We report here that IPO8 deficient patients also display increased prevalence of immunedysregulation features, including intestinal inflammation of early onset and allergies with elevated levels of IgE and higher levels of eotaxin-1 and CC-chemokine ligand 19. We then sought to determine whether *Ipo8*$^{-/-}$ mice might recapitulate the immune phenotypes observed in IPO8 deficient patients. Pointing to spontaneous intestinal inflammation, we found that *Ipo8*$^{-/-}$ mice showed failure to thrive over time and about 80% developed rectal prolapse at a variable time between 12 to 50 weeks whereas wild-type littermates did not. Significant decrease in colon length and splenomegaly in *Ipo8*$^{-/-}$ mice compared to wild-type littermates further confirmed underlying low grade inflammation. Overall, our data suggest an immune relevant role of IPO8, and show that *Ipo8*$^{-/-}$ mice are a valid model to study how dysregulation of the TGF-β signaling dependent on loss of IPO8 predisposes to intestinal inflammation and immune dysregulation, underscoring the prominent role of these pathways in directing immune responses to mucosal antigens.

SIGNAL BRANCHING IN T CELL ACTIVATION

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Immune cells convert environmental signals into complex responses. The molecular machinery underlying this process often involves adapter proteins which, upon activation, engage a collection of protein partners to propagate signals down several pathways. The protein linker for activation of T cells (LAT) is a membrane-associated adapter required for propagating and branching signals from the T cell receptor. The discovery of inducible protein interactions and higher-order assemblies mediated by LAT post-translational modifications and binding surfaces suggests that LAT is a platform for the regulated balance of downstream pathways. To achieve a comprehensive picture of LAT molecular function, we developed a high-content screening approach in which a series of mutants in the LAT protein are analyzed to characterize their effect on T cell activation. Our method employs single-cell genomics to provide a direct readout of the sequential and parallel aspects of T cell activation and associated LAT amino acid sequence dependencies. Measuring the epigenetic, transcriptomic, and cell surface protein dynamics of cells harboring distinct LAT mutants identifies varying degrees of robustness to disrupted upstream signaling across these regulatory layers. Overall, our results expand the collection of LAT sequence regions important for T cell activation and begin to inform a hierarchy of activities with varying sensitivity to LAT mutation. More broadly, we describe an approach for interrogating protein sequence-function relationships across various levels of signal processing throughout the cell.

ALTERED GERMINAL CENTER DYNAMICS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic Lupus Erythematosus (lupus) is a chronic autoimmune disease characterized by the presence of antibodies reactive to self-antigens, such as DNA and chromatin. These autoantibodies form immune complexes within organs such as the kidneys, resulting in inflammation and subsequent tissue damage. Autoantibodies are believed to arise in germinal centers (GCs), sites of T cell-driven selection of B cells within secondary lymphoid organs. GCs are essential for B cell proliferation and survival, as well as affinity-based selection, allowing for their differentiation into memory B and long-lived plasma cells. The GC reaction is an iterative process where B cells cycle through three anatomically defined areas: the dark zone (DZ), grey zone (GZ), and light zone (LZ). In lupus, the GC reaction becomes dysregulated, allowing for aberrant selection of autoreactive B cells to expand and differentiate. However, the breakdown in the GC pathway resulting in the development of autoreactive B cells and subsequent autoantibody production in lupus remains unclear. Our data demonstrate that in murine lupus, the DZ and GZ B cells decrease over disease progression while LZ B cells increase. Despite the temporal increase in LZ B cells, we observed decreased proliferation in both DZ and GZ B cells, suggesting a complication in zonal cycling. Furthermore, we found no change in apoptosis in these GC B cells, suggesting that instead of dying by failed selection, LZ B cells remain and may erroneously differentiate into autoantibody-secreting cells. Temporal transcriptional analysis of LZ B cells throughout disease revealed an altered transcriptional profile, highlighted by a decrease in the transcription factor *Myc*, whose expression delineates positively selected LZ B cells. The decrease in MYC indicates a lack of selective signaling in LZ B cells, suggesting increased diversity and mutational burden. Yet, temporal single cell BCR sequencing of GC B cells from lupus-prone mice demonstrated a loss of BCR diversity and a decrease in mutational burden as disease progressed. Furthermore, as GCs became dysregulated, we found an increase in plasma cells and $CD11c^{+}Tbet^{+}$ B cells during the later stages of disease. Together, these data suggest that malfunctioning GC B cells that would normally be removed are now able to be selected and differentiate. Collectively, our data suggest that as lupus progresses, GC B cells undergo a loss in their ability to undergo proper selection, allowing the propagation and differentiation of autoreactive B cells.

CONTROL OF VIRAL INFECTION BY JC VIRUS EPIGENETIC MIMICS

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Virus-host interaction is commonly viewed as a conflict between the virus-driven subversion of biosynthetic processes in infected cells and the antiviral host program. This focus on biosynthetic processes overshadows non-biosynthetic processes relevant for life-long persisting DNA viruses that replicate in the nucleus and can accumulate there in sub-million particle numbers. The nuclear accumulation of large numbers of assembling and mature viral particles implies the existence of virus-encoded molecules that govern the compartmentalization of the virus in the cell nucleus. It is unknown how nuclear viruses alleviate the spatial constraints imposed by the packed host chromatin, nuclear matrix, and the nuclear membrane. Here we report a mechanism regulating compartmentalization and release of the JC polyomavirus by interfering with the interface between heterochromatin and the nuclear envelope. We found that JC virus, one of the most prevalent chronic human DNA viruses, employs an array of short linear motifs in its nonstructural Agnoprotein (Agno) to bind to heterochromatin protein 1-alpha (HP1α), modulate HP1α-driven liquid-liquid phase separation and interfere with HP1α-nuclear lamina interaction. We will discuss how Agnoprotein-HP1α interaction contributes to the spatial compartmentalization of the virus and its release from the nucleus, and how virus-driven changes in nuclear structure can contribute to the JC virus life cycle and JC virus-induced pathology.

ANTIBODY FEEDBACK REGULATION OF MEMORY B CELL DEVELOPMENT IN SARS-CoV-2 mRNA VACCINATION

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Feedback inhibition of humoral immunity by antibodies was initially documented in guinea pigs by Theobald Smith in 1909, who showed that passive administration of excess anti-Diphtheria toxin inhibited immune responses. Subsequent work documented that antibodies can enhance or inhibit immune responses depending on antibody isotype, affinity, the physical nature of the antigen, and engagement of immunoglobulin (Fc) and complement (C') receptors. However, little is known about how pre-existing antibodies might influence the subsequent development of memory B cells. Here we examined the memory B cell response in individuals who received two high-affinity IgG1 anti-SARS-CoV-2 receptor binding domain (RBD)-specific monoclonal antibodies, C144-LS and C135-LS, and subsequently two doses of a SARS-CoV-2 mRNA vaccine. The two antibodies target Class 2 and 3 epitopes that dominate the initial immune response to SARS-CoV-2 infection and mRNA vaccination. Antibody responses to the vaccine in C144-LS and C135-LS recipients produced plasma antigen binding and neutralizing titers that were fractionally lower but not statistically different to controls. In contrast, memory B cells enumerated by flow cytometry after the second vaccine dose were present in higher numbers than in controls. However, the memory B cells that developed in antibody recipients differed from controls in that they were not enriched in VH3-53, VH1-46 and VH3-66 genes and predominantly expressed low-affinity IgM antibodies that carried small numbers of somatic mutations. These antibodies showed altered RBD target specificity consistent with epitope masking, and only 1 out of 77 anti-RBD memory antibodies tested neutralized the virus. The results indicate that pre-existing high-affinity antibodies bias memory B cell selection and have a profound effect on the development of immunological memory in humans that may in part explain the shifting target profile of memory antibodies elicited by the 3rd mRNA vaccine dose.

MOLECULAR FATE-MAPPING OF SERUM ANTIBODIES REVEALS THE EFFECTS OF ANTIGENIC IMPRINTING ON REPEATED IMMUNIZATION

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The ability of serum antibody to protect against pathogens arises from the interplay of antigen-specific B cell clones of different affinities and fine specificities. These cellular dynamics are ultimately responsible for serum-level phenomena such as antibody imprinting or “Original Antigenic Sin” (OAS), a proposed propensity of the immune system to rely repeatedly on the first cohort of B cells that responded to a stimulus upon exposure to related antigens. Imprinting/OAS is thought to pose a barrier to vaccination against rapidly evolving viruses such as influenza and SARS-CoV-2. Precise measurement of the extent to which imprinting/OAS inhibits the recruitment of new B cell clones by boosting is challenging because cellular and temporal origins cannot readily be assigned to antibodies in circulation. Thus, the extent to which imprinting/OAS impacts the induction of new responses in various settings remains unclear. To address this, we developed a “molecular fate-mapping” approach in which serum antibodies derived from specific cohorts of B cells can be differentially detected. We show that, upon sequential homologous boosting, the serum antibody response strongly favors reuse of the first cohort of B cell clones over the recruitment of new, naïve-derived B cells. This “primary addiction” decreases as a function of antigenic distance, allowing secondary immunization with divergent influenza virus or SARS-CoV-2 glycoproteins to overcome imprinting/OAS by targeting novel epitopes absent from the priming variant. Our findings have implications for the understanding of imprinting/OAS, and for the design and testing of vaccines aimed at eliciting antibodies to evolving antigens.

INTRINSIC AND EXTRINSIC CONTRIBUTIONS OF ENDOSOMAL SORTING FACTOR HD-PTP TO ADIPOSE TISSUE HOMEOSTASIS THROUGH POSITIVE REGULATION OF RECEPTOR ACTIVATION

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Receptor composition within the plasma membrane determines how a cell perceives the extracellular milieu. Upon receptor activation the appropriate cellular response is initiated through signaling effectors, modulated in response to lipid microdomains, and the receptor is trafficked to the endosome. Ubiquitin modified receptors at the endosome are then recognized by the endosomal sorting complexes required for transport (ESCRTs) for incorporation into forming intraluminal vesicles (ILVs) during multivesicular body (MVB) formation. Sorting into ILVs blocks access of cytosolic signaling molecules to the activated receptor tail, thus terminating further signaling. In mammals, the Bro1 family member HD-PTP participates in the MVB formation process by coordinating with the ESCRTs to link cargo recognition with ILV formation. While this pathway is understood at a biochemical and biophysical level, the contributions to normal physiology remain unknown due to the essential nature of the ESCRT genes. Here we show that HD-PTP contributes to maintenance of adipose tissue homeostasis through positive regulation of receptor signaling and extrinsic regulation of macrophage function. HD-PTP homozygous hypomorphic mice (HD-PTP$^{h/h}$) represent the lowest gene dosage compatible with survival and display severe lipodystrophy, defined as a decrease in all white adipose tissue deposits. Proteomics data from HD-PTP$^{h/h}$ mice revealed unexpected decreases in major signaling pathways, which we have traced back to reduced receptor phosphorylation following in vitro ligand addition. Evaluation of cellular cholesterol distribution suggests this is due to disruption of plasma membrane lipid microdomains via intracellular cholesterol accumulation. HD-PTP$^{h/h}$ mice also display defects in primary macrophage function, adipose tissue infiltration, and inflammatory cytokine production, though bone marrow derived macrophages are unimpacted. Our results demonstrate a novel role for HD-PTP in adipose tissue homeostasis through modulation of cellular cholesterol distribution and extrinsic contributions to macrophage function. Elucidation of impacted pathways upon HD-PTP depletion may aid in identification of treatment targets for obesity.

POTENTIAL ROLE OF X-CHROMOSOME INACTIVATION ESCAPEE ZFX IN SEX-BIASED PROINFLAMMATORY MACROPHAGE POLARIZATION

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Macrophages play a key role in the defence against infection and are crucial for the maintenance of tissue homeostasis. Based on signals from their microenvironment, they differentiate across a gradient of polarization states towards broadly defined pro-inflammatory M1 or anti-inflammatory M2 macrophages. This polarization is driven by extensive epigenomic and transcriptomic changes.

To better understand the molecular mechanisms contributing to macrophage polarization, we built an enhancer-mediated gene regulatory network using our tool GRaNIE (https://grp-zaugg.embl-community.io/GRaNIE/), using publicly available RNA-seq and H3K27Ac ChIP-seq data from macrophages stimulated with IFNγ/LPS (M1) or IL-4 (M2). We then applied GRaNPA (https://grp-zaugg.embl-community.io/GRaNPA/), a machine learning framework with feature selection, to predict transcription factors (TFs) important for macrophage polarization. Zinc Finger Protein X-Linked (ZFX) came out as the top TF for predicting gene expression linked to the M1 phenotype. Indeed, we found higher ZFX expression in M1 macrophages compared to M2 and M0, which was consistent across multiple datasets. ZFX is one of a few X-linked genes that resists X-chromosome inactivation and remains biallelically expressed in female cells, presumably leading to higher dosage in females (XX) compared to males (XY). To confirm this, we compared the expression of ZFX in RNA-seq data from male and female macrophages, and found its expression to be significantly higher in females. Moreover, we identified increased ZFX expression in RNA-seq data from synovial biopsies from rheumatoid arthritis patients, which was associated with an increase in M1 macrophages.

We are using inducible CRISPR based knockdown models to investigate the role of ZFX X-linked dosage in modulating M1 macrophage polarization potential between females and males. This will help to dissect the causality between dosage of immunity-related X-linked genes and immune-response between females and males, which remains a critical open question. Our study will contribute to a better understanding of sex-based differences in the immune response, and potentially identify new targets for treatment of infectious- and autoimmune diseases.

ST8SIA6 EXPRESSION IN BETA CELLS MITIGATES ONSET OF AUTOIMMUNE DIABETES IN THE MURINE NOD MODEL

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1.25 million Americans have type 1 diabetes (T1D), where the immune system destroys insulin-producing beta cells in the islets of Langerhans in the pancreas. While islet transplantation has the potential to be curative, it has yet to overcome dual issues of pre-existing autoreactivity to beta cells as well as alloreactivity due to MHC mismatched transplants. Targeted immunotherapy to prevent immune-mediated attack on transplanted islets without systemic immunosuppression would be ideal, but that has not yet been achieved. Additional strategies are needed to block immune-mediated islet attack without requiring general immunosuppression are needed. Non-obese diabetic (NOD) mice spontaneously develop autoimmune diabetes with a well characterized progression with similarities in the multiple genes that influence T1D development in both the NOD model and in patients. Recently, we demonstrated that changes in sialic acid incorporation into cell surface glycans by the sialyltransferase ST8Sia6 were able to effectively protect cancer cells from an immune response by engaging the inhibitory receptor Siglec-E. Here, we hypothesized whether the same mechanism could be used to protect beta cells in autoimmune attack in the NOD mice. To do this, we generated a novel line of genetically-engineered mice to overexpress ST8Sia6 in beta cells in NOD mice in a cre-dependent and dox-regulatable manner ("RIP-cre LNL-tTA ST8Sia6", hereafter called "NOD βST"). The expression of ST8Sia6 in beta cells decreased the incidence of diabetes in female NOD mice by 90%. While there was a similar initiation of insulitis at 8 weeks in female NOD βST mice, insulitis did not progress further in stark contrast to their littermate controls. Autoreactive T cells and B cells are present in NOD βST mice, indicating that this is a peripheral rather than central mechanism of tolerance. Strikingly, shutting off the ST8Sia6 transgene at 20 weeks of age in NOD βST mice maintained protection from diabetes, indicating dominant suppression and durable tolerance had been established. However, when the ST8Sia6 transgene is shut off at 8 weeks of age, NOD βST mice had a similar progression to diabetes as their littermate controls, indicating that there is a window between 8 and 20 weeks where progression from insulitis to immune-mediated destruction is halted, and durable tolerance is established in NOD βST mice. Thus, ST8Sia6 in beta cells provides local protection from immune attack in the NOD model of T1D.

THE ROLE OF CYTOSOLIC EZH2 IN TNBC AND CANCER - ASSOCIATED IMMUNE RESPONSES

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We have previously reported that the histone methyltransferase (HKMT), Ezh2 controls leukocyte migration through interaction with the cytoskeleton remodeling effector VAV, and direct methylation of the integrin adaptor, Talin1. Our recent study further demonstrates that cytosolic Ezh2 promotes cellular transformation and enhances the aggressiveness of murine triple negative breast cancer (TNBC) cells. The role of cytosolic Ezh2 in TNBC and the associated immune signatures in mice during the disease progression are further determined in this current study. Our preliminary investigation revealed that Brca1-deficient TNBC was associated with increased proportion of breast cancer cells expressing high level of cytosolic Ezh2. Hence, we employed an established mouse model for spontaneous TNBC (*P53*$^{+/-}$*;Brca1*$^{f/f}$*;LGB*-cre) with an EYFP reporter. Using this mouse model, we performed immune profiling in blood before, during and after the onset of disease. Our data showed that before the development of any palpable tumors, the percentage of immature neutrophils increased significantly, while the percentages of monocytes and mature neutrophils were reduced. It is likely the development of microscopic neoplasia in the mammary grands of multiparous females contributes to significant change in circulating immune cell profiles, which has the potential to serve as an early non-invasive diagnostic marker. In parallel, the molecular mechanisms controlling subcellular localization of Ezh2 in TNBC will be determined.

ORGANOID-BASED SINGLE-CELL SPATIOTEMPORAL GENE EXPRESSION LANDSCAPE OF HUMAN EARLY EMBRYONIC DEVELOPMENT AND HEMATOPOIESIS

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The engineering of immune cells largely depends on the precise specification of hematopoietic tissues through interactions with embryo proper and extraembryonic tissues. Here we defined the single-cell spatiotemporal gene expression landscape of embryo proper, extraembryonic tissues, and subsequent hematopoiesis in organoids. We established the human embryonic organoid (HEMO), generated from human expanded potential stem cells followed by embryonic body formation and patterning of three germ layers as well as extraembryonic tissues. Combining time-series single-cell RNA sequencing with single-cell resolution spatial transcriptomics, HEMO followed the development of human embryos. Preceded by extraembryonic tissues such as placenta and yolk sac, embryo proper including neural crest, neuroectoderm, and cardiac mesoderm developed. Hematopoietic tissues appeared as hemogenic endothelium and erythroblasts, predominating the HEMO with hematopoietic progenitor cells, erythroid-megakaryocytes, and monocytes in due course. Leveraging cell-cell communication network analysis, we demonstrated that trophoblast-like tissue facilitated WNT signaling in migrating neural crest cells. With single-cell resolution spatial transcriptomics, we defined the yolk sac erythro-megakaryopoiesis niche. Vitronectin-integrin signaling, a major contributor to megakaryocyte maturation, is consistently enriched in HEMO and the yolk sac of human fetal tissues. Overall, our study provides the blueprint for engineering immune cells.

DENDRITIC CELL-INTRINSIC EZH2 EXPRESSION MAINTAINS THYMIC HOMEOSTASIS AND PREVENTS PREMATURE THYMIC INVOLUTION

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Ezh2, a well-known histone methyltransferase and a regulator of transcription, was previously shown to display a cytosolic methyltransferase activity in dendritic cells (DCs). It regulates the integrin-dependent migration of DCs by the methylation of cytosolic protein Talin. As DCs have a large cytosolic compartment, Ezh2 may have additional cytosolic targets to regulate other physiological functions of DCs. Here we found that DC-intrinsic Ezh2 expression regulated the early T cell development in an age-dependent manner. In adult mice, DC-intrinsic Ezh2 deficiency led to a decreased thymic cellularity starting from the early double negative (DN) stages. The thymic architecture was disrupted with the loss of distinction between cortex and medulla. These phenotypes suggest that DC-intrinsic Ezh2 deficiency caused an accelerated thymic involution. Surprisingly, although there was an overall decreased cellularity, γδ T cells and CD8 T cells increased significantly. In addition, several secreted molecules involved in inflammation and tissue remodeling were increased. Interestingly, Ezh2 deletion reduced the tri-methylation level of histone 3 lysine 27 in cDC1 but not in cDC2 and pDC, suggesting that Ezh2 regulates the functions of DCs in a cell type-specific manner via both canonical and non-canonical mechanisms. While thymic DCs are generally known to present self-antigens to mediate the negative selection of self-reactive thymocytes, our study reveals their unexpected involvement in mediating the early stages of T cell development and thymic involution. The reduction in T cell output due to thymic involution is one of the main contributors to the increased susceptibility of old individuals to cancer, infections, and autoimmunity. Our study sheds light on the previously underappreciated role of dendritic cells in mediating thymic involution and may bring us one step closer to the fountain of youth.

UNCONVENTIONAL PH-SENSITIVE PHOSPHATASE STS1 INTERACTS WITH CBL-B TO SUPPRESS T CELL FUNCTION IN ACIDIC ENVIRONMENTS

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T cell responses are inhibited in acidic environments. T-cell receptor (TCR) signaling is transduced by cytoplasmic tyrosine kinases that phosphorylate downstream signaling molecules. To properly regulate the magnitude of the TCR signaling pathway, phosphorylated proteins are subjected to dephosphorylation and ubiquitination. Little is known about how environmental pH affects intracellular signaling pathways. Here, we report that STS1, an unconventional phosphatase which relies on a pH-sensitive histidine to catalyze tyrosine dephosphorylation in its catalytic core, inhibits T cell responses in a Cbl-b dependent manner. Upon TCR stimulation, STS1 inducibly associates with the ubiquitin ligase Cbl-b and dephosphorylates protein substrates bound to Cbl-b to suppress signaling. Deficiency of either STS1 or Cbl-b desensitizes T cells to the inhibitory effects of extracellular acidic pH. Moreover, deficiency in STS1 or Cbl-b in mice inhibits tumor growth and improves survival. These results reveal that a TCR-induced STS1-Cbl-b complex senses environmental acidity and tunes T cell responses.

HUMAN TRANSCRIPTOME IN LONGITUDINAL, DAILY UPPER RESPIRATORY SPECIMENS FROM THE INCIDENCE OF SARS-COV-2 INFECTION

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We have previously performed quantitative measurements of viral loads in different specimen types (saliva, nasal swab, throat swab) prospectively collected daily from individuals starting before or at the incidence of SARS-CoV-2 infection. We identified that viral loads in these three sampling sites exhibit extreme differences in viral load kinetics within the same person (https://www.medrxiv.org/content/10.1101/2022.07.13.22277513v1). These differences have significant implications for the effectiveness of certain diagnostic testing approaches (https://www.medrxiv.org/content/10.1101/2022.07.13.22277113v1). These differences also may be driven by the differences in the mucosal immune response among individuals. To understand these observations in additional mechanistic detail, we are probing the human transcriptome present in these unique, longitudinal specimens via RNA sequencing. Evidence of antiviral responses in each sampling site during the early phase of the infection may provide insight into the primary site of infection, and the relative compartmentalization of the mucosal immune response across respiratory sites. The correlation of immune response signatures to viral load kinetics may also suggest certain pathways associated with effective or ineffective control of local viral replication, and early determinants of disease course.

STORE-OPERATED CALCIUM ENTRY CONTROLS T FOLLICULAR HELPER CELLS DIFFERENTIATION THROUGH UNIQUE CALCIUM REGULATED TRANSCRIPTION FACTORS IN INFLUENZA VIRUS INFECTION

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T follicular helper cells (Tfh) provide help to germinal center B cells, promote clonal selection and affinity maturation, which is critical for humoral immune responses. TCR signaling induces store-operated calcium entry (SOCE) mediated by STIM and ORAI proteins. We previously showed (Vaeth et al., 2016) that SOCE regulates the differentiation of $CXCR5^{+}PD\text{-}1^{+}$ Tfh cells, but the molecular and transcriptional mechanisms by which SOCE controls gene expression, chromatin accessibility and transcription factor (TF) activity in Tfh cells is not completely understood. To investigate how SOCE controls Tfh differentiation *in vivo*, we transferred T cells from *WT OT-II*, *Orai1*$^{fl/fl}$*Cd4Cre OT-II* and *Orai1*$^{fl/fl}$*Orai2*$^{-/-}$ *Cd4Cre OTII* mice into T cell deficient host mice, which were infected with PR8 (H3N2) strain of influenza virus (IAV) engineered to express $OVA_{323–339}$ peptide. Whereas *Orai1*-deficient T cells have partially reduced SOCE, SOCE is abolished in T cells lacking both Orai1 and Orai2 expression, allowing us to assess the quantitative SOCE requirements during Tfh differentiation. We compared the transcriptome and chromatin accessibility in Tfh cells isolated *ex vivo* by RNA-SEQ and ATAC-SEQ and found that partial reduction of SOCE in *Orai1*-deficient had only minimal effects on gene expression and chromatin accessibility. By contrast, abolished SOCE in *Orai1/2*-deficient T cells revealed strong differences at the transcriptome and chromatin landscape level. A comprehensive analysis of differentially accessible regions (DAR) and differentially expressed genes (DEG) combined with known TF motif enrichment analysis, TF pathway analysis and TF footprint analysis shows that several of TFs including NF-κB, NFAT are negatively regulated in *Orai1/2*-deficient T cells, whereas TFs of the E2F family and NRF1 are positively regulated. Our data reveal that SOCE regulates Tfh cell differentiation by controlling the function of a variety of TFs.

IN VIVO CRISPR SCREEN IDENTIFIES SPECIFIC REQUIREMENT OF RETINOID RECEPTORS IN DRIVING MACROPHAGES DIVERSITY AND SPECIALIZATION

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Nuclear receptors (NRs) are broad metabolic sensors and ideal regulators of gene expression and have been identified as attractive targets of therapeutic drugs. Several NRs play pleiotropic functions in the immune system. Most classically, corticosteroids acting via GR are major immunosuppressors. RORγ is a master regulator for the differentiation of Th17, as well as ILCs and gdT cells. Retinoic acid acting via RARα or RARγ affects T cell differentiation. However, there is no comprehensive perspective on the role of NRs in immunologic cell-types. To explore this novel territory, we developed and implemented a cytometry based CRISPR screen on the whole family of NRs in the differentiation of immunocyte lineages in vivo. In a nutshell, Cas9-expressing bone marrow stem cells are transduced with lentiviruses that encode sgRNAs and then transferred into irradiated hosts, where they differentiate into different immunologic cell-types. We identified several uncharacterized NR functions in the differentiation and homeostasis of multiple immunocyte lineages, including B cells, T cells, macrophages, monocytes, and dendritic cells, etc. Most interestingly, we demonstrated differential requirement for specific retinoid receptors (RARs/RXRs) in differentiation and specialization of macrophages in different tissues (i.e., peritoneal cavity, lung, liver, colon, spleen and visceral adipose tissue) and subcutaneous MC38 tumor. Moreover, the agonist/antagonist compounds that selectively manipulate particular retinoid receptors are able to phenocopy the differentiation/homeostasis effects and mimic the transcriptional signatures. Therefore, these selective compounds may be developed as potential drugs to curb autoimmune/inflammatory diseases or to refine anti-tumor responses.

COMBINATORIAL CONTROL OF PERIPHERAL REGULATORY T CELL GENERATION BY *FOXP3* ENHANCERS PREVENTS INTESTINAL TYPE 2 INFLAMMATION

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Regulatory T (Treg) cells, characterized by the lineage-defining transcription factor *Foxp3*, are specialized $CD4^+$ T cells with immunosuppressive function. Treg cells specific for self and foreign antigens are thought to be generated from distinct precursor cells in separate anatomic locations: thymocytes develop into thymic Treg (tTreg) cells in response to self antigens, while environmental antigens of barrier site origin stimulate naïve conventional T cells to differentiate into peripheral Treg (pTreg) cells. Due to a lack of tools that allow for specific identification and manipulation of these cell populations, whether tTreg and pTreg cells represent separate lineages with unique function and properties remains elusive. Therefore, we sought to selectively ablate pTreg cells through deletion of *Foxp3* enhancers responsive to pTreg cell-inducing signals in a temporally controlled manner. By knocking out *Foxp3*-CNS0 immediately before weaning in *Foxp3*-CNS1 deficient mice, the resulting conventional T cells had severely diminished response to IL-2, TGF-β, or retinoic acid signaling in *in vitro* induction assays and failed to express Foxp3. pTreg cell-deficient mice spontaneously developed mucosal inflammation in the large intestine at 3-4 months featuring mastocytosis, expansion of T helper 2 cells, and heightened production of type 2 cytokines. Furthermore, germinal center B cells in the gut-associated lymphoid tissues of pTreg cell-deficient mice preferentially switched to the type 2 immunoglobulin IgG1, and serum IgE titers in these mice were also elevated. In conclusion, intestine-related pTreg cells restrain exuberant type 2 immunity and safeguard gastrointestinal health, dysregulation of which may underlie food allergy.

MITOCHONDRIAL ROS PROMOTES SUSCEPTIBILITY TO INFECTION VIA GASDERMIN D-MEDIATED NECROPTOSIS

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Although mutations in mitochondrial-associated genes are linked to inflammation and susceptibility to infection, their mechanistic contributions to immune outcomes remain ill-defined. We discovered the disease-associated gain-of-function allele *Lrrk2*^{G2019S} (leucine-rich repeat kinase 2) perturbs mitochondrial homeostasis and reprograms cell death pathways in macrophages. When the inflammasome is activated in *Lrrk2*^{G2019S} macrophages, elevated mitochondrial ROS (mtROS) directs association of the pore-forming protein gasdermin D (GSDMD) to mitochondrial membranes. Mitochondrial GSDMD pore formation then releases mtROS, promoting a switch to RIPK1/RIPK3/MLKL-dependent necroptosis. Consistent with enhanced necroptosis, infection of *Lrrk2*^{G2019S} mice with *Mycobacterium tuberculosis* elicits hyperinflammation and severe immunopathology. Our findings suggest a pivotal role for GSDMD as an executer of multiple cell death pathways and demonstrate that mitochondrial dysfunction can direct immune outcomes via cell death modality switching. This work provides insights into how LRRK2 mutations manifest or exacerbate human diseases and identifies GSDMD-dependent necroptosis as a potential target to limit *Lrrk2*^{G2019S}-mediated immunopathology.

NAÏVE B CELLS EXPRESSING ARID3A DEFINE A UNIQUE SUBSET OF CELLS IN AUTOIMMUNE AND HEALTHY INDIVIDUALS.

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ARID3a (AT-rich interaction domain3a) is a DNA-binding protein originally associated with increases in immunoglobulin heavy chain transcription. Constitutive expression of ARID3a in transgenic mouse B lymphocyte lineage cells resulted in autoimmunity. Examination of naïve B cells from patients with systemic lupus erythematosus (SLE) revealed that healthy controls had few ARID3a-expressing cells, while numbers of ARID3a -expressing naïve B cells in SLE patients was dramatically increased and associated with increased disease activity. Others suggested that “activated” SLE naïve cells are precursors of pathogenic autoantibody producing B cells in SLE. Therefore, we performed single cell RNA-seq on sorted naïve B cells from ten SLE patients with varying frequencies of ARID3a-expressing cells. UMAP clustering revealed that ARID3a-expressing cells were limited to three of nine naïve B cell clusters indicating differences in transcriptomes related to ARID3a expression. Data were then binned based on ARID3a transcript expression levels revealing a number of genes associated with immune responses and activation, including TLR7 and HLA molecules. Because these data suggest that ARID3a+ cells may represent activated naïve B cells, we stimulated healthy B cells with anti-Fab’2, the TLR7 agonist R848 and a panel of cytokines and found that ARID3a expression is induced in a subset of “activated” naïve B cells. These data support the hypothesis that ARID3a is a marker of B cell activation in autoimmune cells, and suggest the possibility that transcriptomes associated with those activated naïve B cells are at least in part due to ARID3a expression. Our RNA-seq data also allowed identification of a previously unknown surface marker associated with ARID3a expression and induced in vitro in association with ARID3a production in naïve B cells. Analyses of SLE patient samples confirmed the co-expression of ARID3a with this marker, as well. This marker will allow separation of ARID3a+ and ARID3a- cells for future functional studies. Together, these data define a new subset of “activated” naïve B cells present in SLE. Funded by AI118836.

ENCODING OF ANTIGEN INFORMATION IN T CELLS BY COMBINATORIAL SIGNALING DYNAMICS .

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T cells mount functionally distinct responses to pMHC antigens of varying affinities and doses, but the mechanism by which they sense and interpret different pMHC inputs remains unknown. pMHC binding to the T cell receptor (TCR) activates multiple signaling pathways, including the Ca^{2+}/NFAT and Erk/MAP kinase pathways. Both pathways have been shown to transmit ligand information through their temporal responses in other cellular contexts. As such, they may also function in a dynamic manner in T cells to transmit antigen information within the cell. To test this possibility, we developed an *in vitro* system to simultaneously monitor NFAT and Erk dynamics in single, living T cells over day-long timescales as they respond to various pMHC inputs. We found that both pathways initially activated uniformly in response to varying pMHC affinity and dose, but diverged in their responses over longer timescales (>8 hrs), thereby jointly encoding pMHC input information through their dynamic responses. We then define subsets of genes that are differentially expressed in response to varying pMHC affinity and dose, and further find that their expression profiles arise in part due to distinct logic in their integration of NFAT and Erk signaling. Our findings shed light on how T cells translate differences in pMHC inputs to distinct functional outcomes.

GROUP 3 INNATE LYMPHOID CELL PYROPTOSIS REPRESENTS A HOST DEFENCE MECHANISM AGAINST *SALMONELLA* INFECTION

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Group 3 innate lymphoid cells (ILC3s) produce interleukin (IL)-22 and coordinate with other cells in the gut to mount productive host immunity against bacterial infection. However, the role of ILC3s in *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) infection, which causes foodborne enteritis in humans, remains elusive. Here we show that *S*. Typhimurium exploits ILC3-produced IL-22 to promote its infection in mice. Specifically, *S*. Typhimurium secretes flagellin through activation of the TLR5-MyD88-IL-23 signalling pathway in antigen presenting cells (APCs) to selectively enhance IL-22 production by ILC3s, but not T cells. Deletion of ILC3s but not T cells in mice leads to better control of *S*. Typhimurium infection. We also show that *S*. Typhimurium can directly invade ILC3s and cause caspase-1-mediated ILC3 pyroptosis independently of flagellin. Genetic ablation of *Casp1* in mice leads to increased ILC3 survival and IL-22 production, and enhanced *S*. Typhimurium infection. Collectively, our data suggest a key host defence mechanism against *S*. Typhimurium infection via induction of ILC3 death to limit intracellular bacteria and reduce IL-22 production.

HOMEODOMAIN-ONLY PROTEIN HOMEOBOX (HOPX) REGULATES CD8+ T CELL PROLIFERATION, DIFFERENTIATION, AND AGING

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HOPX is a highly conserved homeodomain protein that plays critical roles in cell differentiation including Treg and Th1 CD4+ T cell survival. However, the role of HOPX in CD8+ T cell differentiation and functions has not been studied. Here, we showed that HOPX expression increases from human naïve (TN) to memory (central, TCM and effector, TEM) CD8+ T cells resembling CD4+ T cells and increases post in vitro activation via anti-CD3/CD28 antibody. Furthermore, we found that human CD8+ T cells express three isoforms (a, b, and c) of HOPX, and HOPXb is a dominant form (60% of HOPX). Ectopically expression of HOPXb in Jurkat cells and primary CD8+ TN cells significantly reduce their growth without increasing cell death. Strikingly, ectopically expressed HOPXb in CD8+ TN cells had comparable levels of HOPXb mRNA and growth post activation in vitro compared with those of TEM cells, suggesting level of HOPXb contributes to the proliferative difference of CD8+ TN and TEM cells. Mechanistically, HOPXb directly binds to the promoters and represses expression of genes regulating T cell proliferation and function, including MYC and NR4A1. We further showed that ectopically expressed HOPXb reduces NR4A1 expression and TCR signaling. Lastly, we found that HOPX expression is higher in activated naïve CD8+ T cells from the old (>70 years) than young (<40 years) healthy donors, correlating with the significant less expansion after in vitro stimulation of CD8+ TN cells from old than from young donors. Collectively, our findings show that HOPX represses activation-induced CD8+ T cell proliferation and contributes to differentiation and aging of CD8+ T cells.

HETEROGENEOUS TUMOR MICROENVIRONMENT MODULATES T-CELL PHENOTYPE AND $CD57^+$ T FOLLICULAR HELPER CELLS HAVE AN ADVERSE EFFECT IN FOLLICULAR LYMPHOMA

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The tumor microenvironment (TME) plays a crucial role in mediating the tumor immune response, thereby affecting patient outcomes in follicular lymphoma (FL). T-lymphocytes are prevalent in the TME of follicular lymphoma (FL). However, the phenotype of T-cells may vary, and the prevalence of certain T-cell subsets may influence tumor biology and patient survival. We therefore analyzed a cohort of 82 FL patients with biopsy specimens collected before first treatment regimen was administered and retrospectively extracted patient clinical parameters. CyTOF was utilized to determine whether specific T-cell phenotypes were associated with distinct TME and patient outcome. We identified 4 immune subgroups with differing T-cell phenotypes and the prevalence of certain T-cell subsets was associated with patient survival. Patients with increased T cells with early differentiation stage tended to have a significantly better survival than patients with increased T-cells of late differentiation stage. In patients with less T cells overall, the T cell phenotypes were more terminally-differentiated, senescent, and exhausted. Patients rich of lymphoma B cells featured with increased number of follicular T helper (T_{FH}) cells. We found that T-cell associated survival only can be seen in low grade lymphoma but not in patients with high grade, suggesting that T cell-mediated anti-tumor immunity is preserved in low grade lymphoma but not in higher grade. We observed that $CD57^+$ T_{FH} cells are highly represented in patients with disease progression and correlate with inferior patient outcomes. These $CD57^+$ T_{FH} cells were cells with late differentiation stage indicated by functionally and phenotypical features. Single cell analysis (CITE-seq) revealed that $CD57^+$ T_{FH} cells exhibited a substantially different transcriptome from $CD57^-$ T_{FH} cells with upregulation of inflammatory pathways, evidence of immune exhaustion ad susceptibility to apoptosis. Taken together, our results show that different tumor microenvironments among FL patients are associated with variable T-cell phenotypes and an increased prevalence of $CD57^+$ T_{FH} cells is associated with poor patient survival.

IDENTIFYING MECHANISTIC DRIVERS OF THE TH17/TREG PARADIGM THROUGH scRNA-SEQ

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Regulatory T cells (Tregs) and T helper 17 cells (Th17s) are subsets of helper T cells that play crucial and orthogonal roles in the adaptive immune system. Canonically, regulatory T cells act as anti-inflammatory regulators that suppress immune responses by inhibiting pro-inflammatory T cell proliferation and cytokine production, while Th17s, characterized by the production of interleukin 17 (IL-17), have both protective and pro-inflammatory phenotypes. Imbalance of Treg and Th17 cells is tied to autoimmunity and has been heavily implicated in autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis.

The differentiation of naïve helper T cells into each subtype depends heavily on the makeup of the extracellular environment. The two cell types have a reciprocal relationship and both need TGF-β for induction. Additionally, both immune cell subtypes have a degree of plasticity that allows for subsequent differentiation after initial polarization. The introduction of additional extracellular cytokines or antigenic stimuli can further increase heterogeneity amongst cell populations, creating “sub-subtypes” of helper T cells, each with a distinct phenotype. Of note are Th17-like Tregs which can be induced in the presence of IL-6 and IL-1β and share gene expression and phenotypic features with both cellular subtypes.

A comprehensive unbiased transcriptional analysis on the various subtypes could lead to improved understanding of knowledge gaps behind the driving mechanisms of Treg/Th17 subtype differentiation. Performing single-cell RNA sequencing (scRNA-seq) on cells differentiated in vitro under Treg and Th17 polarizing conditions will uncover a complete transcriptional signature of each subtype. Since the transcriptional differences between subtypes are often subtle and traditional clustering methods are difficult to assign biological meaning to, we will use Bayesian topic modeling to simultaneously learn cell labels and subtype specific features. These features will then be used to identify actionable pathways that can be manipulated in order to shift subtype proportions within a cell population. Ultimately, this will give a greater understanding of the drivers of autoimmunity and the causes for imbalance between Treg and Th17 cell populations.

ALLOSTERIC INHIBITION OF THE T CELL RECEPTOR BY A DESIGNED MEMBRANE LIGAND

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The T cell receptor (TCR) is a complex molecular machine that directs the activation of T cells, allowing the immune system to fight pathogens and cancer cells. Despite decades of investigation, the molecular mechanism of TCR activation is still controversial. One of the leading activation hypotheses is the allosteric model. This model posits that binding of pMHC at the extracellular domain triggers a dynamic change in the transmembrane (TM) domain of the TCR subunits, which lead to signaling at the cytoplasmic side. We sought to test this model by creating a TM ligand for TCR. Previously we described a method to create a soluble peptide capable of inserting into membranes and bind to the TM domain of the receptor tyrosine kinase EphA2. Here we show that the approach is generalizable to complex membrane receptors, by designing a membrane ligand for TCR. We observed that the designed peptide caused a reduction of Lck phosphorylation of the CD3 ζ subunit. As a result, in the presence of this Peptide Inhibitor of TCR (PITCR), the proximal signaling cascade downstream of TCR activation was significantly dampened in human Jurkat T cells. We observed a reduction of CD69-upregulation in the engineered $CD8^+$ OT1-TCR Jurkat cells stimulated by pMHC-APC as well. Co-localization and co-immunoprecipitation results in DIBMA native nanodiscs revealed that PITCR was able to bind to the TCR. We also observed the colocalization of PITCR and pMHC-TCR complex at the central supramolecular activation cluster (cSMAC) in murine primary $CD4^+$ T cells. We propose that PITCR binds into a crevice present between the TM helices of the CD3 ζ and CD3 epsilon (δ) subunits. Our results additionally indicate that PITCR disrupts the allosteric changes in the compactness of the TM bundle that occur upon TCR activation, lending support to the allosteric TCR activation model. The TCR inhibition achieved by PITCR might be useful to treat inflammatory and autoimmune diseases and to prevent organ transplant rejection, as in these conditions, aberrant activation of TCR contributes to disease.

UNRAVEL THE ROLE OF MACROPHAGES AND T CELLS IN SURGICAL WOUND-MEDIATED IMMUNOSUPPRESSIVE MICROENVIRONMENT USING SINGLE-CELL BASED TCR SIGNALING DETECTION

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Major open surgery has been associated with the induction of post-traumatic immunosuppression with patients' enhanced susceptibility to infections. TCR signaling regulation under such primary immunosuppressive conditions were not fully understood especially at the single-cell level. Mass cytometry allows highly quantitative measurement of up to 50 proteins or protein modifications at single-cell resolution. However, mass cytometry analysis faces a sensitivity limitation requiring the accumulation of ~ 300 metal-tagged antibodies per epitope to produce a detectable signal. This inherent limitation prevents a general application in profiling the low-abundance proteome including TCR signaling nodes

Here, we adopted a novel technology, termed amplification by cyclic extension of DNA oligo (ACED) to amplify isotope-based signals for mass cytometry detection. We established a 31-plex TCR signaling ACED panel that includes p-CD247(CD3ζ), p-CD28, p-ZAP70, p-LAT, p-SLP76, p-PLCγ1 and p-BTK in the classic TCR pathways, p-MEK1/2, p-ERK1/2, p-p90RSK and p-S6 in the MAPK-ERK-related pathways, and many key phosphorylation sites involved in stress, inflammation and cell cycles to comprehensively profile the states and dynamics of the TCR signaling networks.
We acquired patient-derived human wound fluid samples to study (1) the function of each wound fluid in long-term T cell regulation through dye-based T cell proliferation assay, (2) the influences of wound fluid in short-time TCR signaling characterized by ACED, and (3) the causality between short-term and long-term wound fluid effects on the human T cells. We were able to show that wound fluid negatively influences T cell proliferation and further enhances macrophage-dependent T cell suppression. The phosphorylation dynamics of the immunosuppressive wound fluid-treated samples exhibited a decreased peak times and integrals in most of TCR signaling and MAPK/ERK nodes. These data suggested patient-derived wound fluid induces differential signaling effects on T cells and that the influences on short-term TCR signaling dynamics measured by ACED are indicative of the environmental immunosuppression levels.

ZFP36L2 REGULATES PRODUCTION OF IFNγ IN CD8+ T CELLS DURING PROLONGED T CELL ACTIVATION

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CD8+ T cells kill target cells by releasing cytotoxic molecules and inflammatory cytokines, such as TNF and IFNγ. The magnitude and duration of cytokine production is defined by post-transcriptional regulation, and a critical regulator in this fine-tuning are RNA-binding proteins. To date, it is not well understood which RNA-binding proteins drive the cytokine production in T cells. Here, we found that deleting the RNA-binding protein ZFP36L2 specifically in murine T cells (CD4-Cre) regulates the cytokine production in CD8+ T cells in a context-dependent manner. ZFP36L2-deficiency increased the frequency of IFNγ producing T cells, but not of TNF and IL-2. In effector T cells, cytokine production was identical in numbers and level of production between WT and ZFP36L2-deficient CD8+ T cells when reactivated short-term (4 hrs) in vitro and ex vivo. Strikingly, substantial differences were found when T cells were repetitively exposed to tumour cells in vitro or when chronically exposed to tumors in vivo, ZFP36L2-deficient CD8+ T cells displayed substantially higher percentages of IFNγ-producing CD8+ T cells, irrespective of a similar “exhaustion” marker expression profile. Interestingly, tumor-infiltrating CD8+ T cells showed an increased stability of IFNγ mRNA when lacking ZFP36L2, which may thus drive the enhanced protein production. Together, our results show that the RNA-binding protein ZFP36L2 controls the production of IFNγ production in tumor-infitrating CD8+ T cells, which could possibly be harnessed for therapeutic purposes.

OBESITY INDUCES MACROPHAGE FUNCTIONAL CHANGES IN TRIPLE NEGATIVE BREAST CANCER

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Obesity is associated with increased risk for many cancer types, including triple-negative breast cancer (TNBC). In addition to obesity's role in TNBC pathogenesis, it is also recognized as a marker of poor prognosis for survival in pre- and post-menopausal women. However, the mechanism underlying how obesity worsens TNBC progression remains unclear. In this study, we aim to characterize the immune population changes in mammary adipose during the development of obesity and their impact on TNBC progression. First, we utilized a diet-induced obesity mouse model to evaluate tumor growth under obese condition. Four weeks after E0771 (TNBC mouse cell line) mammary fat pad injection, TNBC tumors volume were 1.6-fold larger in obese C57BL/6 mice compared to their lean counterparts. To profile the transcriptome changes induced by obesity, we performed single-cell RNA-Seq on mammary adipose stromal vascular fraction and TNBC allograft from lean and obese C57BL/6 female mice. In TNBC allografts, obesity upregulated genes enriched in the extracellular matrix and epithelial-mesenchymal transition pathways in cancer cells, creating a more aggressive phenotype. Consistent with the heavier TNBC tumor burden, lymphocyte infiltration decreased while myeloid population increased in TNBC allografts from obese mice, which indicated an immunosuppressed tumor microenvironment. Macrophage was the dominant population among Cd45+ cells in tumors, constituting 41.2% in lean and 51.2% in obese mice. An increase of adipose tissue macrophage (ATM) population (2.2-fold increase in obese vs lean adipose; 5.6-fold increase in tumor-bearing vs control adipose) was also observed in the obese or tumor-bearing mammary adipose stroma. Using Gene Set Enrichment Analysis (GSEA), we found that ATM adapted to the obese condition by upregulating fatty acid metabolism and adipogenesis hallmarks while downregulating IFNγ response, allograft rejection, and TNFα signal hallmarks. When encountering TNBC development, ATM downregulated similar proinflammatory pathways, while upregulated the hypoxia and glycolysis pathways. Through integrative analysis, we found that the complement pathway is the primary pathway upregulated in ATM under both obese and tumor growth conditions. Major components of the complement pathway, C1qa and C1qb, were significantly upregulated in ATM by TNBC and obesity. Interestingly, C1qa and C1qb were also up-regulated in bone-marrow-derived macrophage during M2 polarization and when co-culturing with E0771. Taken together, our data showed that the C1qahi/C1qbhi macrophage expanded in mammary adipose tissue during the development of obesity and TNBC, which created an immunosuppressed microenvironment to facilitate TNBC progression. This suggests that the complement pathway may serve as a potential immune therapy target for obese TNBC patients.

MECHANISMS OF IKAROS MEDIATED GENE REGULATION

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Ikaros (IKZF1) is a Zn finger transcription factor essential for B cell progenitor differentiation that is recurrently mutated in human B-ALL with BCR-ABL translocations. IKZF1 is thought to regulate the expression of target genes by competition with transcriptional activators (EBF1, STAT5) and in association with NuRD histone deacetylase and chromatin remodeling complex. How IKZF1 can function as a transcriptional activator of some target genes and as a repressor of others is poorly understood. Here we use a model system where the rapid nuclear translocation of IKZF1 recapitulates many of the gene expression changes observed during the FrC' to FrD transition during pre-B cell differentiation. We combine the identification of IKZF1 interactors by IKZF1 ChIP mass spectrometry with time course experiments that track IKZF1-induced changes in chromatin accessibility, post-translational histone modifications, and chromatin-associated nascent RNA. Together these datasets allow us to investigate and couple the IKZF1-mediated modification of the chromatin landscape to the timing and direction (i.e. repression or activation) of transcriptional response at target genes.

DECIPHERING PREDICTED MHC SPECIFIC IMMUNOPEPTIDOME RESULTS IN THE IDENTIFICATION OF DONOR-SPECIFIC CD4+ T CELLS

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Introduction: Transplantation offers life-saving therapy for end-stage organ failure. However, long-term allograft survival post-transplantation remains a problem in part due to the immunosuppression-resistant T cell-dependent antibody-mediated rejection (AMR). The development of *de novo* donor-specific antibodies (DSA) directed against donor MHC antigens has been described as a major driver of graft dysfunction. Although it is well known that CD4+ T cells help in DSA production, few studies have explored the specificity of alloreactive CD4+ T cells and the antigenic epitopes. Here we aim to identify the alloreactive immunopeptidome and CD4+ T cell responses that can contribute to T cell-dependent AMR in the murine system.
Methods: To predict epitopes recognized by CD4+ T cells, we developed a bioinformatic pipeline using the Immune Epitope Database platform to identify polymorphisms of donor MHC class I and class II (BALB/c: Kd, Dd, Ld, IAd, IEd) that could be broken down into peptides and presented in recipient MHC Class II (C57BL/6: I-Ab). We performed skin grafts from BALB/c donors to C56BL/6 recipients and analyzed CD4+ T cells in response to those predicted peptides.
Results: We identified 132 candidate predicted peptides from MHC polymorphic regions. To test whether these predicted peptides play a role in CD4+ T cell allorecognition, we grafted C57BL/6 mice with Balb/c skin and isolated lymphocytes on day 14 post grafting. Following stimulation with predicted peptides, we found that allo-peptide stimulation resulted in an increased proportion of divided cells ($p<0.001$), production of effector cytokines (TNFα, IFNγ, $p<0.001$), and upregulation of activation markers on CD4+ T cells (CD25, OX40, $p<0.0083$). To identify whether any peptide represented an immunodominant epitope, we used ELISpot to map CD4+ T cell reactivity to each peptide. This analysis resulted in the identification of prominent CD4+ T cell responses against 4 peptides, derived from the polymorphic region of the H2-Kd allele, that we hypothesize to be immunodominant CD4+ T cell epitopes involved in BALB/c skin rejection.
Conclusion: Collectively, these findings provide a novel bioinformatic approach to identify immunodominant T cell epitopes and allo-specific CD4+T cells. By providing a greater understanding of allo-specific CD4+ T cell responses that can contribute to antibody responses in the murine system, similar approaches can be used to identify, track, and mitigate alloreactive CD4+ T cells preventing DSA in human transplantation.

GLUCOCORTICOID SIGNALING AND REGULATORY T CELL COLLABORATE TO MAINTAIN THE HAIR FOLLICLE STEM CELL NICHE

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The maintenance of tissue homeostasis in steady state or under stress is dependent on the proper communication between the stem cells and the supporting cells in their microenvironment or “niche”. In addition to promoting immune tolerance, regulatory T cells (Tregs) have recently emerged as a critical component of the stem cell niche in the hair follicle (HF), injured muscle, bone marrow, and small intestine to support stem cell differentiation or maintain their quiescence. How Treg cells sense the dynamic signals in the niche environment and communicate with stem cells during tissue regeneration is largely unknown. Here, by using HF as a model, we uncover a hitherto unrecognized function of steroid hormone glucocorticoid that instructs skin-resident Treg cells through glucocorticoid receptor (GR) to facilitate hair follicle stem cell (HFSC) activation and HF regeneration. Ablation of GR signaling in Tregs blocked depilation-induced hair regeneration and natural hair growth without affecting Treg’s immune suppressive function. Mechanistic study revealed that GR signaling induces skin-resident Tregs to produce TGF-β3, which directly activates Smad2/3 in HFSCs and facilitates HFSC activation and proliferation. Our study identifies a novel crosstalk between skin-resident Tregs and HFSCs mediated by the GR/TGF-β3 axis, highlighting a new avenue to manipulate Tregs to support tissue regeneration.

STIM1 DIFFERENTIALLY REGULATES TCR- AND IL-12-INDUCED TBX21 EXPRESSION IN T CELLS TO CONTROL ADAPTIVE IMMUNITY TO INFECTION

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The ER Ca2+ sensor STIM1 is critical for store-operated Ca2+ entry (SOCE) through ORAI1 Ca2+ channels, which play important roles in T cell function. Loss-of-function (LOF) mutations in STIM1 or ORAI1 cause severe immunodeficiency with recurrent infections and autoimmunity. Several studies have shown that SOCE contributes to the differentiation of T helper (Th) 2, Th17, T follicular helper (Tfh) and T regulatory (Treg) cells, but whether SOCE is required for Th1 differentiation and the underlying mechanisms are not well understood. Here, we report a patient with an intronic mutation in STIM1 that creates a new splice acceptor site, abnormal *STIM1* mRNA splicing and loss of STIM1 protein expression. The immunodeficiency of the patient is associated with abolished SOCE and reduced expression of Th1 cell-specific genes, including the lineage-specific transcription factor *TBX21* and *IFNG*. Further experiments confirmed that STIM1 deficiency impairs *TBX21* expression in both human and mouse T cells upon TCR stimulation or under IL-12-independent Th1 polarization condition. Mechanistically, TCR stimulation results in STIM1 activation, SOCE and NFAT binding to the distal region of the *TBX21* promoter. NFAT synergizes with STAT1, which is activated by IFN-γ or type I IFN produced by activated T cells or antigen presenting cells (APC), to initiate the transcription of the *TBX21* gene. On the other hand, STIM1 deficiency sensitizes T cells to interleukin 12 (IL-12) signaling by increasing IL-12 receptor expression. Exogenous IL-12 was able to rescue *TBX21* expression and Th1 differentiation in STIM1-deficient T cells. Our study reveals how STIM1 and SOCE differentially regulate TBX21 expression and Th1 differentiation under classical IL-12-dependent and alternative IL-12-independent conditions.

EPIGENETIC SENSING OF ENVIRONMENT CONTROLS INFLAMMATORY RESPONSE IN MACROPHAGES

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Inflammatory response often changes tissue environment, such as pH, oxygen level or composition of cells. Sustained deviation in these variables from homeostasis leads to tissue dysfunction and pathology, as collateral damage of inflammation. Meanwhile, immune cells, such as macrophages, constantly monitor tissue environment and maintain tissue homeostasis. This raises an intriguing question: do changes in tissue environment feedback to modulate immune functions during inflammation?

pH is normally maintained within a narrow homeostatic range. Decrease in pH, or known as acidosis, is associated with diverse inflammatory conditions. Using macrophages, we found that acidic pH modulates their inflammatory response to lipopolysaccharide (LPS, TLR4 agonist) in a gene-specific manner, independent of known pH-sensing receptors. Employing a computational deconvolution algorithm, we systemically characterized how macrophages integrate cues of microbial infections and environmental pH to regulate inflammatory gene expression. This revealed three distinct programs: a group of genes induced by LPS insensitive to the environment (Tnf, Nfkbia), and two groups representing alternative inflammatory activation that are either antagonistic (Il6, Edn1) or synergistic with acidic pH (Ifnb, Adm). Surprisingly, this influence by pH correlates with differential control by NF-kB and IRF3, but is not due to difference in pathway activity. We found that pH-dependent inflammatory response has a delayed activation kinetics, requires chromatin remodeling, and regulates the activity of specific enhancer regions. This is coupled with pH-dependent formation and dissolution of transcription condensates involving BET family proteins and mediators. Perturbations that inhibit functions of BET family proteins or disrupt condensate formation, largely recapitulate the inhibitory effects of acidic pH. Our findings reveal a novel environment-sensitive epigenetic mechanism that elicits qualitatively different inflammatory responses to microbial infections, highlighting a critical role of tissue microenvironment in modulating inflammation.

MAST CELL ACTIVITY INDUCES *FOSB* AS A NEGATIVE FEEDBACK MECHANISM TO LIMIT RELEASE OF PRO-INFLAMMATORY MEDIATORS

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Mast cells (MCs) are innate immune cells with a diverse array of physiologic and pathologic functions due to their capacity to respond to a variety of stimuli and selectively release bioactive molecules ranging from pro- and anti-inflammatory cytokines to growth factors and neurotransmitters. Surprisingly, the transcriptional mechanisms controlling MC responses remain largely unexplored. Based on initial findings that stress and IgE-mediated activation of MCs significantly increased the expression of *FosB*, which encodes the FosB and ΔFosB transcription factors critically involved in long-term modulation of neuronal activity, we hypothesized that *FosB* plays a fundamental role in regulating stimulus-induced MC activation and mediator release. To test this, we crossed the Mcpt5-Cre with the Cre-dependent *floxed FosB* mouse lines, creating the first mice in which *FosB* expression is ablated specifically in MCs (MC^{FosB-}). *In vitro* studies showed that bone marrow derived mast cells (BMMCs) from MC^{FosB-} show increased IgE-antigen induced Ca^{2+} mobilization and release of proinflammatory mediators compared to wild type (WT) BMMCs. Next, we overexpressed ΔFosB or its dominant negative binding partner ΔJunD into WT and MC^{FosB-} BMMCs and found that ΔFosB inhibits stimulus-induced mediator release in both WT and MC^{FosB-} BMMCs while ΔJunD exerts the opposite effect. To uncover regions of ΔFosB binding in MC chromatin we combined CUT&RUN and RNAseq analyses from baseline and IgE-antigen activated BMMCs and found that one of ΔFosB functional targets is the dual specificity phosphatase Dusp4, critical negative regulator of FcER1-pathways involved in MC degranulation. Finally, in vivo experiments showed that, compared to WT, MC^{FosB} mice show exaggerated hypothermic responses, increased plasma inflammatory cytokines, and heightened activation of mesenteric MCs after passive systemic anaphylaxis. Together, these data show that *FosB* products exert an inhibitory effect on MC activation and proinflammatory mediator release *in vitro* and *in vivo* and coincides with binding to DUSP1, providing a novel negative feedback mechanism of MC regulation relevant to disorders associated with exacerbated inflammation.

PEPTIDOGLYCAN FRAGMENT LIBRARY TO INVESTIGATE INNATE IMMUNE RESPONSES

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The human body is covered with bacteria. It has been estimated that for every human cell there are ten bacterial cells living in or on the human body. The bacteria that occupy the human body, which do not cause harm are called commensal bacteria. These bacteria normally provide essential functions for the human body, for example they aid in digestion. However, chronic inflammatory diseases such as asthma, rheumatoid arthritis, and Crohn's disease are thought to arise from an inappropriate response to commensal bacteria. Chronic inflammation has also been shown to lead to a variety of types of cancers including gastric, colon, and lung. The immune system's interaction with bacteria plays a pivotal role in these diseases and in order to develop novel therapies for these diseases, better tools are needed to dissect how the immune system interacts with the microbiome. The field of bacterial activation of the innate immune system is limited to a few select synthetic tools derived from bacterial peptidoglycan. The bacteria are believed to communicate to the human cell through the use of specific carbohydrate polymers. The synthesis of these polymers is extremely demanding and thus the probes are scarce. My research laboratory has focused on developing a combination of chemical and enzymatic synthesis to prepare bacterial cell wall fragments from a number of different types of bacteria. These fragments are then used to investigate how different strains of bacteria initiate an immune response. Here we will discuss our progress in using these tools to probe innate immune recognition pathways.

THE HIGH-ORDER MULTIMERIZATION OF TRANSCRIPTIONALLY ACTIVE AIRE REQUIRES DIRECT INTERACTION WITH P300/CBP

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Autoimmune regulator (Aire) is a transcription factor that plays a critical role in establishing central tolerance. Aire up-regulates the ectopic expression of thousands of peripheral tissue antigens in medullary thymic epithelial cells. This transcriptional activation allows self-reactive T-cells to undergo negative selection or develop into regulatory T-cells. Aire has the unique ability to homo-oligomerize into supramolecular aggregates, which can be visualized as nuclear foci. While we and others have previously shown that Aire foci formation is required for transcriptional activity, the precise mechanisms by which Aire multimerization leads to gene activation remain unclear. To investigate potentially new modes of gene activation by Aire, we focused our attention on Aire's transactivation domain (TAD) in isolation. We first used a combination of genetic and proteomic screens to identify interaction partners that are required for mediating Aire TAD function. One of our screens' top hits was the closely related histone acetyltransferases (HATs) p300/CBP –known co-activators for a variety of other transcription factors. Subsequent structural studies and biochemical analyses confirmed Aire TAD directly interacts with the TAZ2 domain of p300/CBP. Experiments using the p300/CBP inhibitor A-485 revealed that p300/CBP is not only critical for regulating a subset of Aire-dependent genes, but also is required for proper Aire foci formation. In corroboration with these findings, we discovered that deleting the 14aa p300/CBP-binding interface in Aire TAD results in complete loss of Aire foci formation and transcriptional activity. Moreover, we found that even at the initial stages of Aire foci formation, p300/CBP HAT activity was critical for Aire foci formation. In ongoing experiments, we are interested in further elucidating the intimate link between Aire multimerization and p300/CBP-mediated gene activation. We postulate that Aire:p300/CBP interaction is also a means to target Aire to superenhancers where Aire is known to recruit chromatin remodeling factors. Altogether, our data demonstrates that Aire engages with p300/CBP in a novel way: Aire foci formation is not simply due to Aire homo-multimerization, but rather the formation of larger macromolecular structures that build upon Aire multimerization in a manner dependent on p300/CBP interaction and HAT activity.

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RASGRP1 DEFICIENCY IMPAIRS IL-2 PRODUCTION, IL-2R EXPRESSION, PROLIFERATION, AND MAPK SIGNALING IN CD4+ T CELLS.

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Ras guanyl nucleotide releasing protein 1 (RasGRP1) is a guanine nucleotide exchange factor that activates Ras small GTPase by converting GDP to GTP. Patients with mutants in RasGRP1 exhibit impaired T cell expansion, less T cell populations, and are susceptible to viral infection such as Epstein Barr virus infection. It has been reported that RasGRP1-deficient mice have defected T cell development in thymus and less peripheral T cells. However, the roles of RasGRP1 in response to T cell signaling are not clear. The fact that systematic RasGRP1 defect renders T cell to undergo abnormal development in thymus makes it difficult to determine if impaired T cell phonotypes are resulted from RasGRP1 deficiency. To understand the roles of RasGRP1 in T cell biology, we generated CD4-Cre-ERT2 RasGRP1 floxed transgenic mouse line. By injecting tamoxifen, we were able to induce RasGRP1 deletion in CD4+ T cells specifically, allowing T cells to undergo normal development in thymus. Here, we show that RasGRP1 deficiency decreased the number of post-positive selected thymocytes. In periphery, RasGRP1-deficient CD4 T+ cells exhibited impaired IL-2 production and failed to express IL-2 receptor (CD25), causing proliferation failure. Furthermore, RasGRP1-deficient CD4+ T cells failed to induce MAPK/ERK signaling. These results show that RasGRP1 plays a key role in T cell signaling and proliferation.

HISTONE VARIANT H3.3 MEDIATES CELL HOMEOSTASIS BY AVOIDING CHRONIC IFN RESPONSE

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Chronic IFN expression is common cause of inflammatory and autoimmune diseases. To maintain cell homeostasis, it is necessary to strike a balance between proinflammatory and anti-inflammatory responses. Here we show that histone variant H3.3, a nonallelic isoform of canonical histone H3, plays a role in maintaining this immune balance in terminally differentiated bone marrow derived macrophages (BMDMs). H3.3 is critical for early embryonic development and maintaining genome integrity. Nonetheless, its significance in innate immunity has not been fully deciphered. Transcriptome analysis of H3.3 KO BMDMs showed upregulation of interferon stimulated genes (ISGs). To delineate the mechanism behind this ISG induction, we are testing various hypothesis We first examined the possibility that STING pathway is activated in BMDM in the absence of H3.3 where ds-, ss- RNA or DNA may be produced. However, we found that Sting-/-: H3.3fl/fl:LysMcre/cre still induced ISGs at the level comparable with those in H3.3fl/fl:LysMcre/cre. Thus, we ruled out the invovelment of Sting as a mechanism of ISG induction. . Second, we are currently testing the possibility that H3.3 controls expression of Irf7, master ISG inducer. IRF7 is a transcription factor of the IRF family and once activated, it activates a cascade of downstream ISGs. We will present our results obtained form Irf7 KO:H3.3fl/fl:LysMcre/cre mice. Thirdly, it has been reported that H3.3 maintains endogenous retroviral (ERV) silencing in mESCs. H3.3 KO mESCs exhibit ERV derepression when reducing the deposition of H3K9me3 on ERVs, presumably since ss-and ds- RNAs generated from ERVs can lead to activation of ISGs. On the other hand, somatic cells are known to maintain ERV silencing through DNA methylation. We unexpectedly observed upregulation of ERV class II members, possibly resulting in ISG induction. Our results indicate that H3.3 plays a role in silencing some ERV expression which might provoke ISG mediated innate immune responses in differentiated BMDMs. Our work will add to further understanding of the contribution of H3.3 to the transcriptome program of myeloid cells and macrophages.

WBP1L NEGATIVELY REGULATES LEUKOCYTE HEMATOPOIESIS AND THYMUS DEVELOPMENT

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Background: WW domain binding protein 1-like (WBP1L), also known as outcome predictor of acute leukemia 1 (OPAL1), is a transmembrane adaptor protein, expression of which correlates favorable prognosis in childhood leukemia. It has a broad expression pattern in hematopoietic and in non-hematopoietic cells. Our previous data show that WBP1L is a novel regulator of CXCR4 signaling and hematopoiesis.
Methods and Results: Leukocyte hematopoiesis is altered in OPAL1 deficient mice. In the bone marrow, OPAL deficient mice present higher levels of stem cells (Lin-Sca1+c-kit+) and early progenitors (Lin-Sca1+c-kit-). Flow cytometry analysis of hematopoietic stem cell subpopulations show altered levels of multipotent progenitor 4 (MPP4) subpopulation. Competitive transplantation of bone marrow shows increased engraftment and reconstitution of OPAL1-/- cells. Also, OPAL1 deficient mice have larger thymuses, higher number of thymocytes, and altered levels of the double negative and single positive subpopulations. For the mechanism of action, mass spectrometry and co-immunoprecipitation analyses show OPAL1 interaction with multiple NEDD4 family ubiquitin ligases and possible interaction with cullin-RING ubiquitin ligases (CRL). OPAL1 and CXCR4 co-expression shows OPAL1 regulating CXCR4 ubiquitination but OPAL1 seems to have also CXCR4-independent effects on hematopoiesis so different OPAL1 binding partners are being investigated to further explain its function.
Conclusions: Overall, OPAL1 seems to negatively regulate leukocyte hematopoiesis. Further investigation of the mechanisms and underlying pathways by which OPAL1 regulates hematopoiesis will have implications in clinical research.

microRNA-122 AND microRNA-1247 REGULATE THE PATHOGENIC PHENOTYPE OF EFFECTOR CD4+ T CELL SUBSETS IN (AUTO)IMMUNE RESPONSES IN VIVO

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Autoimmune diseases are often associated with an imbalance between CD4+ T cell subsets, namely pro-inflammatory effector cells, including T helper 1 (Th)1 and Th17 cells (IFN-γ- and IL-17-producers, respectively), and anti-inflammatory Foxp3+ regulatory cells (Treg). The differentiation these distinct CD4+ T cell subsets is known to be regulated by microRNAs (miRNAs), small non-coding RNAs that fine tune gene expression at the post-transcriptional level. While various individual miRNAs have been implicated in this process, a holistic approach focused on in vivo immune responses is missing to better understand how miRNA networks shape the CD4+ T cell compartment in pathophysiology.

To address this biological question, we established a triple reporter mouse for Ifng, Il17 and Foxp3, and subjected it to experimental autoimmune encephalomyelitis (EAE), a widely used rodent model of Multiple Sclerosis (MS). We performed miRNA-seq analysis on Th1, Th17 and Treg cells isolated from the spleen and lymph nodes (LNs) at the peak-plateau stage of EAE, and found 110 miRNAs to be differentially expressed between the effector and regulatory subsets. From these, we studied the functional role of 5 candidate miRNAs as they were specifically upregulated in one population versus the others. In vivo miRNA modulation showed that silencing miR-122 (upregulated in Th17 cells) increased the frequency of IL-17A+ cells in the LNs and precipitated the onset of EAE, whereas upregulation of miR-1247 (highly expressed in the Th1 subset) decreased the severity of the disease reducing the number of IFN-γ+ cells in the LNs. We further found that both IL-6 and TGF-β induce miR-122 expression, whereas IL-23 and IL-1β repress its expression. Given that IL-23 and IL-1β are critical to induce Th17-mediated pathogenicity, our data suggests that miR-122 is expressed in a non-pathogenic context. Interestingly, we have observed a pathogenic gene signature in CNS-derived Th17 cells (when compared to peripheral Th17 cells) with concomitant decreased levels of miR-122, suggesting that miR-122 may regulate Th17 pathogenicity. Similarly, we observed that once Th1 cells infiltrate the CNS, their levels of miR-1247 decrease and they produce higher levels of IFN-γ. Furthermore, as we found that this miRNA is induced by the anti-inflammatory cytokines IL-10 and TGF-β, we propose that miR-1247 constitutes an auto-regulatory mechanism of Th1 cells in the periphery, which is disrupted upon CNS infiltration.

Overall, our results suggest that miR-122 and miR-1247 control the pathogenic phenotype of effector Th17 and Th1 cells, respectively, during CNS autoimmunity. These findings may have important implications for autoimmune diseases, which we are now assessing in samples from MS patients.

MULTIPLE-WAY CHROMATIN CONTACT ANALYSIS SHOWED THAT Vα GENES OF T CELL RECEPTOR GENE TCRA COMPETED WITH Jα GENES FOR INTERACTING WITH THE ENHANCER Eα IN DP THYMOCYTES

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High diversity of antigen receptors was generated by V(D)J recombination of antigen receptor genes during T and B lymphocyte development. Usually, V gene segments of antigen receptor genes are far away from DJ gene segments, and spatial proximity is necessary for rearrangement. It has been shown that the enhancer Eα regulates accessibility of Vα and Jα genes by long-range interactions during Tcra rearrangements, but whether Eα mediates directly the interaction of Vα and Jα genes is still lacking evidence. Current 3C-based assays are useful in detecting the spatial proximity of two DNA segments, but it is still difficult to study the spatial proximity of three or more DNA segments. We analyzed 3C-HTGTS, a new one-to-genome interaction assay, and found that it contained sufficient information to analyze multiple-way interactions. We applied this technique to study the three-segment interactions between the proximal Vα gene region, the Jα gene, and Eα in DP cells. We observed a substantial interaction between the proximal Vα gene region and TEA on the Eα viewpoint. However, the viewpoint-SOI analysis showed that contacts with proximal Vα gene segments were significantly disfavored in conformations where the Eα viewpoint interacted with the proximal Jα region, and vice versa. The same competition was observed in the viewpoints of the Trav gene and TEA promoter. The result suggests that the Vα gene and the Jα gene compete for the enhancer Eα. Although Eα regulates both the accessibility of the Jα gene and the proximal Vα gene, it appears to be spatially separate. We analyzed multiple-way contacts in DP thymocytes of EACBE knockout mice, in which the CTCF binding site near Eα was deleted. We found that interactions of the proximal Vα gene region with Eα reduced, which puts the Vα gene segments at a disadvantage in competing with the Jα genes for contact with Eα. Our study reveals the mechanism of the enhancer Eα in Tcra rearrangement, which has implications for understanding the spatial organization of chromatin and gene regulation.

A SYSTEMIC AUTOINFLAMMATORY SYNDROME CAUSED BY NEMO EXON 5 SKIPPING

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NF-κB essential modulator (NEMO, encoded by IKBKG) is an essential gene in immune response, development, and cell death regulation. Mutations in NEMO have been associated with 3 Mendelian phenotypes with developmental defects and/or immunodeficiencies but a systemic inflammation phenotype was rarely reported. Here we identified a group of 20 patients (15 females and 5 males) with systemic inflammation caused by 10 different splice variants resulting in exon 5 skipping of NEMO. Common clinical features include panniculitis with systemic inflammation (100%), ectodermal dysplasia (83%), hepatosplenomegaly (77%) and B-cell lymphopenia (80%). We named this disease as NEMO deleted exon 5 autoinflammatory syndrome (NEMO-NDAS). Cell death was observed in skin and liver biopsies. Moreover, enhanced levels of soluble TNFR1 and TNFR2 were detected in serum compared to healthy controls, which further supported a role of cell death in the pathology. To understand the disease mechanism, U937 cell line with NEMO exon 5 skipping was created by CRISPR editing. This mutant cell line is highly susceptible to TNF induced cell death compared to the wildtype U937 cells. The cell death can only be partially rescued by RIPK1 inhibitor Nec1s but fully rescued by the combinations of Nec1s and CASP8 inhibitor Z-IETD-FMK, which indicates a RIPK1- and CASP8-dependence with additional factors. NEMO is the central hub regulating TNF-induced TBK1 activation, IKKα/β activation and NFκB activation, which are important cell death checkpoints in the TNF pathway. Applying inhibitor for one of the checkpoints can further enhance TNF-induced cell death in mutant U937 cells, which suggests NEMO-NDAS mutation only led to partial loss-of-function in the three cell death checkpoints. Combination of the three inhibitors, however, led to increased cell death in wildtype U937 cells, which is comparable to TNF-stimulated mutant cells without inhibitors and indicates that NEMO-NDAS mutations cause partial loss-of-function in NEMO-mediated TBK1 activation, IKKα/β activation and NFκB target gene expression, which is mimicked by combining the 3 respective checkpoint inhibitors. The cell death in mutant cells can be rescued by TNF inhibitor Adalimumab or anti-TNFR1 antibody in a dose-dependent manner and in some conditions can be further enhanced by co-administration of the RIPK1 inhibitor Nec1s. Our study showed that NEMO exon 5 deletion mutations in NEMO-NDAS patients lead to susceptibility to TNF induced cell death that is RIPK1- and CASP8-dependent and can be rescued by RIPK1 inhibitor, TNF inhibitor, and TNFR1 inhibitor, which provide novel therapeutic options for treating these patients. This work was supported by the NIH IRP of NIAID

IRF7 RESPONSE TO DECITABINE TREATMENT STRATIFIES ACUTE MYELOID LEUKEMIA PATIENT PROGNOSIS VIA INTERFERON-γ MEDIATED IMMUNE RESPONSE

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Decitabine is a DNA methyltransferase inhibitor which is FDA approved for treatment of myeloid malignancies. Despite its efficacy, the molecular mechanism of its induced response in blood cancers is not completely understood. In solid tumors, decitabine response has been attributed to upregulation of transposable elements which triggers viral defense signaling[1]. We study decitabine response in acute myeloid leukemia patients using single cell RNA sequencing of serial samples whilst these patients undergo decitabine treatment. Like solid tumors, decitabine treatment induces upregulation of transposable elements in cancerous cells. However, this upregulation does not stratify patients based on the clinical response. We found that expression of dsRNA receptor DHX9 is upregulated on decitabine treatment in both responsive and unresponsive patients, but interferon-regulator factor 7 (IRF7) showed higher increase in leukemic cells of post-treatment responders making them more conspicuous to immune surveillance. Trajectory analysis in leukemic cells along erythroid differentiation lineage indicated greater upregulation of MHC class II genes in differentiated cells in responding patients. It would contribute to increased recognition of these cells by the immune system and result in the observed greater reduction of these cells. The responding patients showed stronger upregulation of interferon-γ in cytotoxic T cells and natural killer cells. Corresponding interferon-γ response signal is also more enriched in leukemic cells of responders as well. All together our study supports the known drug action of transposable element upregulation on decitabine treatment and extends it to leukemias. However, it establishes the resulting immune response as the key factor which portends patient prognosis.

1. Chiappinelli, K.B., et al. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. Cell 162, 974-986 (2015).

DISSECTING THE IFNγ- VERSUS IL-17-SPECIFIC mRNAomes OF EFFECTOR γδ T LYMPHOCYTES

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The ability of murine γδ T cells to rapidly produce the pro-inflammatory cytokines interleukin-17 (IL-17) or interferon-γ (IFN-γ) underlies their crucial roles in several pathophysiological contexts, from infection to cancer or autoimmunity. This functional capacity stems from a complex process of 'developmental pre-programming' in the thymus, after which a significant fraction of γδ T cells migrate to peripheral sites already committed to producing either IL-17 (γδ17) or IFN-γ (γδIFN). While several studies have studied these γδ T cell subtypes using surface markers that enrich for effector function, we still lack a characterisation of the mRNA transcriptomes that specifically associate with IL-17 or IFN-γ production by γδ T cells. To overcome this limitation, in this study we established a double reporter IL-17-GFP:IFN-γ-YFP mouse strain, which allowed us to isolate pure IL-17+, IFN-γ+ and the remaining IL-17-IFN-γ- (DN) γδ T cell populations from the peripheral lymphoid organs in order to perform RNA-sequencing and identify the subset-specific mRNAomes.
Overall, we detected the expression of 12822 genes in γδ T cells, with a significant number of genes being enriched in γδ17 when compared with γδIFN and γδDN cells. Among these, 936 genes were differentially expressed between the three populations, with γδ17 and γδIFN cells displaying the most distinct mRNAomes, which highlights their functional specialization, and γδIFN being more similar to DN than γδ17 cells. Pathway and gene ontology analyses indicated that γδ17 cells differ from their IFN-γ-producing counterparts in their selective ability to sense and integrate external cues, whereas γδIFN cells stood out in replication, transcription and translation processes. These results highlight how IL-17 versus IFN-γ production is associated with substantially different cellular identities within the γδ T cell lineage. A more detailed analysis of the top 30 differentially expressed genes among the most expressed genes by γδ17 and γδIFN cells revealed that the majority of the signature genes increase their expression levels in the periphery upon their egress from the thymus, suggesting that these effector subsets only terminate their differentiation process at peripheral sites. Notably, γδ17- associated signature genes are specifically expressed in this subset, unlike γδIFN signature genes, which are also often expressed by γδDN T cells, thus suggesting a developmental relationship between these two subpopulations.
Collectively, our data allowed us to identify distinct mRNA signatures directly associated with cytokine expression in γδ T cells, several of which we are now further studying in disease models as to identify potential new roles in pathophysiology.

DEPLETION OF PTPN23 LEADS TO IMMUNOGENIC CELL DEATH IN ACUTE MYELOID LEUKEMIA (AML) CELLS.

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Tumor necrosis factor receptor superfamily members (TNFRSFs) have diverse functions from modulation of T cell activity to control cell survival and, therefore, integrate multiple cell types to initiate immune response against pathogens and malignant cells. In this study, we focused on spatiotemporal control of TNFR1 and showed its dysregulation led to immunogenic cell death in AML cells.

Using a CRISPR screen in AML cells, we identified PTPN23 (Protein Tyrosine Phosphatase Non-receptor type 23) as an essential requirement for cell survival. PTPN23 is an accessory protein for Endosomal Sorting Complex Required for Transport (ESCRT) machinery, which promotes lysosomal degradation of membrane proteins and restrains downstream signaling. Transcriptomic and biochemical characterization revealed that loss of PTPN23 resulted in activation of NF-κB and RIPK3, indicating aberrant activation of death receptors. In addition, we detected inflammasome formation and cleavage of CASP1 and GSDMD, suggesting the involvement of pyroptosis. Genetic deletion of CASP8, RIPK3, and CASP1/GSDMD, to block all three branches of PANoptosis (Pyroptosis, Apoptosis, and Necroptosis), rescued the lethality caused by PTPN23 depletion, thus illustrating regulation of PANoptotic cell death by PTPN23. The PANoptosis is a proinflammatory form of cell death, induction of which has been shown to contribute to protective antitumor immunity, and improve the effectiveness of cancer therapies.

To explore the mechanism of PTPN23 orchestrating multiple cell death pathways, we examined TNFR1, the prototypic death receptor, which has been implicated in the regulation of apoptosis, necroptosis, and pyroptosis. In the absence of PTPN23, we observed both TNF-α-dependent and -independent accumulation of TNFR1. Using the proximity-dependent biotin identification (BioID) approach, we demonstrated that adaptor protein NAP1 interacts with both PTPN23 and TNFR1, suggesting that PTPN23 regulates the sorting of TNFR1 via NAP1. Two ESCRT-accessory proteins, PTPN23 and ALIX, share similar domain organization, both containing a Bro1 domain, V domain, and Proline-Rich Region, but ALIX lacks a phosphatase domain. We show that the V domain confers specificity between PTPN23 and ALIX. The V domain of PTPN23 associates with UBAP1, an ESCRT-I component. Mechanistically, we propose a model in which PTPN23-dependent ESCRT function sustains cell survival through regulation of trafficking of the TNFR1. PTPN23 facilitates TNFR1 sorting, in a phosphatase activity-independent manner, through interaction with NAP1, and engagement of the ESCRT machinery, via UBAP1, to promote lysosomal degradation of TNFR1 and eliminate immunogenic cell death signaling.

GSK3β SHAPES DOUBLE NEGATIVE THYMOCYTES Tcrβ REPERTOIRE AND APOBEC3

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During thymocyte development, double negative (DN) thymocytes go through consecutive rounds of Tcrβ and Tcrα gene recombination that involve the generation of DNA double-strand breaks (DSB), the addition of N-nucleotides between coding ends, and DNA repair with the final goal of enhancing the diversity of the TCR repertoire. How DN thymocytes can survive such a genomic instability during this complex process and generate functional Tcrβ, and Tcrα genes remain unclear. Performing scRNA-seq analyses of isolated DN3 and DN4 thymocytes, we have shown a clear trajectory from DN3 through DN4 stage delineated by a G1 cell cycle checkpoint, but also by a G2M cell cycle checkpoint to help DNA repair during recombination of Tcrβ and Tcrα prior to cell proliferation. We have previously shown that inactivation of GSK3β by phosphorylation on Ser389 occurs selectively in response to DNA DSB to enhance survival during DNA repair. In this study, we show that failure to inactivate GSK3β by Ser389-phosphorylation during the transition from DN3 to DN4 stages compromises Tcrβ and Tcrα repertoire diversity. Inactivation of GSK3β during V(D)J recombination is needed to prevent cell death by necroptosis. Thus, cell cycle checkpoints to ensure proper DNA repair during V(D)J recombination, as well as the use of unique survival pathways to avoid necroptosis during this process, are critical to warrant a large diversity of pre-selection TCR repertoire.

SINGLE CELL BLOOD LEUKOCYTE SIGNATURE OF THE RHESUS CYTOMEGALOVIRUS-BASED VECTOR PROTECTION SIGNATURE OF SIV VACCINATION

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Nonhuman primate models of SIV infection are used to evaluate HIV-vaccine candidates expressing SIV antigens, facilitating in vivo efficacy studies of candidate HIV vaccine platforms. We have shown that approximately 50% of rhesus macaques (RM) receiving strain 68-1 rhesus cytomegalovirus (RhCMV)-based vaccine vector expressing SIV antigens are able to arrest and clear SIV infection from multiple virus challenges in a manner linked with antigen presentation by MHC-E . An interleukin (IL)-15 and immune activation signature including inflammatory, Toll-like receptor, and cell-death signaling programs in whole blood was linked with RhCMV/SIV vaccine protection via bulk mRNA sequencing. We found that genes in the IL-15 response signature are uniquely linked with the interface of innate and adaptive immunity, and were persistently induced/upregulated across the prime-boost vaccine regimen in animals that were protected from SIV infection. To identify the cells in whole blood that harbor this protective signature, we performed single cell RNA sequencing (scRNAseq) on RM PBMC isolated at baseline and pre-challenge (88 weeks post-prime immunization). Two cohorts of RMs were administered the 68-1 RhCMV/SIV vaccine that differed by inoculation route: group O was given the vaccine orally and group S was given the vaccine subcutaneously. A validation group of RMs (group X) who had previously received a modified/nonprotecting version of the vaccine (68-1.2 vector) lacking MHC-E restriction received the 68-1 vaccine subcutaneously, with 50% of the animals similarly protected from SIV infection. Cryopreserved PBMC from eight RMs (4 protected and 4 nonprotected) from each group were evaluated by scRNAseq. We found that genes in the protective IL-15 signature were primarily expressed in monocytes and NK cells. Differential gene expression (DE) analysis between pre-challenge and baseline time points for protected and non-protected cohorts and the validation cohort revealed that blood monocytes were the most transcriptionally responsive cell type to the vaccine, with a distinct set of significant DE genes present in protected animals across the three vaccine groups. These genes were enriched in pathways involved in cell activation and innate immunity within an overall response to IL-15. Thus, the 68-1 vaccine platform links MHC-E restriction and vaccine immunity with blood monocytes, implicating this cell subset in IL-15 production and/or response that imparts immune programming for vaccine protection.

PAG FACILITATES IMMUNE SYNAPSE FORMATION AND PD-1 FUNCTION IN T CELLS

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Cancer remains the second leading cause of death in the US. Immunotherapy seeks to bolster immune cells' ability to target malignant cells and has brought immense improvements in the field. One important inhibitory protein in T cells, Programmed Cell Death Protein 1 (PD-1), has become an invaluable target for cancer immunotherapy. While anti-PD-1 antibody therapy is extremely successful in some patients, in many others, it fails to help or causes complications, including cancer hyper-progression and immune-related adverse events. Study of the inhibitory transmembrane protein Phosphoprotein Associated with Glycosphingolipid Rich Microdomains 1 (PAG), a downstream target of PD-1 signaling, will help us better understand the PD-1 pathway, and offer another, perhaps more nuanced, target to potentially improve response rates and/or avoid immune-related adverse events. As a link between lipid-rich/signaling-protein-rich membrane regions and the actin cytoskeleton, PAG is an exciting and novel target for manipulating immune function. Prior therapeutic methods of immune manipulation all disrupt ligand binding or enzyme function. In contrast, innovative use of an anti-PAG antibody to simply disrupt appropriate PAG localization within the synapse could disturb immune synapse architecture. Synapse organization is tightly regulated to prevent inappropriate immune responses, but the precise interaction between cytoskeletal dynamics and synaptic organization is not fully understood. Investigating the role of PAG in this process provides added clarity. PAG mutated to prevent its link to actin results in disorganized actin architecture and PAG localization within the synapse. It also disrupts Ras signaling, an early signaling mediator downstream of TCR ligation. Determining which PD-1 downstream targets are dependent on the PAG-actin link will provide evidence for whether PAG and PD-1 could serve as good co-targets in cancer therapy regimens. Ultimately, this ongoing study hopes to illuminate crucial control mechanisms associated with T cell synapse organization, opening more avenues of targeting the immune synapse.

A SINGLE-CELL APPROACH TO TCR ACTIVATION IN RESPONSE TO PEPTIDES OF DIFFERING STIMULATION STRENGTH

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Cytotoxic T lymphocytes (CTLs) play a key role in the cell-mediated immune response against virally-infected and tumourigenic cells, killing their targets through the release of cytolytic granules. T cell receptor (TCR) recognition of foreign peptides presented by Class I MHC molecules stimulates naïve CD8+ T cell differentiation to effector cells and triggers the cytolytic activity of effector CTLs. Signal transduction downstream of the TCR is a highly diverse and coordinated network of post-translational protein modifications that ultimately drive transcriptional, translational, metabolic and cytoskeletal changes in the cell. How cytotoxic T cells coordinate their molecular machinery in response to strong versus weak stimuli remains unclear. Utilising single-cell methods including mass cytometry and single-cell RNA-sequencing, we demonstrate how naïve and restimulated cytotoxic T cells coordinate their responses against peptides of differing stimulation strengths.

THESE CELLS ARE ACTIN' WEIRDLY: HOW CDC42 REGULATES KILLER T CELL FUNCTION

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Cytotoxic T lymphocytes (CTLs) are key agents of the adaptive immune system responsible for the recognition and killing of oncogenic or infected cells. CTLs recognise, via their T cell receptors, foreign peptides presented on MHC class I molecules on the surface of target cells. The ensuing signalling cascade results in significant rearrangement of cellular architecture to deliver cytotoxic granules to the target. Key to this rearrangement is the actin cytoskeleton, of which there are many regulators that are known to give rise to primary immunodeficiencies when mutated in humans. One such regulator is cell division control protein 42 homolog (CDC42), a small Rho GTPase, which acts to promote actin filament branching via its activation of Wiskott-Aldrich syndrome protein (WASP), which in turn activates the actin-related protein 2/3 (ARP2/3) complex. CTLs from a Δ*CDC42* patient exhibit reduced killing capacity, the mechanisms for which are incompletely understood. Using CRISPR-cas9 gene editing to target *Cdc42* in primary mouse CTLs, we have examined the role of Cdc42 in the cytolytic function of CD8+ T cells. Using imaging, RNA-seq and FACS we see changes that suggest CDC42 acts to control not only cellular polarisation but also membrane protein trafficking and, via these mechanisms, impacts the ability of CTLs to kill their targets.

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VISITOR INFORMATION

EMERGENCY (to dial outside line, press 3+1+number)	
CSHL Security	**516-367-8870 (x8870 from house phone)**
CSHL Emergency	**516-367-5555 (x5555 from house phone)**
Local Police / Fire	**911**
Poison Control	**(3) 911**

CSHL SightMD Center for Health and Wellness Dolan Hall, East Wing, Room 111 **cshlwellness@northwell.edu**	**516-422-4422** **x4422 from house phone**
Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2000**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400**

GENERAL INFORMATION

Meetings & Courses Main Office
Hours during meetings: 9am – 9pm
After hours – See information on front desk counter
For assistance, call Security at 516-367-8870
(x8870 from house phone)

Dining, Bar
Blackford Dining Hall (main level):
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Blackford Bar (lower level): 5:00 p.m. until late

House Phones
Grace Auditorium, upper / lower level; Cabin Complex;
Blackford Hall; Dolan Hall, foyer

Books, Gifts, Snacks, Clothing
CSHL Bookstore and Gift Shop
516-367-8837 (hours posted on door)
Grace Auditorium, lower level.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail and printing in the business center area
WiFi Access: GUEST (no password)

Announcements, Message Board Mail, ATM, Travel info
Grace Auditorium, lower level

Russell Fitness Center

Dolan Hall, east wing, lower level
PIN#: **(from your room key envelope)**

Photocopiers, Journals, Periodicals, Books

CSHL Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Use PIN# (from your room key envelope) to enter Library after hours.
See Library staff for photocopier code.

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

Local Interest

Fish Hatchery	631-692-6758
Sagamore Hill	516-922-4788
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take CSHL Shuttle OR Uber/Lyft/Taxi to Syosset Train Station
Long Island Railroad to Penn Station (33rd Street & 7th Avenue).
Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681
Executive Limo Service	631-696-8000
Limos Long Island	516-400-3364
Syosset Taxi	516-921-2141
Orange & White Taxi	631-271-3600
Uber / Lyft	

Trains

Long Island Rail Road	718-217-LIRR (5477)
Amtrak	800-872-7245
MetroNorth	877-690-5114
New Jersey Transit	973-275-5555

Ferries

Bridgeport / Port Jefferson	631-473-0286
Orient Point/ New London	631-323-2525

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
British Airways	800-247-9297
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Southwest Airlines	800-435-9792
United	800-241-6522
Virgin American	877-359-9792

CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

Cold Spring Harbor Laboratory is dedicated to pursuing its twin missions of research and education in the biological sciences. The Laboratory is committed to fostering a working environment that encourages and supports unfettered scientific inquiry and the free and open exchange of ideas that are the hallmarks of academic freedom. To this end, the Laboratory aims to maintain a safe and respectful environment that is free from harassment and discrimination for all attendees of our meetings and courses as well as associated support staff, in accordance with federal, state and local laws.

By registering for and attending a CSHL meeting, either in person or virtually, participants agree to:

1. Treat fellow meeting participants and CSHL staff with respect, civility and fairness, without bias based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Use all CSHL facilities, equipment, computers, supplies and resources responsibly and appropriately if attending in person, as you would at your home institution.

3. Abide by the CSHL Meeting Alcohol Policy if attending in person.

Similarly, meeting participants agree to refrain from:

1. Harassment and discrimination, either in person or online, in violation of Laboratory policy based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Sexual harassment or misconduct.

3. Disrespectful, uncivil and/or unprofessional interpersonal behavior, either in person or online, that interferes with the working and learning environment.

4. Misappropriation of Laboratory property or excessive personal use of resources, if attending in person.

DEFINITIONS AND EXAMPLES

Uncivil/disrespectful behavior is not limited to but may take the following forms:

- Shouting, personal attacks or insults, throwing objects, and/or sustained disruption of talks or other meeting-related events

Harassment/discrimination is not limited to but may take the following forms:

- Threatening, stalking, bullying, demeaning, coercive, or hostile acts that may have real or implied threats of physical, professional, or financial harm
- Signs, graphics, photographs, videos, gestures, jokes, pranks, epithets, slurs, or stereotypes that comment on a person's sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or physical appearance

Sexual misconduct is not limited to but may take the following forms:

- Unwelcome and uninvited attention, physical contact, or inappropriate touching
- Groping or sexual assault
- Use of sexual imagery, objects, gestures, or jokes in public spaces or presentations
- Any other verbal or physical contact of a sexual nature when such conduct creates a hostile environment, prevents an individual from fulfilling their professional responsibilities at the meeting, or is made a condition of employment or compensation either implicitly or explicitly

REPORTING BREACHES OR VIOLATIONS

Cold Spring Harbor Laboratory aims to maintain in-person and virtual conference environments that accord with the principles and expectations outlined in this Code of Conduct. Meeting organizers are tasked with providing leadership during each meeting, and may be approached informally about any breach or violation. Breaches or violations should also be reported to program leadership in person or by email:

- Dr. David Stewart, Grace Auditorium Room 204, 516-367-8801 or x8801 from a campus phone, stewart@cshl.edu

- Dr. Charla Lambert, Hershey Laboratory Room 214, 516-367-5058 or x5058 from a campus phone, clambert@cshl.edu

Reports may be submitted by those who experience harassment or discrimination as well as by those who witness violations of the behavior laid out in this Code.

The Laboratory will act as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

Since many CSHL meetings and courses are funded by NIH grants, you may also contact the Health & Human Services Office for Civil Rights (OCR).

See this page for information on filing a civil rights complaint with the OCR; filing a complaint with CSHL is not required before filing a complaint with OCR, and seeking assistance from CSHL in no way prohibits filing complaints with OCR. You may also notify NIH directly about sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported events.

CSHL Campus Map

CSHL Map

COVID-19 POLICIES

Cold Spring Harbor Laboratory (CSHL) will make every effort to ensure the wellbeing of our participants and staff by following current federal and state health guidelines. Measures may include mandatory masking (except when eating or drinking), capping in-person attendance, staggered meal-times, spreading out indoor gatherings (such as poster sessions), and enhanced hygiene protocols.

VACCINATION

All CSHL meeting participants are REQUIRED to provide proof of full vaccination AND first booster (if eligible) with an FDA or EMA approved vaccine. For more information, see COVID-19 Policies and Protocols.

PRE-ARRIVAL TESTING

We ask that all incoming Meeting Participants obtain a negative COVID-19 test, administered within 72 hours of arrival (PCR) or 24 hours (antigen) OR Documentation of Recovery. A self-administered test kit is acceptable. If you test positive, please do NOT travel to CSHL.

TESTING AT CSHL

If you feel unwell during your stay, the CSHL Meetings & Courses office will provide a COVID-19 rapid antigen test kit. ***If you test positive:***

- Notify the Meetings & Courses Office
- You must **isolate for five (5) days**. We will provide you with a private room, box meals, and virtual meeting access
- Place a **privacy sign** on your door to alert housekeeping not to enter

516-367-8346 ▪ meetings@cshl.edu