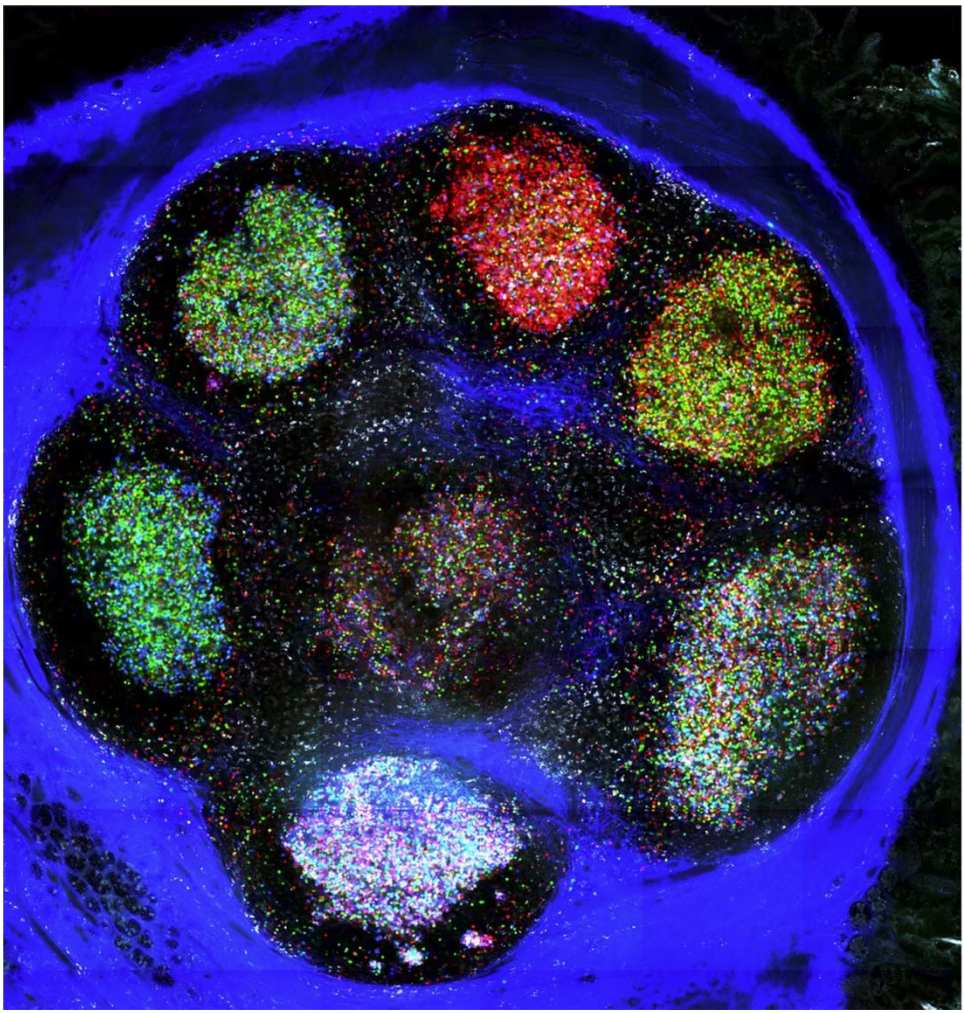
Abstracts of papers presented
at the 2026 meeting on

GENE EXPRESSION & SIGNALING IN THE IMMUNE SYSTEM

March 4–March 8, 2026



CSH Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2026 meeting on

GENE EXPRESSION & SIGNALING IN THE IMMUNE SYSTEM

March 4–March 8, 2026

Arranged by

Jon Kagan, *Boston Children's Hospital / Harvard Medical School*
Richard Locksley, *HHMI / University of California San Francisco*
Daniel Mucida, *The Rockefeller University*
Ellen Rothenberg, *California Institute of Technology*

CSH Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

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GENE EXPRESSION & SIGNALING IN THE IMMUNE SYSTEM
Wednesday, March 4 – Sunday, March 8, 2026

Wednesday	7:30 pm – 10:30 pm	**1** Regulation of Gene Expression
Thursday	9:00 am – 12:00 pm	**2** Cell Lineage and Ontogeny
Thursday	2:00 pm – 5:00 pm	**3** Tissue Immunology
Thursday	5:00 pm	*Wine & Cheese Party*
Thursday	7:30 pm – 10:30 pm	**Poster Session I**
Friday	9:00 am – 12:00 pm	**4** Signaling Intracellularly
Friday	2:00 pm – 5:00 pm	**5** Intercellular Communications
Friday	7:30 pm – 10:30 pm	**Poster Session II**
Saturday	9:00 am – 12:00 pm	**6** Host-Microbe Interactions
Saturday	2:00 pm – 5:00 pm	**7** Immune Responses
Saturday	6:00 pm	*Cocktails and Banquet*
Sunday	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

WEDNESDAY, March 4—7:30 PM

SESSION 1 REGULATION OF GENE EXPRESSION

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

Macrophage reprogramming regulated by temporal and combinatorial signaling codes
Alexander Hoffmann.
Presenter affiliation: UCLA, Los Angeles, California. 1

Not the old ImmGen anymore
Christophe Benoist.
Presenter affiliation: ImmGen Consortium, Boston, Massachusetts. 2

Molecular mechanisms driving αβ and γδ T cell development in human
Lena Boehme, Jolien Van Hulle, Yana Van Droogenbroeck, Imke Velghe, Tom Taghon.
Presenter affiliation: Ghent University, Ghent, Belgium. 3

Vitamin D receptor modulates NF-κB bandwidth to promote thymic epithelial diversity for immunological tolerance
Joshua McKeever, Jason A. Caldwell, Noah Gamble, Olubusayo Bolonduro, Marc Timmers, Michael Downes, Ronald M. Evans, Alexander Hoffmann, Andrew S. Koh.
Presenter affiliation: University of Chicago, Chicago, Illinois; University of Chicago, Institute for Biophysical Dynamics, Illinois. 4

Co-option of the chromosomal passenger complex for site-specific inflammatory gene regulation
Luca Frosio, Federica La Terza, Roza Maria Barouni, Simona Barresi, Stefano Volpi, Tom Fillot, Martina Fiumara, Andrew W. Daman, Steven Z. Josefowicz, Davide Mazza, Samuele Ferrari, Alessandro Aiuti, Luigi Naldini, Marco Genua, Renato Ostuni.
Presenter affiliation: IRCCS San Raffaele Institute, Milan, Italy. 5

Lmo2's native role impacts T cell developmental speed
Samantha J. Chang, Ellen V. Rothenberg.
Presenter affiliation: Caltech, Pasadena, California. 6

THURSDAY, March 5—9:00 AM

SESSION 2 CELL LINEAGE AND ONTOGENY

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

THURSDAY, March 5—2:00 PM

SESSION 3 TISSUE IMMUNOLOGY

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

Dynamic fibroblast-immune states and niches in injury and infection
Ari B. Molofsky.
Presenter affiliation: University of California, San Francisco, San Francisco, California. 30

Goblet-mimetic mTECs enforce central tolerance to peripheral-goblet-cell antigens
Yebin Wang, Chong Zuo, Brooke M. Huisman, Christophe Benoist, Diane Mathis.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 20

THURSDAY, March 5—5:00 PM

Wine and Cheese Party

THURSDAY, March 5—7:30 PM

POSTER SESSION I

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FRIDAY, March 6—9:00 AM

SESSION 4 SIGNALING INTRACELLULARLY

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

A genome-wide CRISPR screen identifies regulatory circuits that restrain TLR7-driven autoimmunity
Gregory M. Barton, Victoria E. Rael, Julian A. Yano, Leianna C. Slayden.
Presenter affiliation: University of California, Howard Hughes Medical Institute, Berkeley, California. 21

Detection of non-self nucleic acids by Toll-like receptors
Veit Hornung.
Presenter affiliation: Ludwig-Maximilians-Universität Munich, Munich, Germany. 22

FRIDAY, March 6—2:00 PM

SESSION 5 INTERCELLULAR COMMUNICATIONS

Chairperson: **Greg Barton,** University of California, Berkeley

FRIDAY, March 6—7:30 PM

POSTER SESSION II

See p. xxi for List of Posters

SATURDAY, March 7—9:00 AM

SESSION 6 HOST-MICROBE INTERACTIONS

Chairperson: **Dan Littman,** NYU School of Medicine, New York

Myeloid expression of mitochondrial genes shapes antimycobacterial immunity
Edwardo L. Martinez, Cory J. Mabry, Aja K. Coleman, Taylor Newbolt, Kristin L. Patrick, Robert O. Watson.
Presenter affiliation: Vanderbilt University Medical Center, Nashville, Tennessee. 35

Species-specific innate immune sensing and inflammatory signaling during Legionella infection
Dario S. Zamboni.
Presenter affiliation: Ribeirão Preto Medical School. University of São Paulo, Ribeirão Preto, Brazil. 36

Social sensing of infection reprograms peripheral immunity in healthy mice
Temitope W. Ademolue, Lena F. Pernas.
Presenter affiliation: UCLA, HHMI, Los Angeles, California. 37

Virus-like particles enable targeted gene engineering and pooled CRISPR screening in primary human myeloid cells
Pascal Devant, Hyuncheol Jung, Carter Ching, Mineto Ota, Jennifer Hamilton, Zachary Steinhart, Wayne Ngo, Luis Sandoval, Da Xu, Meirui An, Takuya Tada, James K. Nunez, Nathaniel R. Landau, David R. Liu, Justin Eyquem, Jennifer A. Doudna, Julia Carnevale, Alexander Marson.
Presenter affiliation: Gladstone Institutes, San Francisco, California. 38

SATURDAY, March 7—2:00 PM

SESSION 7 IMMUNE RESPONSES

Chairperson: **Daniel Mucida,** The Rockefeller University, New York, New York

SATURDAY, March 7—6:00 PM

COCKTAILS and BANQUET

SUNDAY, March 8—9:00 AM

SESSION 8 TISSUE-IMMUNE COMMUNICATIONS (IMMUNOPHYSIOLOGY)

Chairperson: **Ruslan Medzhitov,** Yale University School of Medicine, New Haven, Connecticut

POSTER SESSION I

POSTER SESSION II

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MACROPHAGE REPROGRAMMING REGULATED BY TEMPORAL AND COMBINATORIAL SIGNALING CODES

Alexander Hoffmann[1,2]

[1]UCLA, Microbiology, Immunology, and Molecular Genetics, Los Angeles, CA, [2]UCLA, Quantitative and Computational Biosciences, Los Angeles, CA

Immune sentinel cells must initiate the appropriate immune response upon sensing the presence of diverse pathogens or immune stimuli. To generate stimulus-specific responses, immune sentinel cells have evolved combinatorial and temporal signaling codes using a small number of stimulus-responsive transcription factors.

Our recent studies have characterized how the transcription factors NFκB and IRF/STAT cause large scale changes in the epigenetic enhancer landscape. We identified about 4000 genomic locations that acquire the long-lasting enhancer mark H3K4me1 in response to LPS exposure, but not when macrophages are exposed to immune cytokines IFNβ or TNF. I will describe how this specificity is mediated by temporal and combinatorial transcription factor control.

Half of the epigenomic locations are induced by NFκB, but only when NFκB activity is non-oscillatory. This occurs when it is induced via the MyD88 pathway but not in response to TNF. Using mathematical modeling we identified the mechanism by which nucleosome displacement can be regulated via a temporal code (Science, 372, pp.1349, Cell Reports, 40, 111076). The other half are induced by IRF transcription factors. We found that these locations are induced by LPS but not IFNβ, because IFNβ-activated ISGF3 must collaborate with NFκB-activated IRF1 which displaces nucleosomes. Hence, these locations are regulated by a combinatorial code of IRF/STAT family members (Science Signaling, 18, eado8860).

NOT THE OLD IMMGEN ANYMORE

Christophe Benoist

ImmGen Consortium, Dept of Immunology, Boston, MA

The immunological Genome Project is a collaborative group of Immunologists and Computational biologists who pool expertise and resources to perform a broad and deep dissection of the genome's activity and its regulation in the immune system of the mouse, at a scale that none of us could achieve alone. The project defines gene expression patterns and modules, regulatory motifs and structures in the genome, their connectivity, and the factors that control them. The scope of the effort covers all immunological lineages ex vivo, from mast to memory T cells, as shaped by differentiation, genetic variation, activation and challenges. These data support the computational reconstruction of the genetic regulatory network that underlies immunocyte differentiation and activation. Beyond the discoveries it makes, ImmGen is intended as a public resource. The data and metadata are accessible for direct visualization and exploration through tailored interfaces, aiming to serve knowledge, not merely facts.

We will highlight recent results achieved in the main OpenSource arms of ImmGen, and the tools that you can use to access them. ImmGenMaps, led by the Reina lab, employs multimodal spatial omics at subcellular resolution to map the immune system in all lymphoid and non-lymphoid organs in which immunocytes roam, to decode its wiring across anatomical compartments.

By scRNA/marker/TCR profiling of T cells in >250 organs and conditions, the immgenT Project led by the Zemmour lab shows that T cell diversity converges onto a finite set of recurrent molecular states reused across conditions and proposes a data-driven molecular classification of T cells. More than a change in terminology, immgenT provides a conceptual and practical foundation to frame T cell heterogeneity, which should facilitate communication and precise comparisons across studies,

Finally, we are working to re-generate the entire compendium of mRNA expression using long read sequencing, to establish the landscape of differential splicing, and analyze the "dark transcriptome" of exotic RNA species and endogenous retroviruses.

MOLECULAR MECHANISMS DRIVING αβ AND γδ T CELL DEVELOPMENT IN HUMAN.

Lena Boehme, Jolien Van Hulle, Yana Van Droogenbroeck, Imke Velghe, Tom Taghon

Ghent University, Department of Diagnostic Sciences, Ghent, Belgium

αβ and γδ T cells have distinct roles in the immune system but arise from common precursors in the thymus. Many details about their development are incompletely understood; in particular, how and when the αβ vs. γδ fate decision occurs during human T cell development is still being debated. Using bulk RNA- and ATACseq, we previously demonstrated that thymocytes committing to the γδ but not the αβ lineage undergo pronounced changes in chromatin accessibility. This suggests that the epigenetic drivers responsible for lineage specification may differ between the two developmental trajectories, but owing to the limitations of bulk analysis approaches, the underlying molecular processes have yet to be elucidated. To overcome these constraints, we have now used single cell sequencing technologies to build a high-resolution multi-modal map of human αβ and γδ T cell differentiation. This allowed us to profile gene expression, chromatin accessibility, surface marker levels and TCR locus rearrangement events along the two developmental trajectories to characterize lineage-specific changes in these modalities.

VITAMIN D RECEPTOR MODULATES NF-κB BANDWIDTH TO PROMOTE THYMIC EPITHELIAL DIVERSITY FOR IMMUNOLOGICAL TOLERANCE

Joshua McKeever[1], Jason A Caldwell[1], Noah Gamble[1], Olubusayo Bolonduro[1], Marc Timmers[2], Michael Downes[3], Ronald M Evans[3], Alexander Hoffmann[4], Andrew S Koh[1,5]

[1]University of Chicago, Pathology, Chicago, IL, [2]German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK) Partner Site Freiburg, Heidelberg, Germany, [3]Salk Institute for Biological Studies, Gene Expression Laboratory, La Jolla, CA, [4]University of California, Los Angeles, Microbiology, Immunology and Molecular Genetics, Los Angeles, CA, [5]University of Chicago, Institute for Biophysical Dynamics, Chicago, IL

Self-reactivity of the T cell repertoire established in the thymus varies across individuals to contribute to autoimmunity risk and host defense. Ectopic expression of tissue-specific genes in medullary thymic epithelial cells (mTECs) plays a central role; however, the determinants underlying the variability of this process remain unclear. Here, we investigated the influence of nuclear receptors that sense cell-extrinsic factors – such as nutrients and hormones – on the diversity of self-antigens expressed by mTECs for central tolerance induction. Using a single-cell multiomics approach, we identified unusually high vitamin D receptor (VDR) expression and activity levels in mTECs. Conditional deletion of *Vdr* limited transcriptional diversity across mTECs, causing select cohorts of tissue-specific genes to be prioritized at the expense of hundreds of others becoming silenced. This compromised mTEC diversity led to the escape of self-reactive T cells from thymic selection and multi-organ autoimmunity. Mechanistically, we found VDR-deficiency enhanced NF-κB activity in mTECs, particularly at putative enhancers near the 'prioritized' genes which were selectively enriched with NF-κB target motifs. Moreover, VDR directly bound to and repressed chromatin accessibility at these putative enhancers to inhibit NF-κB localization, allowing redistribution of NF-κB to alternative sites that increased the range of ectopic gene expression for central tolerance induction. Taken together, our findings define VDR as a modulator of mTEC transcriptional diversity with implications for a mechanistic link between vitamin D deficiency and susceptibility to autoimmune diseases.

CO-OPTION OF THE CHROMOSOMAL PASSENGER COMPLEX FOR SITE-SPECIFIC INFLAMMATORY GENE REGULATION

Luca Frosio[1,2], Federica La Terza[1], Roza Maria Barouni[1], Simona Barresi[1], Stefano Volpi[3], Tom Fillot[4], Martina Fiumara[1], Andrew W Daman[5], Steven Z Josefowicz[5], Davide Mazza[2,4], Samuele Ferrari[1,2], Alessandro Aiuti[1,2], Luigi Naldini[1,2], Marco Genua[1], Renato Ostuni[1,2]

[1]IRCCS San Raffaele Institute, SR-Tiget, Milan, Italy, [2]IRCCS San Raffaele Institute, Vita-Salute San Raffaele University, Milan, Italy, [3]IRCCS Giannina Gaslini Institute, Rheumatology and Autoinflammatory Diseases Unit, Genoa, Italy, [4]IRCCS San Raffaele Institute, Experimental Imaging Center, Milan, Italy, [5]Weill Cornell Medicine, Pathology and Laboratory Medicine, New York, NY

Innate immune cells arise through differentiation trajectories that involve proliferation and specialization of immature precursors. Upon reaching terminal differentiation, they acquire enhanced sensing and effector functions while entering a quiescent-like state. The mechanisms by which immune functions are integrated with proliferative signaling remain poorly understood. Here, we found that multiple inhibitors of mitotic Aurora kinases abrogate the induction of type I IFN transcriptional program in different mouse and human immune cells, and in septic mice. Furthermore, scRNA-Seq of PBMCs collected from patients with the interferonopathy SAVI showed a reduced IFN I signature in all immune cell types upon *ex vivo* treatment with the Aurora kinase inhibitor. By genetically depleting Aurora kinases, we revealed that Aurora B-, but not Aurora A-, KO macrophages are deficient in the induction of IFN I response. Stimulation with LPS increased Aurora B chromatin binding, hinting at a direct epigenetic control mechanism. Our ChIP-Seq analyses highlighted that H3S10ph—a modification attributed to Aurora B in mitosis—is an LPS- and IFN I-induced histone mark occurring at the gene body of specific genes concomitantly to transcription and histone acetylation, all being impaired upon Aurora B inhibition. Aurora B exerts its mitotic functions within the chromosomal passenger complex (CPC) together with INCENP, Survivin, and Borealin, whose localization on chromosomes is mainly driven by Haspin kinase-mediated H3T3 phosphorylation. Building on this, we found that both genetic depletion of CPC components and chemical perturbation of Haspin hinder transcriptional induction of IFN I response and H3S10 phosphorylation upon stimulation. We propose that upon differentiation and cell cycle exit macrophages, and virtually all immune cells, co-opt the activity of mitotic CPC to fine-tune inflammatory gene expression. In this scenario, stimulus-induced H3S10 phosphorylation by Aurora B represents a novel layer of gene regulation, possibly underlying the need of an immune cell to balance its proliferative potential with an adequate response to the environment.

LMO2'S NATIVE ROLE IMPACTS T CELL DEVELOPMENTAL SPEED

Samantha J Chang, Ellen V Rothenberg

Caltech, Biology & Biological Engineering, Pasadena, CA

The transcription factor (TF) Lmo2 is well known for its oncogenic role in T cell acute lymphoblastic leukemia (T-ALL), where its abnormal activation alongside other hematopoietic stem progenitor cell (HSPC)-associated factors causes T cell developmental arrest. However, normal T cell development emerges from an existing transcriptional program that is inherited from its HSPC past and includes Lmo2, begging the question: what has changed at later stages of T cell development such that the interactions between HSPC-program members and T cell program members which were once compatible with entry into T cell development are now detrimental to T cell development? To answer this question and reveal Lmo2's true contributions to T cell development, we performed knockout (KO) experiments using the CRISPR-Cas9 system in bone marrow-derived precursors. Using the PVA culture system to first expand the HSPC population, we perturbed cells at a timepoint where Lmo2 is highly expressed and then tracked T cell developmental progression using the canonical OP9-DLL1 co-culture system. Strikingly, we found that loss of Lmo2 alone caused a significant acceleration of the T cell program: while HSPCs grown in PVA culture normally take over a week to reach the DN2 stage on the OP9-Dll1 co-culture system, perturbed progenitors emerged at the DN2 stage as early as day 3 of co-culture and substantially by day 5 of co-culture. Bulk RNA-seq of DN1 stage cells at day 5 of co-culture confirmed this developmental acceleration on a transcriptional level, as the DN1 stage progeny of precursors with loss of Lmo2 were transcriptionally more similar to reference DN2 stage cells than to reference DN1 stage cells. To further uncover the molecular mechanisms impacted by Lmo2, we analyzed input populations and populations at day 3 of co-culture with single cell RNA-sequencing. Initial results reveal that the earliest changes caused by loss of Lmo2 include increased expression of Tcf7, Gata3 and Thy1, and activation of the TCRg and TCRb loci, consistent with bulk RNA-seq results, even before expression of T cell program members Bcl11b and Rag1. Lmo2 knockout input HSPC populations before Notch signaling did not differ from controls as dramatically as day 3 populations, indicating that Notch signaling may influence Lmo2's impact on development. This work opens the door for Lmo2 to be explored not just as a bystanding remnant of the HSPC program but as a direct and crucial player in the early T cell developmental network, even supporting a newfound role for Lmo2 as a kinetic controller of T cell development.

SIGNALING INDUCED BIOPHYSICAL DISRUPTION OF REPRESSED CHROMATIN DOMAINS DRIVES IMMUNE CELL FATE

Alexia Martínez de Paz[1], Ari Melnick[2], Christina Leslie[3], Steven Josefowicz[1]

[1]Weill Cornell Medicine, Department of Pathology and Laboratory Medicine, New York City, NY, [2]Josep Carreras Leukemia Research Institute, -, Barcelona, Spain, [3]Sloan Kettering Institute, Computational and Systems Biology Program, New York City, NY

Cell fate transitions require signal-induced chromatin derepression, yet mechanisms governing transitions from repressed to active chromatin states are poorly understood. We discover, at fate defining genes across immune cell types, a signal-induced histone code, and describe domains of H3 serine 28 phosphorylation (H3S28ph) spanning architectural features, often coincident with repressive H3 lysine 27 trimethylation (H3K27me3). Employing biophysical, single cell, and functional approaches to study signal-induced cell differentiation in the immune system, we uncover epigenomic transitions and cell fate choices precipitated by histone phosphorylation (H3ph). Mechanistically, H3ph overrides Polycomb Repressive Complex 2 (PRC2) chromatin repression, biophysically disrupts polynucleosome compaction, and promotes loss of H3K27me3, while increasing activating H3K27 acetylation and H3K36 dimethylation to drive domain interactivity and stabilize transcription. We demonstrate the activity of H3ph in several cell fate transitions and illuminate biophysical mechanisms enabling rapid signal-activated chromatin derepression, processes with general relevance for cellular differentiation and activation.

DEVELOPMENTAL MODULATION OF CER/SIS CONVERTS Igκ RAG-SCANNING FROM A TWO COHESIN LOOP-BASED TO A ONE LOOP-BASED MECHANISM FOR SECONDARY V(D)J RECOMBINATION

Xiang Li[1], Hongli Hu[1], Yiwen Zhang[1], Tammie Zhu[1], Ying Guan[1], Xin Lin[1], Camille Hebert[1], Himanshu Batra[1], Jenny Zhou[1], Zhaoqing Ba[1], Adam Yongxin Ye[1], Duane R Wesemann[2], Frederick W Alt[1]

[1]Boston Children's Hospital, Howard Hughes Medical Institute, Program in Cellular & Molecular Medicine, Boston, MA, [2]Brigham and Women's Hospital , Department of Medicine, Boston, MA

V(D)J recombination assembles *Igh* and *Igκ* variable region exons from hundreds of gene segments across mega-base *Igh* and *Igk* loci. V, D, and J segments are flanked by conserved recombination signal sequences (RSSs) that target RAG endonuclease. RAG orchestrates *Igh* V(D)J recombination in pre-B cells after capturing J_H-RSSs in the J_H-RSS-based recombination center (RC) and then scans upstream D- and VH chromatin linearly presented by cohesin-mediated loop extrusion. During scanning, RAG utilizes D- or V_H-RSSs in convergent (deletional) orientation with J_H-RSSs. *Igκ* contains four J_Hs (J_H1,2,4,5) and over 100 Vκs in clusters oriented for deletional or inversional joining. Primary Vκ-to-Jκ1 joining occurs first, with loop extrusion moving deletional and inversional oriented Vκs locus-wide past the Cer/Sis CBE-based diffusion platform for short-range diffusional presentation to the closely linked Jκ1-based primary RC. To achieve diffusion-mediated Vκ-to-Jκ1 joining, Igk evolved powerful Vκ and Jκ RSSs. Secondary Vκ rearrangements to Jκs2,4,5 replace primary VκJκ1 rearrangements, expanding Igκ repertoires and mediating central tolerance via receptor editing. We will present studies that elucidate the secondary Igκ recombination mechanism. Primary deletional and inversional VκJκ1 joins delete or displace Cer/Sis, creating a pre-B cell population that harbors secondary VκJκ1-based RCs across the Vκ locus and leaves most unrearranged Vκs immediately upstream of secondary RCs in deletional orientation. Thus, RAG scanning from secondary VκJκ1-based RCs, collectively, extends linearly across the Vκ locus in pre-B cell populations. Studies of iPSC-generated mouse models or cell lines with physiological VκJκ-rearrangements revealed that deletional and (originally) inversional Vκs are mostly captured by Jκ2-5-based secondary RCs in deletional orientation via linear RAG-scanning. Strong Vκ-RSSs contribute to restricting secondary (editing) rearrangements to Vκs immediately upstream of the RC and support low level linear scanning-based inversional Vκ-to-Jκ joining. We implicate Cer/Sis deletion/displacement as a developmental switch that converts the two-loop-based diffusional primary Igκ rearrangement mechanism into a one-loop-based linear scanning secondary mechanism. Additional new studies implicate two pairs of cohesin complex factors in differentially regulating *Igh* versus Igκ long-range V(D)J recombination.

ROLES OF RUNX PHOSPHORYLATION AND LCK INTERACTOME IN THYMOCYTE LINEAGE COMMITMENT

Chihiro Ogawa, Kazuki Okuyama, Junji Harada, Ichiro Taniuchi

RIKEN IMS, Lab for Transcriptional Regulation, Yokohama, Japan

MHC-I and MHC-II selected $CD4^+CD8^+$ precursor thymocytes differentiate into $CD4^-CD8^+$ cytotoxic- and $CD4^+CD8^-$ helper-lineage T cells through induction of lineage-determining transcription factor Runx3 and Thpok, respectively. Runx proteins play a crucial role in this lineage choice by suppression of *Cd4* and *Thpok* gene specifically in cytotoxic-lineage cells via activating their silencers. The evolutionarily conserved WRPY motif at the C-terminus end of Runx family proteins is essential for interaction with TLE co-repressors. We showed that a single amino acid subsitution of the terminal tyrosine (Y) to tryptophan (W) or phenylalanine (F) converts Runx proteins into dominant repressors, re-directing MHC-II selected thymocytes toward the $CD4^-CD8^+$ cytotoxic lineage. Notably, the terminal Y residue of Runx1 is more phosphorylated in $CD4^-CD8^+$ thymocytes and promotes Runx-TLE interaction, indicating that phosphorylation status at this site functions as a molecular switch linking MHC restriction to CD4/CD8 lineage choice. We identified interactions between Runx proteins and Lck/Zap70 in the cytoplasm. Using in vivo proximity-dependent labeling assay, we found that TCR signaling molecules such as LAT associate more frequently in MHC-I signaled $CD4^-CD8^+$ thymocytes than in $CD4^+CD8^-$ thymocytes, suggesting that MHC-I engaged TCR stimulation promotes the formation of a stable signalosome. We are currently investigating how the Lck interactome is remodeled upon by CD4/CD8 co-receptor dissociation and exploring the functional impact of additional tyrosine phosphorylation sites in Runx proteins.

MATERNAL-OFFSPRING IMMUNE PARTNERSHIPS

Ai Ing Lim[1,2]

[1]Princeton University, Molecular Biology, Princeton, NJ, [2]Howard Hughes Medical Institute, Chevy Chase, MD

Mothers and offspring are evolutionary partners. From gestation through lactation, mothers undergo profound immunological transformations that enable tolerance of semi-allogeneic offspring while maintaining protection against environmental challenges. Epidemiological studies increasingly show that maternal exposures, from pathogens to pollutants, can shape offspring immune development, leaving lasting imprints on susceptibility to infection and inflammation. In this talk, I will present our recent findings showing that, despite an overall trend toward systemic immunosuppression during reproduction, maternal barrier tissues undergo remodeling to enhance innate defense against pathogens, a strategy that may have evolved to protect both mothers and offspring in pathogen-rich environments. I will also discuss how maternal helminth infections can promote offspring antiviral immunity through a microbiota-dependent mechanism.

DECODING NAÏVE CD8+ T CELL HETEROGENEITY

Connor Kean[1], Norah L Smith[2], Elizabeth A Fogarty[1], Andrew Grimson*[1], Brian D Rudd*[2]

[1]Cornell University, Department of Molecular Biology and Genetics, Ithaca, NY, [2]Cornell University, Department of Microbiology & Immunology, Ithaca, NY

One of the most enduring mysteries in immunology is why some naïve CD8+ T cells become effectors and die after infection while others survive and become memory cells. The current dogma suggests that a single subset of naïve CD8+ T cells can give rise to various types of effector and memory T cells, depending upon cues encountered during infection (inflammation, antigen). However, our group and others have discovered subsets of naïve CD8+ T cells that have predictable behavior during infection, suggesting cell fates may also be determined prior to infection. In this study, we used paired single cell RNA/TCR-seq to comprehensively characterize the heterogeneity present in murine naïve CD8+ T cells. We uncovered eight transcriptionally distinct subsets of naïve CD8+ T cells and performed CITE-seq and flow cytometry to identify surface markers that could be used to sort purify different subsets. We also investigated the key intrinsic factors (age, developmental origin, TCR usage, and host genetics) that determine the overall abundance of naïve CD8+ T cells and examined how their composition changes after infection. Collectively, our findings reveal the causes and consequences of naïve CD8+ T cell heterogeneity and point towards a new model of T cell diversification after infection.

* Co-senior authors.

WEANING ESTABLISHES A DEVELOPMENTALLY PROGRAMMED CYTOTOXIC CD4$^+$ T CELL COMPARTMENT IN THE INTESTINE

Rohit Gokhale*[1], Marina Schernthanner*[2], Alexandra Thoms[1], Gabriella Reis[1], Marwa Saad[1], Izabela Mamede[1], Sandra Yin[1], Roham Parsa[1], Elaine Fuchs[2,3], Daniel Mucida[1,3], Angelina Bilate[1]

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Intestinal immunity dysregulation contributes to chronic inflammatory diseases, including food allergies and inflammatory bowel disease. Gut-resident lymphocytes are essential for maintaining tolerance to luminal antigens while providing resistance against pathogens. However, how early-life T cell responses shape long-term intestinal immunity remains poorly defined. We investigated how T cells recruited to the intestine during postnatal development contribute to the composition and function of the adult gut immune system. By combining genetic fate mapping with single-cell RNA sequencing and TCR repertoire analysis, we tracked the recruitment, localization, and persistence of mouse T lymphocytes in the intestinal epithelium (IE) and lamina propria (LP) across suckling, weaning, and adulthood. T cell migration to the gut was highest shortly after birth and during weaning and declined progressively with age. Suckling was characterized by an increased proportion of naïve CD4$^+$ T cells in both the IE and LP, followed by the accumulation of activated CD4$^+$ T cells during weaning. Disruption of gut-associated lymphoid tissue formation reduced the representation of naïve T cells and promoted the accumulation of T-bet$^+$ T cells, suggesting that Peyer's patches and isolated lymphoid follicles act as reservoirs of naïve T cells in early life. T cells recruited during suckling or weaning persisted until adulthood but exhibited age-dependent spatial and functional specialization. T cells localized preferentially to crypts during suckling, whereas post-weaning T cells redistributed along the villi in a microbiota-dependent manner. At weaning, LP T cells displayed transcriptional programs associated with both cytotoxicity and tissue remodeling, consistent with controlled inflammatory response under homeostatic conditions. Clonal expansion of CD4$^+$ T cells occurred predominantly in the LP during weaning but shifted to the IE during adulthood, indicating age-dependent compartmentalization of adaptive immune responses. Expanded weaning clones were largely granzyme B–expressing and recognized antigens derived from maternal milk and early-life gut microbiota. Together, these findings reveal that weaning represents a regulated, infection-like immune state that enables functional maturation of intestinal T cell immunity without compromising tissue integrity.

NOVEL UNCONVENTIONAL T CELL SUBSET RECOGNIZING MYCOBACTERIAL ADJUVANT

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Unconventional T cells recognize non-peptidic metabolite antigens presented by monomorphic antigen-presenting molecules and respond rapidly to pathogens and altered self, thereby bridging innate and adaptive immunity. We identified a novel $CD4^+$ cytotoxic T cell subset shared across humans that recognizes the mycobacterial adjuvant, trehalose monomycolate (TMM), which is a ligand for the innate immune receptor Mincle. These TMM-specific T (T_{TMM}) cells are restricted by the monomorphic CD1 molecules, CD1b. Using TMM-loaded CD1b tetramers, T_{TMM} cells were detected in the thymus, PBMCs and cord blood from uninfected donors, and expanded following mycobacterial infection and produced bactericidal effectors. Cryo-EM analysis of five distinct TCR-TMM-CD1b ternary complex structures suggest a molecular basis for the thymic selection of TCRs specific for “previously unencountered” mycobacterial antigens. Together, we present immunological and structural insights into the development and function of this newly identified unconventional T cell subset, T_{TMM} cells.

GATA3 AND MEIS1 REGULATE FIRST-WAVE FETAL T-CELL DEVELOPMENT

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Developmental processes such as cellular differentiation are directed by hierarchical and deterministic gene regulatory networks (GRNs). Variations in lineage potential between progenitors and subsequent developmental outcomes are caused by differences in the underlying GRN. T-cell development is notable for shifts in lineage bias and developmental kinetics throughout the lifespan of the individual. The first wave of T-cell development in the fetal thymus is biased toward the generation of innate-like T cells—including cells bearing invariant γδTCRs that are only ever generated in the fetal thymus from first-wave progenitors—and has expanded potential to develop into ILC2 and LTi cells. Therefore, we have sought to define which aspects of the developmental GRN are different between fetal and adult thymocytes, and how these differences impact ultimate cell fate. Using ATAC-seq and scRNA-seq of fetal and adult thymocytes, we found that the T-cell specification program is launched from different initial regulatory states in fetal and adult progenitors, and that many of these differences are perpetuated through the transcriptional states associated with T-lineage commitment. Although the expression pattern of the main drivers of T-lineage specification are largely consistent, fetal progenitors have a substantial increase in Gata3 expression prior to T-lineage commitment at both the RNA and protein level. Alongside this increase in Gata3 is the activation within DN thymocytes of many of its targets in ILC differentiation, most notably PLZF (*Zbtb16*), and the efficient repression of many core factors within the inherited stem/progenitor program, including known epistatic repression targets of Gata3: *Lmo2*, *Mef2c*, *Bcl11a*, and PU.1 (*Spi1*). This, combined with a massive enrichment of GATA motifs within fetal-specific open chromatin regions and Gata3's known role in ILC specification, suggests that Gata3 is a dose-dependent regulator of T/ILC balance and T-lineage developmental kinetics. Aside from TFs with known roles in T-cell development, there were several factors unique to fetal thymocytes, or whose expression pattern differed between fetal and adult progenitors. One such factor, Meis1, is part of the inherited stem/progenitor program and its expression is turned off early in the ETP stage in adult progenitrs. However, fetal thymocytes retain *Meis1* expression through T-lineage commitment, despite turning off its primary cofactor in hematopoiesis, Hoxa9. Loss of Meis1 causes an acute survival defect in fetal liver progenitors when co-cultured with OP9-DLL1 cells, and experiments are ongoing to determine how Meis1 fits into the overall GRN of fetal T-lineage specification.

PERIPHERAL NEUROIMMUNE ECOSYSTEMS

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Neuroimmune interactions play critical roles in tissue defence and organ homeostasis. Neuronal and immune cell subsets can colocalise in discrete tissue environments, forming neuroimmune cell units that constitute the basis for bidirectional interactions, driving coordinated neuroimmune responses to local and systemic challenges. Here, we will discuss how neuroimmune circuits integrate and interact with other body systems to regulate organismal physiology and immunity.

IL-22 AS AN ORCHESTRATOR OF THE GUT-PANCREAS AXIS

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The intestinal microbiome and immune system do not only affect the gut but can also either prevent or accelerate pathology in distal organs. Here we describe that intestinal microbe-induced IL-22 signaling in the pancreas shifts this organ's function from digestion to antimicrobial defense and cellular regeneration, which can be fatal in the context of pancreatic cancer. Intestinal control of pancreatic immunity via IL-22 represents an unsuspected mechanism of gut-pancreas crosstalk and pancreatic disease etiology.

TUFT CELLS AS EPITHELIAL SENTINELS IN PEYER'S PATCH IMMUNE SURVEILLANCE

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The intestinal epithelium coordinates immune surveillance through specialized epithelial subsets that monitor luminal environments and instruct underlying immune responses. Tuft cells are chemosensory epithelial cells best known for initiating type 2 immune circuits in the villus epithelium through IL-25–dependent activation of innate lymphoid cells. Whether tuft cells contribute to immune surveillance at mucosal lymphoid interfaces has remained unclear.

Here we identify tuft cells within the follicle-associated epithelium (FAE) of Peyer's patches, the small intestine's organized lymphoid structures specialized for antigen sampling. FAE-associated tuft cells are transcriptionally and morphologically similar to villus tuft cells but are positioned within a distinct epithelial architecture enriched for immune–epithelial interactions. Their localization places a canonical chemosensory epithelial program adjacent to antigen-sampling M cells within a lymphoid surveillance domain.

Together, these findings suggest that Peyer's patches coordinate immune surveillance through multiple epithelial cell types, with tuft cells and M cells each employing specialized sensing programs that support complementary immune functions. This work extends the role of tuft cells beyond villus-associated type 2 immunity and highlights epithelial positioning as a key determinant of immune surveillance in the gut.

EFFECTOR IDENTITY AND TISSUE TROPISM OF HUMAN POSTNATAL NON-Vγ9Vδ2 γδ T CELLS ARE ESTABLISHED DURING INTRATHYMIC DEVELOPMENT

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γδ T cells are an unconventional subset of the T lineage that, in humans, are broadly divided into innate-like Vγ9Vδ2+ and non-Vγ9Vδ2 subsets with more adaptive-like roles. It is well known that murine γδ T cells acquire effector functions intrathymically prior to peripheral emigration. In humans, however, intrathymic effector programming has been proposed to be largely restricted to the fetal period and to a small subset of postnatally arising Vγ9Vδ2+ T cells.

Using single cell multiomics, we were able to show that the human infant thymus contains mature non-Vγ9Vδ2 γδ T cells that undergo intrathymic effector programming. In-depth analysis revealed multiple discrete subsets with unique transcriptional programmes associated with cytolytic, NK-like and tissue-repair functions. 166-plex CITE-seq further identified discrete phenotypic signatures for the individual subsets, which we used to design a flow cytometry panel for functional ex vivo characterisation. Corresponding γδ T cell populations were readily detected among primary thymocytes and displayed subset-specific expression of key transcription factors, corroborating the scRNA-seq results. Functionally, most subsets were able to produce cytokines and cytolytic mediators upon stimulation, indicating their advanced maturation status and effector functionality. Notably, integration of our thymic data set with published single cell data of γδ T cells from various peripheral tissues revealed that the thymic subsets can be robustly detected outside of the thymus. Strikingly, the different populations were found to exhibit distinct patterns of organ colonisation, which suggests that both effector identity and tissue tropisms are already established during intrathymic development.

Our work demonstrates that postnatal non-Vγ9Vδ2 T cells can adopt full effector capacity as part of their intrathymic developmental trajectory and comprise discrete lineages that colonise distinct tissues and likely carry out diverse functions associated with wound healing and pathogen defence.

TYPE I INTERFERON SIGNALING IN BRAIN HOMEOSTASIS AND PATHOLOGY

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Interferons (IFN) are canonically involved in antiviral responses but increasingly appreciated to have homeostatic and developmental roles, including in the brain. We described a population of Type I interferon-responsive microglia in the developing murine brain that were actively engulfing a subset of neurons, and were required for healthy brain development and behavior (Escoubas et al. Cell 2024). These microglia emerge in response to MAVs-dependent sensing of dsRNA from neurons, expand in the setting of developmental stress, suggesting that nucleic acid sensing by microglia is a molecular pattern that drives IFN-I responses during healthy development. In ongoing work, we are examining the role of the IFN-pathway and IFN-responsive in behavioral disturbances that arise after early life viral infection, a major risk factor for neuropsychiatric diagnoses later in life. We are also examining this pathway in models of neurodevelopmental disease, and have developed mouse and zebrafish tools that enable detection of IFN-responsive cells across tissues.

GOBLET-MIMETIC mTECs ENFORCE CENTRAL TOLERANCE TO PERIPHERAL-GOBLET-CELL ANTIGENS

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Goblet cells are essential modulators of mucosal barrier immunity, dynamically responding to microbial and inflammatory stimuli. How immunological tolerance to goblet-cell-antigens is established and maintained remains unclear but its importance is underlined by the fact that autoantibodies against goblet cells are a hallmark of inflammatory bowel diseases (IBD). Single-cell transcriptomic and histological analyses of medullary thymic epithelial cells (mTECs) revealed an mTEC subtype that transcriptionally and morphologically mirrored peripheral goblet cells. Lineage-tracing analyses indicated that these thymic goblet–mimetics appear to differentiate through an Aire^{+} mTECs. Specific ablation of goblet mTECs by thymus-specific knockout of Nlrp6 led to the spontaneous emergence of autoantibodies against goblet cells, accompanied by T cell infiltration of goblet-cell–enriched tissues such as the colon and lung. Serum immunoprecipitation followed by mass spectrometry revealed that some of the autoantibodies targetted mucus-associated proteins that are induced in mature goblet cells in response to microbes. In contrast, deletion of Aire^{+} mTECs did not elicit comparable autoreactivity to goblet-cell antigens. Thus, thymic goblet mimetics are specialized in tolerizing to a set of goblet self-proteins that are abundantly induced in peripheral tissues in response to microbes and are not adequately represented in Aire^{+} mTECs. To our knowledge, this is the first example of a functionally critical "division of labor" between Aire+ mTECs and mimetic mTECs in T-cell tolerization. Ongoing and future studies aim to assess the overlap of autoantigen targets between mice lacking goblet mTECs and humans with IBDs, and to elucidate their relevance to disease pathogenesis.

A GENOME-WIDE CRISPR SCREEN IDENTIFIES REGULATORY CIRCUITS THAT RESTRAIN TLR7-DRIVEN AUTOIMMUNITY

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Nucleic acid-sensing Toll-like receptors (TLR) 7/8 and 9 enable broad recognition of pathogens while also posing the risk of self-detection. Despite the structural and functional similarities between these receptors, the single-stranded RNA sensor TLR7 is distinctive in its capacity to drive autoimmunity. For example, a single gene duplication of TLR7 is sufficient to drive spontaneous lupus in mice, whereas overexpression of TLR9 has no effect. Recent studies from our group and others have identified human patients with monogenic systemic autoimmunity caused by hyperactivation of TLR7. Cumulatively, these studies underscore the importance of tightly regulating TLR7 activation. Here, we report an unbiased genome-wide CRISPR-KO screen in murine macrophages designed to reveal novel negative regulators of TLR7. Validated negative regulators of TLR7 include numerous pathways responsible for biogenesis, trafficking, and cargo sorting within the endolysosomal network. Strikingly, nearly all validated negative regulators of TLR7 appear to oppositely regulate TLR9, with most genetic backgrounds tested conferring TLR9 hypo-responsiveness. Cumulatively, our results support a model wherein multiple core cell biological pathways enforce a regulatory axis that favors select nucleic-acid sensing TLRs at the cost of others. Our mechanistic studies indicate that the array of negative regulators we have uncovered act through distinct mechanisms to fine-tune TLR7 signalling, with some genes altering receptor levels and localization during homeostasis, while others may restrict TLR7 dynamically during receptor activation.

DETECTION OF NON-SELF NUCLEIC ACIDS BY TOLL-LIKE RECEPTORS

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A central function of the innate immune system is the detection of microbial pathogens through their nucleic acid genomes or through signatures of transcriptional and replicative activity. In mammals, this task is mediated by pattern recognition receptors (PRRs) that enable the discrimination of non-self from self nucleic acids. Over the past years, substantial progress has been made in defining the sensing and signaling pathways that underlie nucleic acid recognition, with Toll-like receptors (TLRs) representing the first class of PRRs identified to function as nucleic acid sensors. TLRs are transmembrane receptors whose ligand-binding domains face either the extracellular space or the lumen of intracellular compartments. A distinct evolutionary subset of TLRs localizes to the endolysosomal compartment, including human TLR7, TLR8, and TLR9. While TLR9 recognizes single-stranded DNA enriched in unmethylated CpG motifs, features that are suppressed in the host genome, TLR7 and TLR8 have evolved to detect RNA-derived ligands. In the endolysosomal compartment, both exo- and endonucleases play a critical role in processing RNA into fragments that can be sensed by these receptors. Importantly, these nucleases do not merely function as degradative enzymes but actively shape ligand availability and specificity through substrate preference and the generation of defined RNA fragments. Despite extensive work on RNA-sensing TLRs, how these receptors reliably discriminate between non-self and self RNA remains incompletely understood, particularly given the abundance of host RNA within endolysosomal compartments. In this talk, I will provide an update on our recent work and present new insights into how endolysosomal RNA processing and receptor recognition are functionally coupled, and how TLR7 and TLR8 have evolved to sense these nuclease-generated ligands to achieve self–non-self discrimination.

CONSTITUTIVE IFN-KAPPA PRODUCTION BY EPIDERMAL KERATINOCYTES PROVIDES BROAD CUTANEOUS ANTIVIRAL PROTECTION VIA CANONICAL AND NON-CANONICAL MECHANISMS

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Type I interferons (IFNs) are essential cytokines for antiviral immunity, with some family members produced in response to infection and others, such as IFN-kappa (IFN-κ), constitutively expressed in specific cell types. While the antiviral role of inducible type IFNs (e.g., IFN-α/β) is well recognized, the function of tonic IFN-κ production in human skin remains unclear. Here, we used CRISPR/Cas9 gene editing and bulk RNA sequencing to demonstrate that constitutive *IFNK* expression in human epidermal keratinocytes drives a distinct transcriptional signature compared to other type I IFNs. Keratinocytes lacking *IFNK*, but not *IFNB1*, displayed markedly increased susceptibility to diverse cutaneous viruses, including vesicular stomatitis virus (VSV), Venezuelan encephalitis virus (VEEV), and herpes simplex virus 1 (HSV-1). Control of VSV by IFN-κ required canonical JAK-STAT signaling, while IFN-κ-mediated protection against HSV-1 was independent of TYK2, JAK1, and STAT1/2, indicating a non-canonical mechanism of restriction. This non-canonical antiviral effect of keratinocyte-derived IFN-κ extended to dermal fibroblasts in a human skin organoid model of HSV-1 infection. Our results together establish IFN-κ as a key mediator of broad antiviral defense in human skin through both canonical and non-canonical mechanisms.

TREGS SELECTIVELY RESTRICT CELLS PRESENTING THEIR COGNATE ANTIGEN

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Peripheral regulatory T cells (pTregs) are essential for restraining effector CD4 positive T cell responses and maintaining immune homeostasis. Effector mechanisms such as Il10 secretion, Il2 sequestration and CTLA4 mediated reduction of co-stimulation are known to contribute to Treg function. These mechanisms were long thought to act in a bystander manner, largely based on in-vitro studies that suggested that Treg cells suppress nearby cells irrespective of antigen specificity. Recent work indicates instead that Treg cells may act selectively on cells that present cognate antigen through immune synapse interactions. Yet, a systematic dissection of which suppressive functions rely on immune synapse interactions has remained out of reach.

I will present a new approach to address this question, which involves the generation of chimeric mice composed of two donor populations with distinct MHC class II loci, IA-b and IA-d/IE-d, termed MHChimeric mice. Because a T Cell Receptor (TCR) recognizes antigen only in the context of an MHC class II molecule, a T cell is expected to form primary and subsequent immune synapse interactions exclusively with cells that share the same MHC haplotype. Therefore, these chimeric mice are designed to contain two parallel antigen specific networks between T cells and antigen presenting cells.

We and others recently discovered that intestinal pTreg differentiation is mediated by RORγt+ antigen presenting cells. Building on this, we have now generated mutant MHChimeric mice in which IA-b positive donor cells lack the ability to present antigen in RORγt+ cells. One network is therefore deprived of pTreg cells, while the other remains intact. This strategy provided us with a unique opportunity to tackle the long standing question of how Treg cells execute their suppression in-vivo and whether their activity relies on immune synapse interactions. For the first time, we now have strong evidence showing that Treg cells suppress inflammatory responses with remarkable precision, acting only on cells that present their cognate antigen while leaving others unrestricted.

IFNγ-INDUCED MEMORY IN HUMAN MACROPHAGES IS NOT SUSTAINED BY EPIGENETIC CHANGES BUT THE DURABILITY OF THE CYTOKINE'S SIGNALING ITSELF

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Macrophages, as key sentinel cells of the innate immune system, can retain memory of prior stimulus exposure. Interferon gamma (IFNγ) plays a central role in maintaining trained immunity in vivo and can induce potent memory in macrophages. Such memory is associated with the formation of de novo enhancers that alter gene expression responses to subsequent stimuli. However, how such enhancers are maintained after cytokine exposure remains unclear. We report that durable IFNγ-induced enhancers can last for days after cytokine washout, yet the underlying persistence mechanism is not cell-intrinsic. IFNγ-treated macrophages continue to exhibit JAK/STAT signaling days after cytokine removal. Blocking IFNγ signaling with a JAK inhibitor or anti-IFNγ neutralizing antibodies after cytokine removal is sufficient to reverse IFNγ-induced enhancers and erase the potentiated state of the treated macrophages. Our findings suggest that epigenetic changes in macrophages do not inherently encode innate immune memory or a "potentiated" macrophage state, but in fact are themselves dependent on ongoing signaling from cytokines sequestered at the cell surface. These findings suggest new possibilities for pharmacologic interventions to reverse aberrantly trained immune states associated with pathology.

pH-DEPENDENT INNATE IMMUNE CIRCUITRY CONFERS TOLERANCE TO SYSTEMIC INFLAMMATION

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PH homeostasis is essential for physiological function, and disruptions in pH are a common feature of inflammation and disease. Immune cells monitor pH changes through specialized receptors, including the proton-sensing G-protein-coupled receptor GPR65. It is widely expressed on tissue-resident immune cells and detects subtle pH variations within the physiological range (pH 7.0-7.4).
We identify GPR65 as a critical regulator of tolerance to systemic inflammation. GPR65-deficient mice exhibit markedly increased lethality following systemic bacterial infection, despite normal pathogen burden and clearance. This heightened sensitivity is recapitulated during LPS-induced sepsis. We found that late-phase IL-17A production from peritoneal γδ T cells is both necessary and sufficient to drive the enhanced lethality in $Gpr65^{-/-}$ mice. Strikingly, IL-17A levels are controlled by a “dual-safe” myeloid- γδ T cell circuit: GPR65 limits myeloid-derived IL-1β and IL-23 that are required for activating γδ T-cell IL-17A production, while simultaneously constraining the intrinsic responsiveness of γδ T cells to inflammatory cues. Thus, GPR65 signaling in either compartment of the myeloid–γδ T-cell axis is sufficient to maintain tolerance to systemic inflammation. Mechanistically, GPR65-mediated pH sensing regulates both the magnitude and kinetics of IL-10 expression in myeloid cells, which is crucial for restraining IL-1β and IL-23. In γδ T cells, GPR65 dampens cytokine sensitivity through intrinsic rewiring of transcriptional programs. Together, this work reveals that GPR65 enforces a “dual-safe” innate immune circuit that calibrates the balance between protective immunity and inflammatory pathology, establishing pH sensing as a fundamental environmental checkpoint in immune-mediated disease.

DENDRITIC CELLS IN IMMUNITY TO CANCER

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Innate and adaptive immunity work concertedly in vertebrates to restore homeostasis following pathogen invasion or other insults. Like all homeostatic circuits, immunity relies on an integrated system of sensors, transducers and effectors that can be analysed in cellular or molecular terms. At the cellular level, T and B lymphocytes act as an effector arm of immunity that is mobilised in response to signals transduced by innate immune cells that detect a given insult. These innate cells are spread around the body and include dendritic cells (DCs), the chief immune sensors of pathogen invasion and tumour growth. At the molecular level, DCs possess receptors that directly sense pathogen presence and tissue damage and that signal to control antigen presentation and/or to regulate a plethora of genes encoding effector proteins that regulate immunity. The lecture will focus on understanding how DCs integrate environmental signals to drive immunity to cancer, with applications in immunotherapy.

ROLES OF SPECIALIZED ANTIGEN PRESENTING CELLS IN INTESTINAL IMMUNE HOMEOSTASIS

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Immediately following birth and throughout life, tissues along the gastrointestinal tract encounter enormously diverse novel antigens derived from both diet and commensal microbiota. Potentially damaging inflammatory adaptive immune responses are kept in check by peripheral regulatory T cells that are induced by the harmless antigens. Defects in the induction of such pTreg cells can result in allergic conditions or in inflammatory bowel diseases mediated by various types of effector T cells. Using model systems and genetic approaches, we are characterizing the antigen presenting cells dedicated to directing the diverse programs adopted by naïve T cells specific for food and microbiota antigens. We have identified a rare dendritic cell subset uniquely equipped to induce pTreg cells and a non-DC subset that induces colitis-promoting microbiota-specific pathogenic Th17 cells. We are characterizing the ontogeny of these antigen presenting cells, their transcriptional regulatory networks, their interactions in homeostatic cellular networks, and their localization and dynamics in tissue and draining lymph nodes. A comprehensive understanding of the cells will provide new insights into allergic and inflammatory bowel diseases

DYNAMIC FIBROBLAST-IMMUNE STATES AND NICHES IN INJURY AND INFECTION

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Fibroblasts collaborate with immune cells to promote healthy organ regeneration and prevent scarring after injury. Although fibroblasts display organ-specific states and functions, emerging evidence suggests conserved functional states that cross-regulate diverse immune cells and are governed by organ spatial cues. Here, I will focus on our recent work in the brain and lung using models of sterile and infectious injuries. First, I will discuss recently published work on conserved fibroblast-immune response to sterile brain injury, in which we found that early pro-fibrotic “myofibroblasts” infiltrated brain lesions at subacute times and were coordinated by fibroblast TGFβ signaling, pro-fibrotic macrophages and microglia, and perilesional glia that activated TGFβ via integrin αvβ8. Myofibroblasts subsequently transitioned into multiple late states, including brain border leptomeningeal states and de novo lymphocyte-interacting fibroblasts that supported long-term brain type 1-skewed lymphocytes. Interruption of this spatiotemporal response impaired brain wound healing and led to persistent neuroinflammation, disrupted CNS lymphocyte niches, and increased stroke mortality, highlighting an unexpected role of fibroblasts as dynamic, conserved regulators of CNS healing and neuroinflammation after brain injury. In the second portion of my talk, I will compare our findings in the brain with unpublished work focused on understanding how lung fibroblasts and their dynamic states cooperate with immune cells during viral infection with influenza A virus, including efforts to understand how TGFβ signaling regulates lung regulatory T cell ‘niches’ to ultimately tune the balance of inflammation and resolution after lung infection

A NOVEL MECHANISM FOR RESTRAINING AUTOIMMUNE γδ T CELLS: REPROGRAMMING INTO ILC1s

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Group 1 innate lymphoid cells (ILC1s) comprise heterogeneous populations with respect to their developmental origins, functional properties, and tissue distribution. Here, we identify a previously unrecognized subset of ILC1s that is enriched in the small intestine and whose generation depends on an intact Tcrd locus. Analysis of Tcrd and Tcrg gene rearrangements in these ILC1s revealed predominantly productive Vδ6 and Vγ1.1 segments, suggesting a developmental origin from Vδ6.3$^+$Vγ1.1$^+$ γδ T cells. TCR signaling induces upregulation of Id3, which suppresses E protein–mediated activation of T cell–specific genes, including that encoding Vδ6.3. Consistent with this mechanism, Id3 ablation results in a massive expansion of Vδ6.3$^+$Vγ1.1$^+$ T cells and leads to severe autoimmunity, characterized by immune cell infiltration in tissues such as the salivary glands and lungs, as well as autoantibody production. We further demonstrate that increased numbers of Vδ6.3$^+$Vγ1.1$^+$ T cells promote follicular helper T cell differentiation, which may contribute to elevated levels of germinal center B cells and aged B cells, often associated with autoimmunity. Together, these findings reveal a reprogramming of T lineage cells into a distinct cell type, a process that plays a critical role in restraining autoimmune-promoting T cells.

ONTOGENY AND TRANSCRIPTIONAL REGULATION OF THETIS CELLS

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Thetis cells (TCs), a recently identified family of RORγt$^+$ antigen-presenting cells (APCs), comprise four subsets, including the TC IV subset that instructs tolerance to gut microbiota and food antigens. A developmental wave of TCs creates a window of opportunity for establishing intestinal tolerance during early life. However, the ontogeny of TCs and the cues that determine their abundance and heterogeneity are not known, limiting our ability to interrogate their functional roles and exploit their therapeutic potential. Here, we identify a RORγt$^+$ TC and lymphoid tissue inducer (LTi) cell progenitor (TLP) that differentiates into the immediate RORγt$^+$ TC progenitor (TCP) and the LTi progenitor (LTiP), with the transcription factor PU1 determining TC fate. Despite their similarity to myeloid derived classical dendritic cells (cDCs), we found that TCs were descended from the common lymphoid progenitor (CLP) with deletion of the plasmacytoid DC (pDC) lineage-determining transcription factor TCF4 leading to expansion of TLP and TCs, suggesting a shared developmental precursor with pDCs. TLP is enriched in mouse fetal liver, but in contrast to LTi cells, TCs emerge postnatally, pointing to a role for developmentally-timed environmental cues in promoting TCP differentiation. We identify one such cue–provision of RANKL by lymphoid tissue organizer cells–which was essential for TC I differentiation. Together, these findings identify the ontogeny of TCs and define the transcription factors that promote TC differentiation and heterogeneity. These insights will facilitate future investigations of these enigmatic cells, which have potential therapeutic applications in promoting tolerance.

TERMINALLY DIFFERENTIATED KERATINOCYTES, RATHER THAN ANTIGEN-PRESENTING CELLS, PROVIDE ESSENTIAL IL-23 IN PSORIASIFORM INFLAMMATION

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Interleukin-23 (IL-23) is a heterodimeric cytokine composed of IL-23 p19 and IL-12 p40 and is essential for maintaining skin and mucosal barrier integrity by sustaining type 17 immunity. In plaque-type psoriasis, excessive IL-23 production drives uncontrolled activation of the IL-23–IL-17 axis; however, the dominant cellular source(s) of pathogenic IL-23 remain debated, in part due to the difficulty of comprehensively identifying IL-23–producing cells *in situ*. Here, we mapped IL-23–producing populations in imiquimod-induced psoriasiform dermatitis, a well-established mouse model of human plaque-type psoriasis, using an Il23a-Venus reporter strain generated in our group. We identified three principal IL-23–producing populations in inflamed skin and draining lymph nodes: keratinocytes (KCs), Langerhans cells, and bone marrow–derived $CD11c^+$ antigen-presenting cells. Genetic and functional analyses revealed that KC-derived IL-23 is indispensable for inducing downstream IL-17 responses and driving psoriasiform skin inflammation, whereas IL-23 from Langerhans cells or $CD11c^+$ antigen-presenting cells is largely dispensable. Using a newly generated Il12b-tdTomato reporter, we further show that terminally differentiated KCs express *Il12b* (encoding IL-12 p40) at the late stage of cornification, enabling assembly of bioactive IL-23 heterodimer together with *Il23a* (encoding IL-23 p19). Furthermore, therapeutic blockade of IL-17—conventionally positioned downstream of IL-23—reduced KC IL-23 production in vivo, indicating that IL-17 can augment KC-derived IL-23 and establish a positive feedback circuit during inflammation. Finally, reanalysis of human psoriasis transcriptomic datasets revealed conserved features of IL-23–linked, terminally differentiated KCs across species. Although *IL12B*-expressing cells are infrequent in single-cell datasets—likely reflecting the transient and fragile nature of terminally differentiated KCs—our cross-species analyses support a shared IL-17–associated program coupled to IL-23 production. Collectively, our findings uncover terminally differentiated KCs as a previously unrecognized, functionally essential source of IL-23 that amplifies type 17 inflammation, providing mechanistic insight into psoriasis pathogenesis and highlighting epithelial IL-23 as a potential therapeutic target.

DIFFERENTIAL SIGNALLING MEDIATING GUT γδ TCR RESPONSES TO INNATE AND ADAPTIVE LIGANDS

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Barrier sites are constantly challenged by microbial and environmental threats, requiring specialised immune surveillance and repair. γδ T cells are central to these processes, yet the mechanisms regulating their homing and activation remain incompletely defined. Elucidating these pathways is important for both fundamental immunology and therapeutic discovery.

γδ T cells show marked tissue-specific TCR usage: mouse and human gut intraepithelial lymphocytes (IEL) predominantly express Vγ7 and Vγ4 TCR chains, respectively. This tropism is controlled by butyrophilin-like (Btnl) proteins. In mouse gut, enterocyte-expressed Btnl1+6 heterodimers govern the establishment of the Vγ7 compartment, while BTNL3+8 perform an analogous function in humans. Btnls engage germline-encoded Vγ residues to drive nonclonal, innate-like responses to healthy tissue. In contrast, adaptive responses triggered by stress-induced antigens, such as MHC-I-like protein T22, engage CDR3 regions. Here, we investigated how γδ TCR signalling discriminates between ligands representing healthy versus stressed states.

Compared with α-CD3 stimulation, Btnl1+6 engagement elicited an attenuated signal that failed to activate ZAP70, Akt and mTOR, resulting in reduced protein synthesis and proliferation. Using mutant mice, we identified FceRIγ and PI3K as required for Btnl-induced signalling. However, constitutively active PI3K did not restore responsiveness, suggesting that γδ IEL activation was regulated by PI3K inhibitors. Indeed, pharmacological inhibition of phosphatases identified SHIP-2 as a key regulatory checkpoint.

To directly compare innate and adaptive responses, we generated retrogenic mice expressing a dual-reactive Vγ7 γδ TCR recognising Btnl1+6 (via CDR2γ and HV4γ) and T22 (via CDR3δ). These cells closely mirrored endogenous IEL in phenotype and tissue tropism. Using this model, we found that while Btnl and T22 signalling shared common pathways, T22 upregulated co-stimulatory receptors, potentially enabling robust effector responses in the presence of stress-induced co-stimulators (e.g. 4-1BBL).

In summary, gut IEL engage their TCR in mechanistically distinct ways, yielding different outcomes. This layered signalling architecture is tightly regulated by lipid phosphatases, providing new insights into γδ T cell biology and informing their potential therapeutic exploitation.

MYELOID EXPRESSION OF MITOCHONDRIAL GENES SHAPES ANTIMYCOBACTERIAL IMMUNITY

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Genome wide association studies have repeatedly identified mitochondrial gene mutations that confer susceptibility to mycobacterial infection. These mutations occur in genes with diverse functions: maintenance of the mitochondrial genome (POLG, TFAM), controlling mitochondrial fission and fusion (MFN2, OPA1, LRRK2) and licensing mitochondrial turnover (PARK2, PINK1, PARL). The common mechanistic features of mitochondrial dysfunction that hamper antimycobacterial immunity remain ill-defined. We recently carried out scRNA-seq of CD45+ immune cells isolated from the lungs of Mtb-infected mice at day 21 and 77 post-infection. In mice with more advanced disease, we observed global downregulation of genes that encode components of the mitochondrial electron transport chain (ETC) in myeloid lineage cells. We hypothesize that loss of ETC gene expression limits MF effector functions and exacerbates Mtb disease. To gain mechanistic insight into how ETC disruption impacts antimycobacterial immune responses, we stably knocked down individual ETC Complex members in iBMDMs. We found that knocking down each complex elicited distinct phenotypes, including but not limited to, hyperinduction of inflammatory cytokine expression, macrophage activation, and decreased surface expression of MHCII.. Based on these data, we propose that loss of mitochondrial respiratory capacity (1) accompanies progression of Mtb disease in vivo (2) can be accelerated in MFs harboring mitochondrial mutations and (3) can inhibit crucial antimycobacterial functions in MFs. We predict that failure to maintain active mitochondrial respiration in myeloid cells represents a key tipping point in the host response to Mtb and may serve as an intervention opportunity, whereby replenishing energetically and functionally exhausted MFs could boost immunity in TB patients.

SPECIES-SPECIFIC INNATE IMMUNE SENSING AND INFLAMMATORY SIGNALING DURING LEGIONELLA INFECTION

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Legionella pneumophila is a Gram-negative intracellular bacterium and the causative agent of Legionnaires' disease, a severe form of pneumonia. Its flagellin is detected by NAIP proteins, leading to activation of the NLRC4 inflammasome and a robust innate immune response. Activation of the NAIP/NLRC4 inflammasome promotes caspase-1–dependent responses, including pyroptotic cell death and pro-inflammatory cytokine release, thereby restricting L. pneumophila replication in mouse macrophages and limiting bacterial burden in vivo. By contrast, another clinically relevant species of the genus, Legionella longbeachae, is highly virulent and often lethal but does not express flagellin, and therefore, the involvement of inflammasomes in host defense against this pathogen remains unclear. Data presented show that L. longbeachae is largely refractory to inflammasome activation, poorly activates the caspase-11–mediated non-canonical NLRP3 inflammasome, and that activation of these platforms does not account for the restriction of bacterial replication in macrophages or in vivo. Accordingly, L. longbeachae freely multiplies in macrophages and in vivo independently of inflammasome signaling. In addition, we demonstrate that, unlike L. pneumophila, L. longbeachae expresses a capsule that is dispensable for intracellular replication in macrophages but is critical for bacterial pathogenesis in vivo. Our data further show that this capsule plays a major role in driving inflammatory processes, promoting robust neutrophil recruitment and NET-mediated inflammation during infection. Together, these findings reveal that L. longbeachae employs a disease strategy fundamentally distinct from that of L. pneumophila. Specifically, L. longbeachae evades or fails to engage inflammasome activation, enabling unrestricted replication in macrophages and in vivo, while relying on a capsule to promote virulence by amplifying inflammatory pathology rather than intracellular fitness. The identification of a capsule-dependent, neutrophil/NET-centered inflammatory axis uncovers an underappreciated mechanism of Legionella pathogenesis and advances our understanding of how closely related bacterial species differentially interact with innate immune sensing pathways, with implications for host restriction, immunopathology, and bacterial disease outcomes.

SOCIAL SENSING OF INFECTION REPROGRAMS PERIPHERAL IMMUNITY IN HEALTHY MICE

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In plants and insects, social immunity enables individuals to detect infection in neighbors and mount protective, community-level responses. Whether mammals possess analogous mechanisms remains unknown. Here, we asked how the presence of sick cage-mates influences the physiology of uninfected neighbors. We found that healthy mice co-housed with conspecifics infected with the non-communicable murine pathogen Toxoplasma gondii undergo a shift in peripheral immune responses that results in a primed immune state. Immune priming in healthy animals induced physiological resilience against a sublethal lipopolysaccharide (LPS)-inflammatory challenge. Thus, our findings show that immune state in healthy mammals can be primed by exposure to infected conspecifics.

VIRUS-LIKE PARTICLES ENABLE TARGETED GENE ENGINEERING AND POOLED CRISPR SCREENING IN PRIMARY HUMAN MYELOID CELLS

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Primary human myeloid cells are promising candidates for immunotherapy, yet efficient and scalable technologies for genetic engineering and screening in these cells are limited. Here we present a virus-like particle (VLP)-based toolkit that delivers diverse CRISPR genome editing modalities to human monocytes, macrophages, and dendritic cells with high efficiency while preserving viability and innate immune responsiveness. VLP-mediated delivery of ribonucleoprotein payloads supports gene knockout, base editing and epigenetic silencing, and enables site-specific integration of large DNA sequences when combined with AAV donors for homology-directed repair. Leveraging sgRNA delivery via lentivirus combined with Cas9 protein delivery via engineered virus-like particle (eVLP) treatment (“SLICeVLP”), we performed the first pooled loss-of-function screens in primary human macrophages. We uncovered regulators of TNF production as well as cell surface expression of the co-stimulatory molecule CD80 in human macrophages, converging on the gene TNFAIP3 (encoding the NF-kB regulatory protein A20) as a central regulator of inflammatory polarization. Genetic ablation of TNFAIP3 promoted a pro-inflammatory cell state that was resistant to suppressive polarization, and augmented cytotoxicity of engineered HER2 CAR-macrophages. Taken together, this technology platform enables unbiased discovery and characterization of functional gene targets primary human myeloid cells

ENVIRONMENTAL TIMING OF GUT IMMUNE MATURATION IN THE TUNICATE, *CIONA*, REVEALS CONSERVED LINKS BETWEEN GENE REGULATION, BARRIER FORMATION, AND HOST–MICROBE NEGOTIATION

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The gut is the dominant interface between animals and the external world, yet how microbial exposure is integrated with developmental programs that build mucosal barriers and calibrate immunity remains incompletely defined. We use the tunicate *Ciona robusta*, a transparent and experimentally tractable invertebrate chordate, to dissect how precisely timed environmental encounters shape gene expression and signaling during gut maturation. Ciona develops in microbe-rich seawater, undergoes rapid metamorphosis, and initiates filter-feeding when the nascent (and naïve) gut first encounters complex microbial and dietary antigens.

Using stage-resolved RNA-seq spanning early metamorphosis through early juvenile stages, we profiled transcriptional responses to defined microbial challenges delivered during discrete developmental windows. We observe coordinated programs that link barrier construction and remodeling (epithelial differentiation, junctional and extracellular matrix dynamics, and mucus/chitin-associated factors) with innate immune sensing and effector deployment (immunoglobulin and lectin domain effectors, complement, antimicrobial peptides, and redox/stress responses), as well as developmental and neuroendocrine signaling associated with tissue remodeling and onset of feeding. These responses are strongly stage-dependent, consistent with a critical window during which early challenges shift the timing and amplitude of barrier and immune programs, potentially via early innate priming that imprints mucosal circuits (GPCR-MyD88/TGF-beta axes) to promote commensal tolerance while maintaining vigilance to pathobionts.

Together, these findings position Ciona as a minimal chordate system to uncover conserved gene-regulatory logic connecting microbial exposures to developmental state. This comparative framework enables mechanistic studies using electroporation-based genetic perturbation, defined microbiota, and spatially resolved profiling, and it provides conceptual links to human contexts where early-life barrier immaturity intersects with inflammatory disease.

REWILDING MICE PRIMES INNATE TYPE 2 IMMUNE RESPONSES AGAINST LUNG MIGRATING HELMINTHS.

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The consequences of major environmental change on type 2 immune responses are poorly understood. Here, we rewilded wildtype and IL-4/IL-13 double knockout mice (dKO) in an outdoor enclosure and found that these type 2 cytokines did not play a significant role in the alteration of circulating immune cell populations in response to rewilding, apart from the basophil population. Circulating cytokine levels were also unaffected by type 2 deficiency during rewilding. However, single cell RNA sequencing (ScRNA-seq) analysis of the lung immune cells showed cell-type specific activation dependent on type 2 cytokines. When rewilded wildtype mice were subsequently challenged with the lung migrating nematode parasite, *Nippostrongylus brasiliensis*, we found a more rapid type 2 response that was coupled with improved lung tissue repair and enhanced parasite clearance. Furthermore, we observed that at day 2 post exposure to *N. brasiliensis* (Nb), rewilded mice demonstrate a more regulated type 2 immune profile with significantly reduced neutrophil infiltration compared to laboratory mice. Hence, transient rewilding primed the mice towards a type 2 response allowing them to respond rapidly to lung migration and tissue damage during helminth infection.

OXIDIZED PHOSPHOLIPID DAMAGE SIGNALS MODULATE IMMUNITY

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Phospholipids are major constituents of cellular membranes and are enriched in pulmonary surfactant. During cellular stress, these lipids undergo oxidation, generating a complex mixture of oxidized phospholipids that accumulate rapidly during inflammation and infection. Yet how oxidized phospholipids regulate innate and adaptive immune responses remains unclear. Here we show that influenza virus infection drives an acute increase of oxidized phospholipids in the lung, reprogramming neutrophils into a highly activated state that suppresses adaptive immunity. Compared to wild-type mice, transgenic mice capable of neutralizing oxidized phospholipids maintain robust antiviral antibody and $CD8^+$ T cell responses. Neutrophil depletion eliminates this disparity, establishing neutrophils as required mediators of oxidized phospholipid-dependent immune suppression. Mechanistically, these lipid damage signals amplify neutrophil oxidative programs and lipid peroxidation, while neutrophils themselves contribute to systemic oxidized phospholipid burden, revealing a feed-forward loop that sustains immune dysregulation. We further show that tumor outgrowth in a lung metastasis model mirrors these infection-associated circuits, with oxidized phospholipid induction and neutrophil activation accompanying metastasis. Neutralizing oxidized phospholipids improves protection from lethal influenza virus infection and limits metastatic burden, identifying an oxidized phospholipid-neutrophil axis as a targetable checkpoint that governs immune setpoints across infectious and cancer-associated disease.

CLONAL AND CELLULAR DYNAMICS OF THE ANTIBODY RESPONSE

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The average affinity of specific antibodies increases dramatically over the course of an immune response. This increase is the result of a Darwinian process in which B lymphocytes undergo iterative cycles of random hypermutation of their immunoglobulin genes, followed by selective proliferation of clones bearing affinity-enhancing mutations. This evolutionary process takes place in highly dynamic microanatomical structures known as germinal centers, which arise within secondary lymphoid organs upon infection or immunization. Our work combines intravital multiphoton microscopy with mouse genetics to study how the dynamics of B and T lymphocytes within germinal centers shapes the evolution of the high-affinity antibodies that are crucial to protection from infectious disease.

REGULATION OF CD4 T-CELL RESPONSE DIVERSITY, AVIDITY AND PROMISCUITY THROUGH PREFERENTIAL INHIBITION OF ANTIGEN-EXPERIENCED T CELL PROLIFERATION

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After antigen (Ag) encounter, specific T cells proliferate extensively before decreasing in number and becoming memory cells. A diverse TCR repertoire should be recruited into the response to allow responding to mutating pathogens. How these processes are controlled is still not understood, especially for CD4 T cells which, in contrast to CD8 T cells, need to be restimulated every 1-2 cell division to keep proliferating. Given also the long lifespan of MHC class II peptides complexes, the current dominant paradigm regulating T cell proliferation which is based on competition for Ag should lead to narrowing of the TCR repertoire with selection of high affinity TCRs as it does for B cells. As this is not observed experimentally, alternative mechanisms should be sought.

We previously proposed that Ag-specific T-T interactions regulate CD4 T cell proliferation through a mechanism involving grabbing MHC:peptides complexes from the APC to be presented to CD4 T cells of the same specificity leading to an Ag specific inhibition of Ag-experienced T cells (Helt et al, Blood, 2008). We then showed that this inhibition was mediated by SOCS1 (Sutra Del Galy et al, Science Immunology, 2021).

Here, inducible inactivation of SOCS1 in T cells allowed us to experimentally prevent the downstream events following the T-T interactions, giving us the tool to test how these T-T interactions impact repertoire diversity, TCR affinity and promiscuity (ability to recognize peptide variants of the original Ag) during a polyclonal response. Here,

1. We directly evidence Ag specific T-T interactions using Dby (male Ag) specific uLipstick donor CD4 T cells and uLipstick acceptor CD4 T cells,
2. Using mathematical modeling of CD4 T cell responses, we show that the T-T interactions preferentially inhibit Ag-experienced CD4 T cell proliferation
   o help to maintain TCR repertoire diversity,
   o lead to a non-monotonic relationship between TCR avidity and clonal expansion, with the highest abundance of medium–high avidity clones.
3. These predictions were experimentally verified by performing single-cell RNA and TCR sequencing of >32,000 Dby-specific $CD4^+$ T cells from 20 mice, quantitative functional and binding avidity measurements of 77 re-expressed TCRs, and systematic testing the reactivity of 90 TCRs against 54 Dby peptide variants (5,400 TCR–peptide interactions).
4. We show that the diversity of WT repertoires provides broader recognition of Dby peptide variants than SOCS1-deficient repertoires suggesting that T-T interactions preserve TCR repertoire diversity to allow recognition of Ag variants.

Altogether, we provide a new and general view on the mechanisms regulating CD4 T cell responses allowing a better fitness of the response.

REGIONAL T REGULATORY CELL CIRCUITS CONTROL GUT INFLAMMATION

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Maintenance of immune homeostasis at mucosal barrier sites requires a careful balance between pro-inflammatory anti-pathogen mechanisms and immunosuppressive tolerance. Failure to maintain this balance leads to infectious or inflammatory colitis. An important unexplained clinical feature of colitis is its spatial features: both ulcerative colitis and checkpoint blockade colitis stereotypically involve the descending colon, only encompassing the ascending colon in a subset of patients with severe disease. In this study we investigate how the geography of Treg cell centered immunoregulatory networks may drive spatial aspects of colonic inflammation. We recently described the process of terminal differentiation of IL-10-expressing effector Treg cells essential for suppressing colonic inflammation in adult life. Here we discover that these cells are enriched in the proximal compared to distal colon in mice and humans. By labelling cells in direct contact with colonic Treg cells, we characterize the macrophage subsets regulated by Treg cells in a contact dependent and independent manner. Selective depletion of IL-10-expressing Treg cells led to monocyte infiltration specifically in the proximal colon accompanied by region-specific differential transcriptional responses of colonic macrophage subsets. Recapitulating the regionality of human colitis, modelling colitis in mice with DSS administration resulted in a distal colon bias of myeloid cell infiltration. Notably depletion of IL-10 T reg cells sensitized the proximal colon, increasing infiltration by neutrophils and monocytes. In sum we propose that the proximal colon enrichment of IL-10 expressing effector T reg cells, acting on macrophages in a contact independent manner, imposes a tissue immunoregulatory circuit which relatively protects the proximal colon from inflammation.

A COMMENSALLY REGULATED IMMUNE RHEOSTAT FINE-TUNES SKIN BARRIER FITNESS

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At the skin's surface, the epidermis must balance stem cell renewal with barrier maintenance to withstand environmental stress and shield against pathogens. Here, we identify a microbial-immune–epithelial feedback mechanism that integrates environmental information into stem cell regulation. Specifically, we show that Langerhans cells—an intra-epithelial macrophage population— orchestrate this circuit by producing prostaglandin E_2, which restrains stem cell proliferation, promotes epidermal differentiation and maintains barrier integrity during homeostasis. Upon pathway disruption, stem cells become overactivated, impairing differentiation and compromising barrier function. Upstream, Langerhans cell activity is tuned by the local microbial environment in a rheostat-like fashion, coupling commensal sensing to stem cell control. Our findings provide a general framework for how barrier tissues achieve adaptive homeostasis amid continual external challenge.

DIE ANOTHER DAY: STEMNESS AND SURVIVAL OF LONG-LIVED PLASMA CELLS

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Long lived humoral/antibody immunity is exclusively and directly dependent on the survival of long-lived plasma cells (LLPC). Despite this well-known axion, our understanding of the components driving LLPC generation and maintenance remains largely incomplete. We developed a novel genetic fate mapping tool and a surgery system that directly tracks long-lived plasma cells (LLPCs) longitudinally in individual mice for extended time periods. Surprisingly, Long-lived PC (LLPC) of different isotypes (e.g. IgM, IgG, IgA) utilize distinct survival programs, and interactions with accessory cells. IgA LLPC survival is largely dependent on antigen-receptor signaling whereas IgM LLPC exclusively express stemness factors. These survival programs are primarily hard-wired in the isotype program and appear conserved across tissues/niches. The compartmentalization of survival regulators suggests that LLPC should be studied in an isotype-dependent manner and with a particular focus on the critical genes expressed by and functionally relevant to each LLPC subset.

IL-27 INDUCES A DISTINCT, T-BET-DRIVEN CYTOTOXIC CD4+ T CELL PROGRAM UNCOUPLED FROM CANONICAL TH1 IMMUNITY

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Cytotoxic CD4+ T cells are emerging as critical effectors in antiviral and anti-tumor immunity, yet the signals governing their differentiation remain unclear. Here, we demonstrate that the pleiotropic cytokine IL-27 acts as a non-canonical driver of a robust cytotoxic program in naive CD4+ T cells. Notably, while these cells express the transcription factors RUNX3 and T-BET to levels comparable to CD8+ effectors and Th1 cells, respectively, they define a state that is proteomically and functionally distinct. Consistent with this unique programming, these cells effectively eliminate cognate target cells in co-culture assays. Establishing the physiological necessity of this pathway, deletion of *Il27ra* during murine cytomegalovirus (MCMV) infection resulted in a specific loss of Granzyme B in liver-infiltrating antigen-specific CD4+ T cells, but not in their CD8+ counterparts. Mechanistically, we show that unlike Th1 development, which relies on autocrine IFN-γ feedback, IL-27-driven cytotoxicity is STAT1-dependent but IFN-γ-independent. Using CRISPR-Cas9 to target key transcription factors, we define a distinct hierarchy wherein a primary driver is essential for program induction, whereas secondary contributors synergize to maximize the magnitude of the cytotoxic phenotype. Finally, we identify TGF-β as a repressor of this transcriptional module, thereby preventing the acquisition of the cytotoxic potential. Collectively, these data demonstrate that IL-27 induces a unique cytotoxic program in CD4+ T cells, suggesting a distinct regulatory mechanism and cell state from CD8+ T cells or Th1 cells.

CELL EXTRINSIC STRESS RESPONSE

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Macrophages play a key role in maintaining tissue homeostasis. Cellular and tissue-level stress responses are induced by extreme deviations of homeostatic variables from their setpoint values. Different cell types vary in their ability to cope with cellular stresses. Here, I will discuss how cells that have trouble managing cell-intrinsic stress are assisted by macrophages to maintain their fitness.

COMPLEX IMMUNOREGULATORY ROLES OF STAT1 AND STAT4 IN AUTOIMMUNITY AND IMMUNODEFICIENCY.

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Signal transducers and activators of transcription (STATs) provide a paradigm for rapid responses of cytokines, interleukins, interferons, and hormone-like factors. Genetic deletion models and humans with loss-of-function mutations provide revealing key insights to function. Additionally, human somatic and systemic activating mutations provide novel aspects of STAT action and mechanisms of disease; identified gain-of-function variants include: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. Human STAT1 GOF is a paradigmatic interferonopathy presenting with diverse disorders that also exhibits paradoxical immunodeficiency. We generated a mouse model that conditionally expresses a common variant T385M and use prototypical pathogen models to elucidate mechanisms underlying aberrant phenotypes including co-existing immunodeficiency and autoimmunity. Despite increased basal IFN-γ production in STAT1 GOF mice with autoimmune features, early robust IFN-γ production during infection is impaired due to disruption of normal actions of STAT4 due to the STAT1 GOF allele. Moreover, infection is associated with lethality and prolonged multi-cytokine production, whereas early administration of exogenous IFN-γ to infected Stat1 GOF mice rescues lethality and exaggerated cytokine response. Probing an alternative viral challenge, we employed LCMV and using CD4-cre mediated T cell expression of STAT1 with impaired effector differentiation associated with misinterpretation of IFN signaling. While normal activation of T cells is associated with integrated stress response (ISR), ubiquitous expression mutant STAT1 is associated with severe stress and apoptosis and STAT1 directly binds ISR genes including ATF family members and CHOP (Ddit3). Efforts are ongoing to identify strategies to mitigate ISR. Additionally, we identified systemic activation mutations of STAT4 that underlie pansclerotic morphea; how these STATs contribute to autoimmunity remain enigmatic.

A NEW FLING WITH AN OLD FLAME: FOXP3 AND STAT5 COOPERATIVITY

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Transcription factor Foxp3, with its singular role in differentiation and function of regulatory T (Treg) cells, is thought to modulate their responses to activatory extracellular cues. However, the underlying mechanisms remain largely unknown. Besides Foxp3 induction during Treg differentiation, STAT5 activation downstream of interleukin-2 (IL-2) signaling is required for fitness, activation, and suppressive capacity of mature Treg cells. We found that IL-2 induced phospho-STAT5 binds to the Foxp3 forkhead domain, endowing Foxp3 with a new binding specificity and enabling its recruitment to GAS motif-enriched cis-regulatory elements. Using acute chemogenetic and pharmacologic protein degradation, we found that Foxp3 selectively modulates STAT5-dependent transcriptional responses of activated Treg cells to IL-2, including a Myc-dependent gene module. Unrestrained Myc expression in activated Treg cells perturbs their features and impairs their fitness. Thus, Foxp3-STAT5 cooperativity fine-tunes responses of Treg cells to IL-2 signaling to support their functionality. These findings suggest a novel molecular mechanism by which Foxp3 controls functionality and fitness of activated Treg cells through a direct modulation of their IL-2 induced transcriptional program during immune responses highlighting a generalizable mode of integration of environmental cues into cell type-specific programs defined by lineage-specifying transcription factors.

TRANSCRIPTIONAL CONTROL OF IMMUNE CELL ADAPTATION IN HUMAN TISSUES

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Transcription factors (TFs) are central regulators of gene expression that govern immune cell development, identity, and function. Transcriptional programs require tight control as progenitors diversify into specialized lineages shaped by development, tissue environment, and functional demands. Many cells remain quiescent until activation triggers rapid network reprogramming. To support this plasticity, chromatin is often maintained in a primed state, permitting swift TF binding and lineage-specific gene induction.

Understanding how TFs coordinate immune cell behavior across tissues and ages is therefore essential. Single-cell multimodal technologies now allow simultaneous measurement of gene expression, surface proteins, and chromatin accessibility, enabling integrative analyses of how TF activity shapes cell identity and functional plasticity in vivo.

Applying this approach, we examined tissue adaptation in human macrophages. Multimodal single-cell profiling across mucosal and lymphoid tissues revealed macrophage-specific gene and protein programs shaped by tissue of residence. Analysis of chromatin accessibility in lung and jejunum macrophages demonstrated that tissue-specific transcriptional programs are mirrored by distinct regulatory landscapes, implicating differential TF usage in establishing and maintaining stable tissue-adapted identities while preserving responsiveness to activating stimuli.

Parallel analysis of memory T cells revealed age-dependent regulation. Infant and adult spleen memory T cells displayed distinct transcriptional and chromatin states, with infant cells adopting a stem-like tissue-adapted program accompanied by constrained effector function. Using joint chromatin and transcriptional analysis with CRISPR-Cas9 perturbation, we identified Helios (IKZF2) as a key regulator of the infant-specific program and revealed shifts in its influence during adult memory T cell maturation.

Together, these studies demonstrate that investigating TF regulation provides a framework to understand immune cell behavior, including adaptation and specialization across tissue microenvironments and the human lifespan. By integrating transcriptional and chromatin measurements at single-cell resolution, our work reveals how dynamic TF-chromatin interactions shape and maintain immune cell identity and plasticity.

CIRCADIAN PROGRAMMING OF EFFEROCYTOSIS IN GUT MACROPHAGES

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Intestinal epithelial cells undergo circadian waves of cell death, yet the mechanisms ensuring timely clearance of dying epithelial cells remain unclear. Here, we show that intestinal macrophages synchronize efferocytosis with epithelial cell death through coordinated retinoic acid receptor (RAR) signaling and an intrinsic circadian clock. Gut macrophages display daily rhythms in efferocytic activity, peaking in phase with apoptosis. This vitamin A dependent efferocytosis program includes the engulfment receptor TIM-4 and is dependent upon RAR signaling and the circadian clock. TIM-4^{+} macrophages selectively clear apoptotic epithelial cells, thereby preserving intestinal barrier function. Disruption of macrophage RAR signaling or the circadian clock uncouples efferocytosis from apoptosis, causing apoptotic cell accumulation and impaired barrier integrity. Thus, a temporally gated efferocytic program synchronizes macrophage efferocytosis with circadian epithelial cell death to maintain intestinal homeostasis.

SPATIOTEMPORAL ORCHESTRATION OF TISSUE RESIDENT T CELL IMMUNITY

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Tissue-resident memory CD8+ T cells (TRM) play a pivotal role in protecting mucosal tissues, ensuring fast and efficient immune responses against pathogens, maintaining peripheral tolerance, protecting from cancerous cells, and coordinating tissue remodeling. TRM cells exhibit differences both across and within tissues. Small intestinal TRM cells consist of at least two distinct subpopulations: effector-like TRM cells with a higher cytotoxic potential and stem-like TRM cells, which exhibit heightened multifunctionality and memory potential. The small intestine is a complex cellular ensemble that forms distinct microanatomical niches with specialized functions, including crypts for intestinal stem cell preservation and villi that establish a highly selective absorptive epithelial barrier. Despite the critical role that TRM cells play for barrier immunity and homeostasis, and the known heterogeneity of intestinal TRM cells, it remains unclear whether immune cell heterogeneity (in phenotype, function, memory potential, signaling, or transcription factor dependence) arises due to differential induction events at distinct anatomical locations.

To investigate this question, we performed spatial transcriptomics of the mouse small intestine. For this, we adoptively transferred antigen-specific CD8+ T cells (P14 cells) into naive mice and followed the response to acute LCMV Armstrong infection. To assess transcriptomic changes by cell types across infection time (effector and memory time points) and location, we calculated anatomical axes (proximal-distal, crypt-villi and distance to epithelium) and developed computational frameworks to visualize spatiotemporal distribution of cell types and gene-expression.

We found that positional gene-expression differences are unique to cell types found in the small intestine. Specifically, we were able to show that antigen-specific CD8+ T cells display a significant gradient of gene expression along the crypt-villi axis, with an enrichment of TCF-1high stem-like cells at the crypts and more cytotoxic TRM cells at the top of the villi. Similarly, known homeostatic cytokines such as IL-15 and IL-18 show strong graded expression across the crypt-villi axis, with yet unexplored functions in the maintenance of TRM subsets. Notably, similar findings are observed in the liver. Further, by perturbing TRM-inducing signaling pathways, we could show that loss of retinoic acid signaling as well as deletion of TGFβRII in CD8+ T cells results in the reorganization of antigen-specific T cell location in the small intestinal tissue structure. In summary, we developed a new framework for the study of TRM cells in the small intestine and show how anatomical axes, structures, and niches define TRM cell state, function, and differentiation, demonstrating that T cell location and maturation state are inseparably interwoven.

FUNCTIONAL BORDER-ASSOCIATED MACROPHAGES LIMIT ALZHEIMER'S DISEASE PROGRESSION

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Brain-resident macrophages are known to play numerous roles in the progression of Alzheimer's Disease (AD). However, the relative contribution of microglia and border-associated macrophages (BAM) to AD pathogenesis has been difficult to disentangle. We recently identified Maf, a newly described AD GWAS gene, as essential for BAM, but not microglial, survival. By crossing BAM depleted mice with the 5xFAD AD model, we found stark evidence of cerebral amyloid angiopathy (CAA), increased overall β-amyloid burden, accelerated markers of neurodegeneration, and early memory deficits. In the healthy brain, BAM take up more β-amyloid per cell than microglia. However, as disease progresses, both in human AD patient samples and model AD mice, BAM number is reduced, and the remaining BAMs display impaired endocytic capacity, and show signs of metabolic exhaustion at an earlier age than microglia. Thus, strategies to preserve or restore BAM function could represent a novel therapeutic avenue for AD and CAA.

ACETYL-COA GENERATION BY ACLY DISTINCTLY REGULATES CD4 T CELL PROGRAMMING DURING MALARIA

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Malaria is a disease triggered by *Plasmodium* parasite infection, causing over 200 million cases and ~600,000 deaths each year. Notably, infected individuals do not establish long-lived sterilizing immunity and can become reinfected. The differentiation of CD4 helper T cells into either T helper 1 (Th1) or T follicular helper (Tfh) cells is critical for parasite control and clearance of infection. However, whether specific disease-associated extrinsic factors within the infected host influence cell-intrinsic programming of Th1 and Tfh cells has not been investigated. We found that CD4 T cell subsets responding to malaria exhibit distinct energetic demands. These energetic dependencies are supported by metabolic pathways that may differentially represent available metabolites, including Acetyl-CoA (Ac-CoA), which sits at a central node between cytosolic and mitochondrial metabolism. Based on the distinct energetic demands of CD4 T cell subsets, different levels of Ac-CoA may be present in the cytosol regulating acetylation and differentiation. Therefore, we aim to identify the mechanisms by which malaria-associated perturbations regulate the generation of cytosolic Ac-CoA, thus impacting the programming of CD4 T cells following infection, which may contribute to this lack of sterilizing immunity following re-exposure.

Our scRNA-seq data show that *Acly*, which converts cytosolic citrate into Ac-CoA, is expressed in malaria-specific CD4 T cells. While published data suggest ACLY impacts CD4 T cell function *in vitro*, the *in vivo* contribution of ACLY in Th1 and Tfh differentiation has not been investigated. We hypothesize that ACLY is required for optimal CD4 T cell programming during malaria. To test our hypothesis, we engineered a conditional genetic system to excise *Acly* from mature, peripheral *Plasmodium*-specific CD4 T cells. We employed systems of co-adoptive transfer and flow cytometry to assess the phenotype of WT and *Acly*-KO cells. Loss of *Acly* restrains Th1 differentiation and promotes Tfh development. SCENITH revealed that *Acly*-KO Th1 cells exhibit reduced mitochondrial dependence with a reciprocal increase in glycolytic capacity, with no observed changes in Tfh cells. Mitotracker staining showed that *Acly*-KO Th1 cells exhibit no change in mitochondrial mass but exhibit reduced mitochondrial activity. Functionally, *Acly*-KO cells produce less IFNγ.

These data establish a role of ACLY in supporting the generation of cytosolic Ac-CoA that is critical for Th1 programming during malaria. Our studies also provide an opportunity to unravel metabolic programs and regulatory nodes that are critical for Th1 development but dispensable for Tfh differentiation.

MACROPHAGE REGULATION OF INFLAMMATION IN TYPE 2 DIABETES

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Type 2 diabetes is a metabolic disease characterized by chronic, low-grade inflammation that leads to comorbidities that significantly impact quality of life, such as susceptibility to infections and non-healing wounds. In mouse models, macrophages from diabetic contexts exhibit hyper-inflammatory states, but the mechanisms behind this phenomenon are poorly explored and have not yet been investigated in human macrophages. To study the response of diabetic macrophages that contributed to ineffective resolution of inflammation, we isolated and cultured bone marrow-derived macrophages from WT and db/db mice , and stimulated them with maturation (CSF1), inflammatory (LPS), and resolving (IL-4) cues. We assessed their timeline of maturation through changes in cell surface markers, their functional inflammatory response through phenotypic marker changes and cytokine release, and their sensitivity to metabolic flux by culturing in the presence or absence of extracellular glutamine. Notably, relative to healthy macrophages, diabetic macrophages exhibited aberrant expression of CSF1R and CD11b markers but eventually acquire a similar level of mature cell surface markers by day 7-8 in culture. In contrast to previous studies, we found that both healthy and diabetic mature macrophages displayed similar levels of inflammatory response cytokines, inflammatory gene expression changes, and phenotypic marker expression, suggesting that differentiation in culture may change phenotypes acquired in vivo. Based on these results, we are now isolating human monocytes from PBMCs from patients with type 2 diabetes and age-matched healthy controls to explore the cell-mediated inflammatory aberrations in type 2 diabetes and may reveal targetable mechanisms to improve patient outcomes.

HDAC5 CONTROLS THE EFFECTOR FUNCTION OF CD8$^+$ T CELLS

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The incidence of complex T cell-mediated immune disorders is on the rise worldwide. Dysregulated CD8$^+$ T cell responses contribute to many T cell-mediated disorders, including Celiac Disease and Type I Diabetes, where CD8$^+$ T cells promote tissue destruction. While disease onset depends on a complex interplay between host genetics, environmental factors, and inflammatory signals within the tissue, it is also dependent on T cell-intrinsic factors that poise the cells to rapidly execute effector programs following their activation. Here, by combining transcriptional analyses with assessment of human CD8$^+$ T cell function, we demonstrate that both the baseline expression of activation-associated genes and the ability to rapidly respond to tissue inflammatory cues, such as IL-15, is tightly regulated by the class II histone deacetylase, HDAC5. Knock down of HDAC5 using small interfering RNA (siRNA) in cell lines of primary human cytotoxic CD8$^+$ intraepithelial lymphocytes (IE-CTLs) significantly altered the transcriptional profile of the cells, resulting in reduced expression of effector molecules, surface receptors (including immune checkpoint receptors), signaling molecules, and key transcription factors such as *BHLHE40*, *IRF4*, *NR4A1* and *TBX21*, which are associated with cell fate and effector function. The effect of HDAC5 on the transcriptional program of the IE-CTLs was evident both at steady state and following stimulation with IL-15. One mechanism by which IL-15 promotes dysregulation of CD8$^+$ T cells is through upregulation of the activating NK receptor NKG2D, which allows for TCR-independent cytolysis. We found that loss of HDAC5 significantly reduced expression of NKG2D on IE-CTLs and resulted in lower GZMB expression. Overall, these results suggest that HDAC5 contributes to a poised activation state in IE-CTLs. Interestingly, the effect of HDAC5 is not unique to IE-CTLs, as loss of HDAC5 also altered the transcriptional profile and early activation of human *ex vivo* circulating memory CD8$^+$ T cells. The circulating memory CD8$^+$ T cells demonstrated a shift towards a central memory phenotype following HDAC5 knock down, as measured by CCR7 expression, suggesting that the presence of HDAC5 may be important for sustaining an effector phenotype in both the periphery and tissue. Overall, this work identifies HDAC5 as a critical enzyme regulating the activation of CD8$^+$ T cells, including within tissue environments. It highlights the potential of targeting HDAC5 to modulate CD8$^+$ T cell responses in inflammatory disorders and paves the way for novel therapeutic strategies aimed at adjusting T cell activation thresholds.

ANTIGEN-DRIVEN TURNOVER ENDOWS REGULATORY T CELLS WITH A KINETIC ADVANTAGE

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Effective immune responses must act rapidly to control pathogens. Immune tolerance mechanisms must act even faster to intercept inappropriate activations. An established model of immune tolerance is that self-activated T cells (Tconv) produce interleukin-2 (IL-2) upon antigen recognition, which drives the proliferation of local regulatory T cells (Tregs) that suppresses Tconv expansion. In this view, IL-2 acts as the central mitogenic signal. However, in this model Treg expansion necessarily acts on the same time scale as Tconv proliferation raising the question of how suppression can be deployed rapidly enough to pre-empt inappropriate responses.
Here, we use a quantitative in vitro suppression assay, mathematical modelling and in vivo analyses to disentangle the signals that control Treg survival, proliferation and suppressive function. We find that IL-2 is not a mitogen for Tregs but instead acts as a survival signal. Restricting IL-2 leads to rapid Treg loss, while adding IL-2 sustains Tregs long-term. By contrast, we found that T cell receptor (TCR) signaling regulates Treg proliferation and suppressive capacity, consistent with recognition of self-antigens.
These findings imply a revised logic of Treg homeostasis. Self-antigens are abundantly presented to Tregs in vivo, driving them into the cell cycle and their survival depends on stochastic ‘bursts’ of IL-2 produced by self-activated Tconvs. This model predicts rapid Treg turnover sustained by transient, localized IL-2 availability, consistent with prior observations of high Treg proliferation and apoptosis in vivo.
Why maintain such energetically costly dynamics? Using mathematical modelling, we show that continuous cycling confers a kinetic advantage by enabling Tregs to rapidly accumulate and form suppressive microenvironments immediately following antigen encounter, outpacing the activation and expansion of Tconvs. In this revised model, tolerance is enforced not through IL-2–driven expansion, but through antigen-driven cycling combined with survival gating.
Together, these findings highlight Treg turnover as a design feature of immune regulation and suggest that selective modulation of Treg survival, rather than global inhibition of suppressive function, may provide a route to tune local tolerance, including in contexts such as tumor immunity.

ENVIRONMENTAL IMPRINTING OF THE POSTNATAL INTESTINE

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At birth, barrier sites such as the intestine undergo an abrupt transition from the sterile womb to a complex microbial world, rendering these immature tissues uniquely vulnerable. The postnatal intestine must complete several critical maturation processes in the presence of diverse environmental stimuli while rapidly adapting to balance absorptive function, barrier integrity and immune responses. Remarkably, the postnatal intestine has evolved not only to accommodate microbes during this sensitive period, but in fact requires the presence of microbes or microbial products for proper maturation and long-term homeostasis. Disruptions to microbial colonization during early life are associated with increased risk for chronic conditions including inflammatory bowel disease, food allergy, and obesity. However, the biological basis for the heightened sensitivity of the early-life intestine to microbial cues remains poorly understood. Here, we take a tissue-centric approach to define how microbial signals and host developmental programs converge to shape intestinal maturation. We generated a comprehensive transcriptomic dataset of the small and large intestine across postnatal development under both specific pathogen-free (SPF) and germ-free (GF) conditions. These data confirmed significant alternations in intestinal development in the absence of microbes and revealed tissue-specific responses to microbial signals across time. Notably, many early life expression patterns were robust to microbial status, highlighting conserved programs of development in the absence of microbes. Furthermore, by probing the consequences of asynchronous microbial colonization on developmental programs using gnotobiotic mice, we identified microbe-dependent tissue adaptations that were irrevocably lost despite the presence of microbes later in life. Together, these findings uncover temporally coordinated interactions between microbial cues and the gene expression programs shaping intestinal development. Understanding the mechanisms underlying such time-restricted plasticity will provide key insights into how early-life disruptions lead to long-term tissue dysfunction, and may inform therapeutic strategies for reversing disease risk rooted in early-life microbial disruption.

CONVERGENCE OF THE NF-κB PATHWAYS INCREASES HIV-1C TRANSCRIPTIONAL FITNESS

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HIV-1 subtype C (HIV-1C) accounts for the majority of global infections and exhibits a distinct promoter architecture characterised by an expanded repertoire of NF-κB binding motifs. Central to this architecture is the C-κB motif, a genetically distinct site absent in all other HIV-1 subtypes, defined by specific A-to-G and T-to-G transitions at positions 4 and 6, respectively. Our laboratory has previously established that this unique motif functions synergistically with the proximal Sp1III element—which itself harbours subtype-specific variations—to drive viral proliferation. Since such core sequence polymorphisms are known to dictate the selective recruitment of Rel family transcription factors, we hypothesised that these modifications allow HIV-1C to exploit the non-canonical (alternative) NF-κB pathway, distinct from the classical RelA-mediated induction required by other subtypes.

To test this, we dissected pathway responsiveness using lineage-specific signaling cues. In $CD4^+$ T cells, we selectively activated the alternative NF-κB axis using the SMAC mimetic AZD5582 to induce NIK-dependent processing of p100 to p52. Complementarily, to model signalling in myeloid and microglial reservoirs, we utilised the physiological ligands Lymphotoxin α1β2 and CD40L. Immunoblotting across these models confirmed robust accumulation of nuclear p52/RelB complexes without concurrent IκBα degradation, establishing pathway specificity.

Functional analysis using subtype-specific reporter viruses revealed that HIV-1C LTR activity is significantly upregulated by alternative pathway activation, whereas the HIV-1B LTR remains refractory to p52/RelB signalling. Mechanistically, we provide direct chromatin-level evidence using CUT&RUN and EMSA that p52 physically engages the HIV-1C LTR, with high-affinity recruitment localised to the subtype-specific C-κB motif.

These findings define the HIV-1C LTR as a dual-input logic gate capable of integrating both canonical and non-canonical NF-κB signals. Building on this specificity, we are currently optimising an immunotherapeutic strategy utilising OX40 agonists to selectively engage the alternative signalling axis. This approach aims to achieve potent latency reversal in HIV-1C reservoirs while minimising the off-target pro-inflammatory effects associated with broad classical NF-κB activation.

CAMPYLOBACTER JEJUNI REPROGRAMS THE NEUTROPHIL EPIGENOME TO SUSTAIN INFLAMMATORY SIGNALING

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Diarrheal diseases are one of the global leaders of morbidity and mortality with approximately 550 million infections and 33 million healthy life years lost annually, with *Campylobacter jejuni* being one of the major causes. While often self-limiting in immunocompetent individuals, several post-infectious disorders are increasingly emerging among adult populations, suggesting that the host immune response contributes substantially to the pathologies seen during and after infection. During *C. jejuni* infection, neutrophils are robustly recruited and can promote tissue damage through hyperinflammatory effector functions, including neutrophil extracellular trap (NET) formation. We previously identified a *C. jejuni* sirtuin-like protein, SliP, that is translocated into neutrophils, localizes with histone H3, and drives *Campylobacter*-dependent NET formation. This work also showed that neutrophil histone H3 is deacetylated in a SliP-dependent manner, implicating epigenetic regulation in the neutrophilic response. However, the global impact of *C. jejuni* infection on the neutrophil epigenome remains unknown.

To address this, we profiled histone H3 and H4 post-translational modifications in primary human neutrophils following infection and assessed downstream effects on chromatin structure and gene regulation and expression. H3K27ac ChIP-seq analysis revealed over 5,000 infection-specific regions of differential histone binding across promoters, introns, and exons. ATAC-seq demonstrated widespread chromatin accessibility in uninfected neutrophils, which was constricted to a highly restricted and conserved set of accessible regions upon infection. Direct comparison of these datasets identified 120 loci with both increased chromatin accessibility and differential histone binding during infection, including genes involved in inflammatory signaling and cellular fate regulation. Notably, the transcription factor RUNX1 and its downstream pro-apoptotic target NOXA were identified as infection-induced regulation targets. Transcript analysis revealed a significant induction of RUNX1 upon infection, leading us to hypothesize that neutrophil-induced inflammation is driven partly by repression of NOXA to delay apoptosis and keep neutrophils in their hyper-inflammatory state.

Together, these findings demonstrate that *C. jejuni* infection induces extensive epigenetic and chromatin remodeling in neutrophils, activating regulation mechanisms that delay apoptosis and prolong inflammatory signaling.

THE PHOSPHATASES TCPTP, PTPN22, AND SHP1 PLAY UNIQUE ROLES IN T CELL PHOSPHOTYROSINE MAINTENANCE AND FEEDBACK REGULATION OF THE TCR

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The protein tyrosine phosphatases (PTPs) TCPTP, PTPN22, and SHP1 are critical regulators of the activating phosphotyrosine (pY) site on the initiating T cell kinase, Lck^{Y394}. Still, the broader implications of these phosphatases in T cell receptor (TCR) signalling and T cell biology remain unclear. By combining CRISPR/Cas9 gene editing and mass spectrometry, we evaluate the protein- and pY-level effects of TCPTP, PTPN22, and SHP1 in the Jurkat T cell model system. We find that deletion of each phosphatase corresponds to unique changes in the proteome of T cells, with few large-scale changes to TCR signalling proteins. Notably, PTPN22 and SHP1 deletions have opposing effects on pY abundance globally, while TCPTP deletion modestly elevates pY levels. Finally, we show that TCPTP is indirectly involved in Erk1/2 positive feedback to the TCR. Overall, our work provides evidence for alternative functions of three T cell phosphatases long thought to be redundant.

ACSS2-DRIVEN METABOLIC AND EPIGENETIC REGULATION OF MACROPHAGE ACTIVATION IN COLITIS

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Ulcerative colitis (UC) is a chronic inflammatory bowel disease driven by persistent intestinal inflammation due in part to the high abundance of leukocytes within the colonic tissue. While the etiology of the disease is multifactorial, including genetic polymorphisms, immune dysfunction, and microbiome dysfunction, little is known about how the host epigenome regulates the host-microbe axis to drive inflammation during UC. Previously, it has been shown that leukocytes within the lamina propria during murine UC possess increased histone acetylation patterns. One mechanism which may underlie this epigenetic state is through the activity of acetyl-CoA synthetase 2 (ACSS2). In the nucleus, ACSS2 utilizes acetate to generate acetyl-CoA, resulting in rapid histone acetylation and gene expression. Importantly, has been shown to drive proinflammatory processes in leukocytes. To determine if ACSS2 plays a role in UC, we challenged WT and mice lacking ACSS2 ($ACSS2^{KO}$) mice with dextran sodium sulfate (DSS) to model UC. We demonstrate $ACSS2^{KO}$ mice are resistant to DSS-induced colitis. Through RNA sequencing of leukocytes from DSS mice, we found ACSS2 promotes inflammatory transcriptional signatures, including the expression of interferon (IFN) response genes and proinflammatory cytokines. To identify the leukocytes which lose their inflammatory transcriptional signature, we performed single cell RNA sequencing of leukocytes during disease. Through this, we discovered that macrophages lose their inflammatory transcriptional signature in DSS-fed $ACSS2^{KO}$ mice. Fascinatingly, while WT macrophages become more inflammatory during disease, $ACSS2^{KO}$ macrophages retain a homeostatic and pro-tissue repair phenotype. In macrophages, we have found ACSS2 to localize strictly to the nucleus and fascinatingly, is chromatin-bound. To understand how chromatin-bound ACSS2 coordinates IFN response in macrophages, we stimulated WT and $ACSS2^{KO}$ HoxB8 macrophages with IFNγ and performed RNA sequencing. Here, we observed that $ACSS2^{KO}$ significantly cannot upregulate IFN stimulated genes as highly as WT macrophages. To test if this phenotype is UC-specific, we challenged mice with *Clostridioides difficile*, a major enteric pathogen. Similarly to DSS-induced colitis, $ACSS2^{KO}$ mice showed decreased disease severity and improved pathogen clearance. We hypothesize that during colitis, macrophages respond to IFN rapidly and robustly due to chromatin-poised ACSS2. As acetate is produced by the gut microbiota, ACSS2 can rapidly use this substrate to promote histone acetylation, opening chromatin, and driving proinflammatory gene expression. Through these studies, we seek to target ACSS2 as an upstream target of inflammation to ameliorate multiple forms of colitis. As such, we hypothesize that ACSS2 is central to the metabolic-epigenetic axis in macrophages in response to IFN during colitis.

COMPETITION BETWEEN FOXP3+ TREG CELLS

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Regulatory T (Treg) cells restrain autoimmunity and maintain homeostasis. Defined by the transcription factor FoxP3, Treg cells control the activation of conventional lymphocytes, and of inflammatory responses more generally, through complex mechanisms. However, when Treg with different FoxP3 variants coexist, the regulatory principles operating within their population remains unclear. Serendipitous observations in heterozygous female mice with different pairwise combinations of modified FoxP3 alleles uncovered competitive dynamics between Treg populations. One “winner” population gains dominance over the other (“loser”), in terms of numerical representation, activation and maturation to different effector states. Winner/loser states are context-dependent, a loser becoming a winner in a different combination. These competitive phenomena seem anchored in differential representation of FoxP3, but translate into extensive differences in transcriptomes, organization of the genetic regulatory network, and chromatin configuration. We will discuss our mechanistic explorations of the winner/loser competition and its relationship to Treg cell homeostasis in normal, inflammatory and tumor contexts. These findings suggest suppressive behavior within the Treg cell population, establishing a “competition effect”, a concept which may have broad immunological implications.

MEMORY CD4 T CELL DERIVED TNF IS CRITICAL FOR PROTECTION FROM REINFECTION BY PATHOGENS

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The innate immune system acts as first line of defense against invading pathogens and relies on pathogen recognition receptors (PRRs) to detect microbes and initiate inflammation. The innate immune system can also instruct cells of the adaptive immune system to mount pathogen specific responses that provide long lasting immunity. This immunological memory facilitates a more rapid and potent response upon re-exposure to the same antigenic determinants. While memory CD4 T cells provide protection through production of key effector cytokines, whether they have evolved other mechanisms to specifically combat virulent pathogens has never been explored. We have previously shown memory CD4 T effector (TEM) cells use specific TNFSF ligands to engage myeloid cells resulting in innate inflammation which drives autoimmune pathology and allograft rejection. In this study, we have discovered that TEM cells can protect mice from virulent Listeria infections, and such protection is critically dependent on TNF produced by pathogen specific CD4 memory T cells. Furthermore, our findings indicate that antigen specific TEM cells facilitate clearance of the pathogenic Listeria challenge independent of canonical pattern recognition receptor activation. T cell derived TNF was not only critical for regulating pathogen burden but also protected mice from lethality. These data provide compelling evidence to support the notion that TEM cells might have evolved the ability to drive innate activation as a protective mechanism against pathogens with the ability to evade innate immune recognition. Future work will dissect the specific molecular and cellular mechanisms by which TEM-derived TNF drives innate inflammation, defining whether protective and pathogenic programs are mediated by overlapping or distinct signaling pathways.

DISTINCT HOMO- AND HETERODIMERS DETERMINE THE BINDING AND FUNCTIONAL SPECIFICITIES OF IKAROS FAMILY TRANSCRIPTION FACTORS IN B CELLS

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The Ikaros and Aiolos transcription factors (TF) are major regulators of B cell development and function. They function as homo- and heterodimers, but the contribution of individual dimer pairs to gene regulation is unknown. Using a novel bimolecular epitope complementation approach, we show that the Ikaros-Ikaros, Ikaros-Aiolos and Aiolos-Aiolos dimers are not equivalent for either binding or function. At early stages of differentiation, Ikaros homodimers bind and regulate a small but highly specific set of genes related to cell adhesion and migration, predominantly as transcriptional repressors. In contrast, Ikaros-Aiolos heterodimers and Aiolos homodimers are sequentially required at later stages to first repress and then activate 10-fold larger target gene repertoires involved in lymphocyte activation and function. Mechanistically, the Aiolos subunit enables more permissive motif recognition than Ikaros, which depends on a recently evolved amino acid variation in the DNA binding domain. These results illustrate how transitions between TF homo- and heterodimers can markedly impact binding specificity to direct distinct biological outcomes.

LEVERAGING HIGHLY RESOLVED MULTIOMICS DATASETS TO ELUCIDATE CROSS-SPECIES CONSERVATION IN THYMIC EPITHELIAL CELLS

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The thymus is the site of T-cell education and mediates central tolerance induction in developing thymocytes. Thymic epithelial cells (TECs) eliminate self-reactive developing thymocytes in order to prevent autoimmunity. In particular, the thymus is notable for the development of specialized TEC's that adopt transcriptional characteristics of cells typically found in the periphery and are believed to be critical for tolerance induction. A better understanding of the regulatory mechanisms underlying the development of these TECs in the thymus can therefore elucidate the origins of autoimmunity in a clinical setting. Due to a scarcity of accessible human thymus tissue, studies of thymic epithelial cells have predominately been conducted in mice. However, maximizing the translational impact of thymus research requires a clear understanding of the differences between mouse and human TECs. Furthermore, cross-species studies of TECs have largely been restricted to qualitative comparisons of celltype composition and marker genes. We collated highly-resolved scRNA and scATAC seq datasets of thymic epithelial cells, including two human and mouse multiomics datasets, and conducted a cross-species comparison of the transcriptional and regulatory programs of the thymic epithelial compartment. We leveraged the multiome data to identify regulatory networks consisting of celltype-driving transcription factors and target genes specific to distinct human and mouse celltypes. We found that few celltype-defining transcription factors are conserved across species but in both human and mouse, genetic regulation in TECs is mediated by the upregulation of pathways associated with their peripheral counterparts.

MEMORY REGULATORY T CELLS REPROGRAM INTO PROTECTIVE TFH-LIKE EFFECTORS IN RECURRENT MALARIA

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Although people living in malaria-endemic areas experience repeated infections with Plasmodium, the role of regulatory T cells (Tregs) in recurrent malaria remains poorly understood. During a primary infection with Plasmodium, Tregs suppress protective immunity by inhibiting germinal center (GC) reactions, thereby impeding the control of parasitemia. In contrast, we demonstrate here that memory Tregs (mTregs) remaining after the clearance of initial Plasmodium infection acquire protective functions upon recall. Relying on longitudinal studies in humans and mice, we show that mTregs undergo antigen-driven expansion and inflammation-induced epigenetic reprogramming during reinfection to transition from Foxp3+ immunosuppressive cells to Bcl6+ follicular T helper (Tfh)-like effectors. These mTreg-derived Tfh-like cells enhance GC responses and the generation of Plasmodium-specific antibodies, ultimately facilitating Plasmodium control. Precluding such mTreg-to-Tfh differentiation abolished protection. Our findings reveal a previously unrecognized adaptive plasticity in canonical mTregs that enables a context-dependent functional switch from immunoregulatory to protective effectors during recurrent infections.

INVESTIGATION OF INTERFERON-β-MEDIATED EPIGENETIC MEMORY IN HUMAN MONOCYTES

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Innate immune cells, such as monocytes, can exhibit enhanced responses to secondary stimulation long after a primary encounter with an immunological stimulus, a phenomenon known as "trained immunity" or "innate immune memory". Accumulating in vivo evidence indicates cytokines, such as interferons, have been implicated in mediating such memory effects via epigenetic reprogramming, yet the underlying molecular mechanisms in human monocytes remain incompletely characterized.

We are interested in the epigenetic mechanisms by which type I interferon priming establishes long-lasting chromatin states that influence subsequent immune responses. To address this, we utilize an in vitro system in which primary human monocytes are primed with interferon beta (IFNβ) for 24 hours, followed by washout and a 5-day resting period, and are then stimulated with LPS after differentiation into macrophages. This approach enables us to investigate whether transient IFNβ exposure induces persistent epigenetic changes after the initial stimulus is removed. Using RNA-seq, ATAC-seq, and CUT&RUN profiling of histone modifications, we aim to identify chromatin states that are gained or maintained during the resting phase and to determine how these states influence macrophage responses upon secondary challenge. Understanding these chromatin dynamics may reveal the molecular basis of innate immune memory and provide insights into therapeutic strategies for modulating inflammatory responses in human systems.

CELL-TYPE SPECIFIC NFκB SIGNALING IN HEMATOPOIETIC STEM CELLS IN HEMATOPOIETIC AGING

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Hematopoietic aging is characterized by chronic inflammation, hematopoietic stem cell (HSC) dysfunction, and hematopoietic bias toward myeloid lineages, leading to less efficient immune responses and increasing incidence of hematologic neoplasia. Aged HSCs show diminished replicative capacity and high quiescence, a reversible G0 state of dormancy, correlated with elevated NFκB, a central regulator of inflammation and cellular stress responses. Yet, it has been difficult to distinguish the effects of age-associated inflammation from the impacts of other age-associated cellular damage on hematopoiesis. We employed an experimental model of elevated NFκB activity termed "IκB–" (*Nfkbia*$^{+/-}$*Nfkbib*$^{-/-}$*Nfkbie*$^{-/-}$) to dissect the role of NFκB-mediated chronic inflammation in hematopoietic aging phenotypes.

We found distinct roles for NFκB in the hematopoietic compartment vs. the bone marrow milieu. NFκB activity correlates with transcriptomic quiescence in aged and young HSCs on a per-cell basis, and experimentally increased NFκB activity functionally impairs bone marrow reconstitution. In contrast, NFκB activity in the bone marrow milieu is sufficient to induce HSC epigenomic re-programming and myeloid-biased hematopoiesis. Evaluation of aged murine and human HSC scRNA-seq datasets support a conserved association between HSC-intrinsic NFκB activity and quiescence, but not myeloid bias (Chia et al. Cell Rep 2025). Math modeling further reveals that both milieu-driven transcriptomic bias and myeloid progenitor proliferation together underlie myeloid bias in aging and clonal myeloid neoplasms (Singh, Chia et al. Blood 2025). These findings delineate separate regulatory mechanisms that underlie the phenotypic hallmarks of hematopoietic aging.

NFκB is expressed ubiquitously, yet can exert different effects in distinct cell types, such as innate immune responses in macrophages and proliferation in B-cells. However, it is unknown whether HSCs may possess a distinct set of NFκB target genes, and whether these may promote HSC quiescence. In on-going studies, we are evaluating for cell-type-specific NFκB target genes in HSCs, macrophages and B-cells, aiming to define HSC-specific NFκB targets and how these may impact HSC quiescence. These studies are anticipated to provide new insights into the effects of chronic inflammation on HSC regulation, with broad relevance to hematologic conditions including clonal hematopoiesis, myeloid neoplasia, lymphoma/leukemia, and bone marrow failure disorders.

TRYPTOPHAN METABOLIC PATHWAY DEPENDENT IMMUNOMODULATORY MECHANISM OF HUMAN RECTUM DERIVED MESENCHYMAL STROMAL CELLS

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Mesenchymal Stem/Stromal Cells (MSCs) derived from bone marrow, adipose tissue and umbilical cord are widely being investigated as allogeneic cellular therapy to mitigate bowel inflammation and perianal fistula in patients with inflammatory bowel diseases (IBD). It is of translational interest to define the anti-inflammatory potency mechanism of MSCs derived from human gut for their application as autologous cellular therapy. In the present study, we investigated the mechanism of action of MSCs derived from human rectal biopsies from seven independent consented human subjects. Human rectum derived MSCs (R-MSCs) display ex vivo growth and metabolic functionality, suggesting their feasibility for cellular therapy. R-MSCs dose dependently inhibit T cell proliferation in the cocultures of activated peripheral blood mononuclear cells (PBMCs). Multiplex technology based secretome analysis of the cocultures of R-MSCs and PBMCs identified both directly and inversely correlative secretome signature that predict immunosuppression of R-MSCs. Mechanistic analysis showed that activated PBMCs induce the tryptophan-metabolizing immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO), but not other enzymes in the same pathway, in R-MSCs. Exogenous addition of recombinant human IFN-γ faithfully recapitulated IDO induction in R-MSCs, suggesting that IFN-γ produced by activated PBMCs is responsible for IDO induction in R-MSCs. Blocking of IDO expression in R-MSCs through siRNA knockdown methodology abolishes their immunosuppressive potency on T cells. Altogether, our immunological investigations of human R-MSCs provide a mechanistic insight on their immunosuppressive properties and thus further investigations are warranted for their use as cellular therapeutics for IBD management.

STRUCTURE OF GUT MICROBIAL GLYCOLIPID MODULATES HOST INFLAMMATORY RESPONSE

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Commensal microbes in the intestine continuously shape the immunological landscape by producing diverse immunomodulatory molecules. Among these, outer membrane glycolipids play a pivotal role in host–microbe communication. Lipidomic analyses revealed that symbiotic microbes possess lipid A species varying in acyl chain number and phosphorylation, whereas pathobionts display limited diversity, typically producing hexa-acylated and diphosphorylated lipid A. However, the compositional complexity of these glycolipids across species has hindered detailed structure–activity studies.

To define the immunological impact of symbiotic lipid A structures, we utilized chemically synthesized lipid A analogs that mimic those found in commensals. These analogs, differing in acylation and phosphorylation, induced distinct transcriptomic signatures in dendritic cells (DCs). Notably, under-acylated (tri- and tetra-acylated) and monophosphorylated analogs strongly upregulated type I interferons (IFNs) and IFN-stimulated genes, whereas penta-acylated lipid A exhibited minimal activity. This structure-dependent response reflects preferential engagement of endosomal TLR4 by specific lipid A species, resulting in sustained IFN-β production and intracellular lipid droplet accumulation. The lipid A–driven IFN-β response is critical for inducing colonic RORγt+ regulatory T cells (Tregs) while suppressing Th17 differentiation and limiting gut inflammation. Consistent with these findings, lipooligosaccharides (LOS) purified from a Bacteroides fragilis strain engineered to overproduce tetra-acylated lipid A showed enhanced IFN-β–inducing activity compared to LOS from the wild type. Intriguingly, while penta-acylated lipid A species dominate in Bacteroidetes, they fail to elicit IFN-β responses; instead, the less abundant tetra-acylated species trigger sustained IFN-β production that supports RORγt+ Treg homeostasis.

Collectively, our work defines a mechanistic link between commensal glycolipid structure and host immunomodulatory responses. Subtle structural variations in symbiont-derived lipid A dictate TLR4 signaling and IFN-β induction, thereby maintaining colonic Treg populations and promoting intestinal immune balance.

m^6A mRNA MODIFICATION PROMOTES OXIDATIVE STRESS RESILIENCE IN THP1 MACROPHAGES

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Macrophages activate an inflammatory response to tissue injury or infection. Reactive oxygen and nitrogen play a central role in the inflammatory response, and thus, macrophages must be able to tolerate the oxidative stress that they themselves induce. Furthermore, macrophages often enter tissue environments that are low in oxygen and other metabolites, leading inflammatory macrophages to rely on glycolysis as their energy source. This further exacerbates the redox stresses under which macrophages must function. Here, we ask how the mRNA post-transcriptional modification N-6 methyladensoine (m^{6A}) catalyzed by METTL3 affects macrophage signaling in hypoxia. Depletion of m^6A leads to a striking four to five-fold downregulation of metallothioneins and components of the phagocyte NADPH oxidase complex. In the absence of m^6A, physiological hypoxia leads to increased transcriptional readthrough, a hallmark of oxidative stress. From these results, we infer that m^6A promotes reactive oxygen tolerance during inflammation. m^6A depletion leads to impaired expression of IL6 and IL1B in hypoxia suggesting that m^6A is required for implementation of downstream inflammatory signaling in these conditions. We discuss the implications for mounting an effective inflammatory response, consequences for cell survival, and the mechanistic basis for m^6A control of a reactive oxygen load.

MEMORY B CELLS AND PLASMA CELLS OF THE IgE RESPONSE

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IgE antibodies are critical mediators of allergic diseases, yet IgE production is highly regulated. IgE cell differentiation has unique characteristics: most IgE-producing cells are plasma cells, and the production of high affinity IgE occurs through the sequential switching of non-IgE cells, mainly IgG1 memory B cells.

We identified a population of IgG memory B cells that forms under type 2 immune conditions and is characterized by expression IL-4/IL-13 regulated genes, including CD23, IL4R and germline *IGHE*. This type 2 memory B cell population contains high affinity allergen-specific clones and is poised to switch to IgE, representing a reservoir of high affinity IgE plasma cells precursors. In recent work using experimental allergy models, we determined that IgE plasma cells, which for long being have been considered short-lived, undergo maturation and some become long-lived, residing preferentially in secondary lymphoid organs like the spleen in addition to the bone marrow. These findings suggest that type 2 IgG memory B cells and long-lived IgE plasma cells play a critical role in the persistence of allergies.

ENGINEERING CYTOKINE-INDEPENDENT CSF1R SIGNALING FOR iPSC-DERIVED MACROPHAGE DIFFERENTIATION AND SURVIVAL

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Engineered macrophages are emerging as promising therapeutic agents due to their ability to integrate into tissues, respond to local signals, and perform context-dependent functions. In several diseases, tissue-resident macrophages promote harmful inflammation, motivating strategies to deplete these cells and replace them with engineered counterparts.

Depletion of these tissue-resident macrophages, by inhibiting colony-stimulating factor 1 receptor (CSF1R), allows the engineered macrophages to fill this empty niche and reset cell function. However, engineered macrophages remain dependent on CSF1R signaling for differentiation and survival, making them vulnerable in vivo to environments where cytokine availability is limited or CSF1R signaling is delayed, spatially restricted, or pharmacologically blocked, impairing cell lineage commitment.

CSF1R signaling engages the MAPK pathway and controls macrophage proliferation and function. While cytokines can be supplemented during in vitro differentiation, engineered macrophages deployed in vivo must function when CSF1R signaling cannot be assumed. Several activating CSF1R mutations have been identified that confer cytokine independence, inhibitor resistance, or both, suggesting a potential strategy to sustain macrophage survival and differentiation under these constraints.

We combined a doxycycline-inducible PU.1 and CEBPα differentiation circuit (iMAC) with constitutively expressed CSF1R variants in human iPSCs, enabling rapid and reproducible induction of macrophage gene expression while independently modifying receptor signaling. We tested two activating CSF1R mutations: D802V in the activation loop and G936S in the C-terminus, hypothesizing that these variants could support macrophage differentiation and survival in the absence of exogenous cytokines.

To assess dependence on CSF1R-activating cytokines IL-34 and M-CSF, cells were differentiated under four conditions: IL-34 + M-CSF, IL-34 or M-CSF alone, or neither. After seven days, parental iMAC cells showed reduced viability in the absence of cytokines, whereas both mutants maintained high viability, indicating functional cytokine independence during early differentiation. Consistent with these observations, immunoblotting revealed phosphorylation of ERK1/2 in mutant CSF1R lines regardless of cytokine presence, while parental iMAC cells exhibited ERK activation only under full cytokine conditions.

Together, these preliminary results suggest that activating CSF1R mutations can sustain downstream MAPK signaling and cell viability independent of exogenous ligand during macrophage differentiation. Ongoing work is focused on validating CSF1R expression, assessing protein stability, and determining whether these variants confer resistance to CSF1R inhibitors. This approach provides a framework for engineering macrophages capable of surviving in cytokine-limited or CSF1R-inhibited environments.

EOSINOPHIL-EPITHELIAL CELL CROSSTALK REGULATES FOOD ALLERGY OUTCOMES.

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The intestinal epithelium is a primary site of post-prandial food allergy antigen exposure, and communication between intestinal epithelial cells (iECs) and mast cells is required for allergic diarrhea and avoidance behavior. In this project, we aimed to uncover additional iEC-immune communication circuits regulating food allergy outcomes, and employed the BALB/c OVA-ALUM food allergy model to do so. After each OVA oral challenge (OVA-OC), eosinophils were acutely but transiently recruited to the intestinal epithelium. To track physical interactions between eosinophils and iECs, we generated villin-uLIPSTIC mice, a tool in which iECs covalently label physically interacting immune cells with biotin. Surprisingly, the primary OVA-OC did not induce iEC-eosinophil interactions despite the robust recruitment of eosinophils to the epithelium. Rather, iEC-eosinophil interactions were only observed after repeated OVA-OCs, and these interactions correlated with the onset and severity of allergic diarrhea. Confocal imaging confirmed the transient recruitment of eosinophils to the epithelium after OVA-OC, and revealed an enrichment of eosinophils at the base of the crypt-villus axis, suggesting that eosinophils communicate with the stem cell niche. To test this possibility, we generated Defa6-uLIPSTIC mice, a tool in which Paneth cells label interacting immune cells, and observed Eosinophil-Paneth cell interactions after OVA-OC. To gain insights into the function of Eosinophil-iEC communication, sensitized mice were treated with an anti-CCR3 antibody to deplete eosinophils. Eosinophil depleted mice exhibited significantly more severe diarrhea after OVA-OC. After four OVA-OCs, epithelial cells were sorted from eosinophil depleted mice and RNA-sequencing was performed. In the absence of eosinophils, we observed a reduction in the expression of genes associated with tuft cells and transit amplifying cells, and an increase in the expression of genes associated with mature enterocytes. These differences in epithelial composition were confirmed by imaging. Coculture of epithelial interacting eosinophils with intestinal organoids induced a tissue repair program in the epithelial cells, demonstrating the ability of eosinophils to directly modulate epithelial cell growth and gene expression. Collectively, these data demonstrate food allergy specific physical communication between eosinophils and iECs that regulates the repair and remodeling of the intestinal epithelium in food allergy.

CHARACTERIZATION OF THE ACTIVATION OF PROGRAMMED CELL DEATH PATHWAYS IN MACROPHAGES AFTER *MYCOBACTERIUM TUBERCULOSIS* INFECTION

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, primarily infects the human lungs, where macrophages serve as its major replication niche. Mtb can modulate macrophage cell death programs to promote its survival, yet the specific pathways engaged in THP-1 and human monocyte-derived macrophages (hMDMs), and their potential crosstalk, remain poorly defined. Our work demonstrates that none of the four major programmed cell death pathways: apoptosis, pyroptosis, necroptosis, and ferroptosis, is significantly involved in Mtb-mediated cell death induction. Instead, we observed extensive release of extracellular DNA (eDNA) from dying THP-1 cells and hMDMs. Further analyses revealed that this eDNA contains minimal myeloperoxidase activity and exhibits low levels of Histone H3 citrullination, indicating that it is distinct from classical NETosis. Confocal microscopy imaging demonstrated a close association between eDNA and extracellular Mtb. Removal of eDNA using DNase reduced Mtb-induced cell death but did not affect bacterial viability. Additionally, DNA release did not require NINJ1, caspase-4, or caspase-5 in host cells. Together, our findings identify an atypical form of DNA release that may reflect a noncanonical necrotic pathway with potential biological relevance during Mtb infection.

PROXIMITY LABELING IDENTIFIES JUNB-INTERACTING PROTEINS THAT MODULATE TH17 CELL PLASTICITY

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IL-17A-producing T helper 17 cells (Th17 cells) are critical to barrier immunity and clearance of extracellular pathogens. However, Th17 cell plasticity towards a Th1-like effector profile with IFNγ expression has been implicated in various autoimmune conditions, including inflammatory bowel disease and multiple sclerosis. Thus, understanding Th17 cell plasticity holds important implications for amelioration of autoimmune disease. JunB is an AP-1 transcription factor that regulates Th17 plasticity by both promoting Th17 cell characteristics, such as *Il17a* expression, and restricting acquisition of a Th1-like phenotype in inflammatory contexts, such as by limiting T-bet and IFNγ expression. JunB regulates Th17 cell identity, in part, by forming protein complexes with transcriptional coregulators that modulate the Th17 cell effector profile. However, few JunB-interacting coregulators have been identified in Th17 cells. We hypothesized that the JunB protein interactome contains novel transcriptional regulators of Th17 cell plasticity. To define the JunB interactome in mouse Th17 cells, we generated TurboJunB: a JunB fusion protein to TurboID, a biotin ligase that rapidly labels proximal proteins. We validated the JunB functionality of TurboJunB by confirming its capacity to repress IFNγ production in JunB-knockout Th17 cells and its ability to localize to known JunB-bound loci. We delivered TurboJunB to Th17 cells *in vitro* and identified biotinylated proteins with quantitative mass spectrometry. After comparing against TurboID controls, we identified 106 unique significant interactors with TurboJunB in Th17 cells, including known JunB interactors that regulate Th17 cell identity, such as BATF and HDAC4. Notably, TurboJunB also associated with IRF2BP2, a transcriptional cofactor that has not been previously reported to interact with JunB or regulate Th17 cell plasticity. To characterize the role of IRF2BP2 in regulation of Th17 cell identity, we performed CRISPR/Cas9-mediated knockdown (KD) of IRF2BP2 in *in vitro*-differentiated mouse Th17 cells. We observed a significant increase in IL-17A expression and a significant decrease in IFNγ expression in Th17 cell cultures with IRF2BP2 KD relative to control cultures, suggesting that IRF2BP2 promotes Th17 plasticity towards a Th1-like effector profile. In future work, we will further characterize the role of IRF2BP2 in Th17 cell plasticity and perform CRISPR/Cas9-mediated KD screening of TurboJunB interactors to identify other novel regulators of Th17 cell plasticity.

TRANSPOSABLE ELEMENTS AND THEIR CONTROLLERS AS REGULATORS OF IMMUNE SIGNALING: A SCREEN-BASED APPROACH

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Transposable elements (TEs) exert profound influences on inflammation, partaking in processes from early antiviral responses to immune recognition of cancer cells. KRAB-Zinc Finger Proteins (KZFPs), which constitute the largest human transcription factor family, evolved to regulate TE expression; however, their involvement in immune processes remains largely unexplored. We hypothesize that KZFPs, in their function as TE binders and beyond, add a species-specific layer of control to the fast-evolving immune landscape.

To search for immune-modulating KZFPs, we first integrate existing datasets – including >300 KZFP ChIP-seq profiles, transcriptomes from primary immune cells, and a genome-wide Perturb-seq screen – to identify family members that bind near core immune genes, such as nucleic acid sensing components, or that display immune-responsive expression. We then deploy two complementary experimental arms: (i) systematic depletion of human transcription factors to reveal gene losses that trigger spontaneous immune signaling; and (ii) construction and deployment of a pooled, doxycycline-inducible KZFP overexpression library, with dual demultiplexing through genomic DNA sequencing and microproteomic mass spectrometry. This novel platform will identify KZFPs whose overexpression modulates cellular responses to stimulus-induced immune activation.

The combination of in-silico data mining, genetic perturbation of endogenous transcription factors, and pooled KZFP overexpression in immune-activated settings will deliver the largest functional dataset to date on KZFPs, and uncover the role of this fast-evolving transcription factor family in immune regulation. More broadly, resolving pooled candidates by either DNA barcodes or peptide signatures can be used to identify KZFPs involved in regulating any other cellular function of interest.

BACK TO COLEY: RETHINKING BACTERIA AS AN ANTICANCER AGENT.

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Here we uncover a new paradigm for cancer immunotherapy that leverages immune responses to bacterial species colonizing tumors. First, we used an attenuated strain of Listeria monocytogenes (ΔactA, Lm) that does not express tumor antigens to explore the immunological response to Listeria itself in the context of intravenous (IV), intratumoral (IT), or a combination of IV+IT administration into tumor-bearing mice. Unexpectedly, we found that Lm delivered IT persisted long-term in tumors of immune competent mice. Furthermore, IT Lm alone led to the recruitment of immunosuppressive neutrophils that promoted tumor growth. In contrast, IV Lm followed by IT Lm controlled tumors. IV Lm vaccination generated a pool of anti-Lm cytotoxic CD8+ T cells that killed Lm-infected immunosuppressive cells in tumors, produced cytokines that limited tumor cell proliferation, and enhanced the cross-presentation of tumor antigens by type I dendritic cells (cDC1s) to promote the tumor-specific T cell response.

Second, this tumor control was completely dependent on Lm triggering of an innate immune sensor, Toll-like receptor 2. We discovered that TLR2 signaling in cDC2s upon IT Lm injection had a unique capacity among all TLRs to reduce regulatory T cell (Treg) frequency and Foxp3 expression. Reduced Treg immunosuppression in tumors underlaid the enhanced cross-presentation of tumor antigen on cDC1s and stronger antitumor CD8+ T cell responses within tumors with IV+IT Lm. Thus, while Toll-like receptors (TLRs) were known to link innate and adaptive immunity by supporting T cell priming in lymphatic tissues, here we uncovered how TLR2 signaling also affects immune effector responses locally within cancer.

Finally, our findings using Listeria were recapitulated with a distinct species of bacteria, Shigella flexneri. Shigella also persisted long-term in tumors and IV+IT Shigella administration led to potent tumor control. Since Shigella also invades the cytosol of host cells like Listeria, we tested the importance of intracellular invasion by bacteria for therapeutic efficacy. IT administration of ΔpurA Listeria, an alternatively attenuated strain of Listeria that disrupts its capacity to convert between intracellular and extracellular niches in the tumor environment completely abolished its therapeutic efficacy. Overall, our findings reveal a potent and multifactorial immunostimulatory potential of bacteria for cancer immunotherapy and provide a novel mechanism for treating cancer by targeting bacteria colonizing tumors.

MYCOBACTERIAL RECOGNITION BY INFLAMMASOMES IN HUMAN AND MURINE MACROPHAGES

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Mycobacterium tuberculosis (Mtb) has been known to evade host innate immunity by manipulating macrophage function. Interleukin-1β (IL-1β) is a cytokine secreted by macrophages as a consequence of inflammasome activation. Mtb can inhibit activation of the NLRP3 and AIM2 inflammasomes and subsequent pyroptosis. The capacity of Mtb to manipulate other types of inflammasomes is unknown. In this study, we investigated whether Mtb or the nontuberculous mycobacteria Mycobacterium kansasii (Mkan), and Mycobacterium smegmatis (Msmeg) can inhibit the NLRP1 or pyrin inflammasomes. We show that none of the mycobacteria consistently inhibit the NLRP1 inflammasome after it is activated by priming with LPS and treatment with 1G244 in human macrophages. Similarly, neither Mtb nor the NTMs inhibit the pyrin inflammasome after it is activated by priming with LPS and treating with TcdB toxin from Clostridium difficile in human macrophages. In murine macrophages, Mkan shows inhibition only at 3 hours post-infection and Msmeg only at 24 hours post-infection, whereas no inhibition was observed at any time point for Mtb. In conclusion, our main findings are that Mtb is unable to inhibit the NLRP1 and the pyrin inflammasomes in human macrophages.

MicroRNA-MEDIATED POST-TRANSCRIPTIONAL REGULATION OF PERIPHERAL γδ T CELL EFFECTOR FUNCTIONS

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γδ T cells are unconventional lymphocytes able to rapidly produce large quantities of interleukin-17A (IL-17A) or interferon- γ (IFNγ), which often have major effects on the pathophysiology of diseases such as cancer or infections. Yet, we do not fully understand how the thymic-derived effector γδ T cell subsets making either IL-17A (γδ17 cells) or IFNγ (γδIFN cells) are activated and function in peripheral tissues. Here, we established an Il17a-GFP:Ifng-YFP double-reporter mouse strain to analyse at unprecedented depth the microRNA transcriptomes of pure γδ17 versus γδIFN cell populations from peripheral lymph nodes, and uncover new mechanisms of miRNA-mediated post-transcriptional regulation of γδ T cell effector functions. We found 103 differentially expressed microRNAs between γδ17 and γδIFN cells, from which we selected 10 candidates likely to target members of the transcriptional networks underlying IL-17A of IFN-γ production, to test their effects on γδ T cell differentiation or effector functions. Retroviral gain-of-function approaches in vitro highlighted a plethora of context-dependent microRNA-specific effects on the differentiation or expansion of γδ17 or γδIFN cells, and respective cytokine production. Furthermore, a detailed analysis of each candidate miRNA expression throughout ontogeny showed that in some cases it was imprinted in the thymus, whereas in others it was peripherally induced. This led to the observation that miR-181a-5p, highly expressed in early thymic γδ T cell subsets, favours thymic γδ17 cell commitment, while conversely promoting peripheral γδIFN activation and proliferation in response to TCR stimulation in vitro and upon malaria infection in vivo. On the contrary, we found miR-128-3p to act as a peripheral regulator of IFN-γ, preventing aberrant IFN-γ production upon TCR stimulation. These findings unravel a new layer of regulation of γδ T cell effector functions, which we are further characterizing with additional molecular assays.

BYSTANDER EPITHELIAL CELLS CONTRIBUTE TO INFLAMMATORY RESPONSES BY SECRETING IL-8 DURING SALMONELLA INFECTION

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Salmonella enterica serovar Typhimurium (STm) causes inflammatory gastroenteritis by activating host signaling pathways that induce pro-inflammatory cytokine release, neutrophil recruitment, and intestinal inflammation. Interleukin-8 (IL-8) is a key mediator of this response, yet the mechanisms regulating its induction during STm infection remain incompletely defined. Here, we investigated the host and bacterial factors that control IL-8 expression in HeLa cells during STm infection. Using ELISA and fluorescence in situ hybridization techniques, we found that IL-8 induction is influenced by both host and bacterial determinants. IL-8 production was higher in HeLa cells at lower passage numbers, increased with infection time, and correlated with bacterial invasion efficiency. Quantitative analysis of RNAScope imaging data revealed that IL-8 transcript levels were higher in bystander cells than in infected cells, particularly in cells proximal to infected neighbors, whereas uninfected control monolayers exhibited low basal expression. The inflammasome machinery is weak in HeLa cells and the two major drivers of IL-8 induction, IL-1β and IL-18, are produced in low levels under most conditions. RNAscope analysis showed some increase in IL-1β and IL-18 expression in STm infected HeLa cells. Also, treatment of uninfected HeLa cells with supernatant from infected monolayers induced IL-8. Nevertheless, neutralizing antibody depletion IL-1β and IL-18 had no significant impact on IL-8 induction. Finally, inhibition of caspase activity partially reduced IL-8 secretion. Together, these findings indicate that IL-8 induction during STm infection is driven by multiple mechanisms and highlight an important role for epithelial bystander cells in shaping the inflammatory response.

ROLE OF SRC-3 IN REGULATORY T CELLS AS A MODULATOR OF ACTIVATION

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While Steroid Receptor Coactivator 3 (SRC-3) is best known for its role in promoting proliferation of breast cancer cells, our group has recently identified a key role for SRC-3 in regulatory T cells (Tregs). Treg specific knockout (KO) of SRC-3 results in the long-term eradication of primary tumors in a syngeneic model of triple negative breast cancer. SRC-3 KO Treg mice also can resist rechallenge with a second dose of tumor cells. This long-term anti-tumor immunity points to the potential that SRC-3 KO Treg cells are differentiating into T memory cells. Our data shows that SRC-3 KO Tregs express higher levels of the T memory cell markers CD44 and CD62L than wild type Tregs and that SRC-3 KO Tregs downregulate inducible T-cell co-stimulator (ICOS), c-MAF, interleukin 10 (IL-10), and IL-13. Additionally, KO of c-MAF in Tregs causes SRC-3 expression to drop. This data suggests a role for SRC-3 in the regulation of Treg activation and provides mechanistic insight into how SRC-3 KO Tregs are able to exert their anti-tumor effects. Further work is ongoing to elucidate the molecular connection between ICOS/c-MAF and SRC-3.

T HELPER CELL SUBTYPES ADOPT A SHARED TRANSCRIPTIONAL IDENTITY DURING INFLAMMATION VIA BATF-DEPENDENT 3D CHROMATIN REPROGRAMMING

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INTRODUCTION: T helper cell identity is maintained by lineage-defining transcription factors (TFs) that operate within insulated chromatin architectures to ensure target gene specificity. In chronic inflammatory conditions such as Crohn's disease (CD), these constraints are disrupted, enabling pathogenic transcriptional reprogramming. BATF is a pioneering TF essential for T cell differentiation and inflammatory Th17 responses and has been linked to higher-order chromatin regulation. Here, we define how inflammation reshapes BATF chromatin occupancy, alters target gene specificity, and reprograms gut-resident T helper cells in CD.
METHODS: We performed single-cell RNA sequencing of gut-resident $CD4^+$ T cells from healthy individuals and CD patients to define lineage-specific and disease-associated states. To identify upstream epigenetic regulators, we integrated RNA-seq and ATAC-seq across purified Th1, Th17, and regulatory T cell subsets. BATF-driven programs were perturbed using CRISPR-mediated gene deletion. We used HiChIP to detect lineage-specific and inflammation-induced changes in 3D chromatin architecture.
RESULTS: Gut-resident $CD4^+$ T cells from healthy individuals exhibited well-defined lineage-specific transcriptional and chromatin programs, whereas T helper subtypes in CD converged toward a shared pathogenic state. BATF expression and activity were increased across all subsets in CD. Under homeostatic conditions, BATF occupancy was restricted to lineage-specific regulatory elements; inflammation expanded BATF occupancy across accessible chromatin and decreased enhancer-promoter specificity. Multi-omic analyses identified BRD4 as an epigenetic co-activator of BATF-dependent programs, and BRD4 deletion markedly attenuated their expression. HiChIP revealed inflammation-driven reorganization of 3D chromatin, increasing enhancer-promoter interactions at BATF/BRD4 target loci and weakening lineage-specific insulation.
CONCLUSIONS: These findings identify inflammation-driven expansion of BATF chromatin occupancy, loss of target specificity, and BRD4-dependent chromatin remodeling as core mechanisms underlying altered T helper cell identity in Crohn's disease. This pioneer TF-epigenetic co-activator axis reorganizes 3D genome topology and enforces a shared pathogenic transcriptional state across $CD4^+$ T cell lineages. More broadly, chronic inflammation repurposes pioneer transcription factors to override lineage-specific chromatin architecture.

DYNAMIC FOXP3-CHROMATIN BINDING IN CONTEXT-DEPENDENT GENE REGULATION AND TREG CELL FUNCTION

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Regulatory T (Treg) cells are essential for maintaining immune tolerance toward self-antigens and unharmful foreign antigens. They also suppress anti-tumor immune response, posing a barrier for immunotherapy. The development and function of Treg cells depend on the key factor Foxp3. However, the principles and mechanisms by which Foxp3 operates in Treg cells remain elusive, specifically whether and how it integrates environmental signals to modulate Treg function accordingly. This also points to a fundamental question regarding whether Foxp3-dependent gene regulation and resulting Treg fate and function are a static or dynamic process. Foxp3 has traditionally been considered a transcription factor, because it binds DNA in vitro and chromatin in vivo. Here, we showed that Foxp3 binds to chromatin depending on the cellular contexts. It is dynamically regulated by TCR and IL-2 signaling, cell activation states, or tissue environment. We propose that Foxp3 acts as a transcriptional co-factor whose chromatin binding is predominantly determined by other transcription factors such that Foxp3 "senses" extracellular signals and cellular activity—such as TCR and cytokine receptor signaling—by forming complexes with context-dependent transcription factors, which then recruit Foxp3 to chromatin. This model is consistent with the result that a Foxp3 mutant lacking DNA-binding ability (Foxp3-DBD) still drives canonical Treg gene expression, including Il2ra (CD25) and Ctla4. This further suggests that DNA-binding stabilizes Foxp3 complex rather than determining the target specificity. Together, these results reveal a previously unrecognized mode of Foxp3 that dynamically regulates gene expression and Treg function through cooperative chromatin targeting according to the environmental contexts.

GERMINAL CENTER B CELLS REGULATE THEIR METABOLIC STATES FOR MIGRATION.

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Germinal centers (GCs) are anatomical sites where GC B lymphocytes receive signals to proliferate rapidly and mutate their antibody genes. Because GCs serve a vetting mechanism for the antibodies that have high affinity and specificity for invading pathogens or vaccines, it is critical to understand how the GC dynamics are regulated. There are two spatially separated regions in a GC, called light zone (LZ) and dark zone (DZ). In LZ high-affinity antibodies, or B-cell receptors (BCRs), on the cell surface confer GC B cells with a proliferation advantage due to the enhanced antigen presentation to the cognate follicular helper T (Tfh) cells, which provide GC B cells with the signals to grow and divide. Activation of mTORC1 and expression of Myc in the GC B cells that received such signals initiate both their cell cycle and migration to the DZ, where they can undergo several cell divisions before they return to the LZ for more rounds of the “selection” by Tfh cells and the clonal expansion by migrating between the two zones. What signals GC B cells to return to the LZ is unclear. We hypothesized that the rapid cell division of GC B cells causes changes in their metabolic states as they cannot replenish their metabolites as quickly as metabolites are consumed and diluted. GC B cells may sense such metabolic changes, which trigger their migration to the LZ. To study how the metabolic states are changing in the dividing GC B cells, we utilized a mouse model that allowed us to track cellular history of division and performed RNA sequencing and metabolomic profiling on the GC B cells that finished multiple cell division and ready to migrate to the LZ, and the GC B cells that received the signals from Tfh cells and were metabolically charged but had not yet divided. We reasoned that because completion of cell division is closely related to migration, the comparison of the divided cells and the undivided cells would provide us with insights into the signals that trigger migration. We observed changes in metabolites involved in fatty acid oxidation, TCA cycle, and branched amino acid metabolism, which indicated altered mitochondrial activity and oxidative stress. We are investigating a possible negative feedback loop involving oxidative stress and transcription factor Foxo1, which is a critical regulator of DZ program in GCs. This study provides an understanding of how cellular metabolites influence cell migration in GCs.

PERIPHERAL REGULATORY T CELLS ARE A NONREDUNDANT LINEAGE THAT SECURES HOST TOLERANCE

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Regulatory T (Treg) cells, central to maintaining immune tolerance and preventing lethal autoimmunity, have dual origins: the thymus and the periphery, where tTreg and pTreg cells are generated, respectively. A widely held view holds that autoimmune responses against “self” are controlled by tTreg cells while pTreg cells restrain reactivity against “non-self” - food and commensal microbiota-born antigens. Yet the extent and the full spectrum of unique vs. redundant functions of the two Treg cell subsets remain poorly understood. We explored the functional divergence between them using mice which allowed for the lineage tracing of pTreg cells and the selective depletion of either pTreg or tTreg cells. Unexpectedly, pTreg cells prevented lethal autoimmunity in the absence of tTreg cells. Furthermore, we found pTreg cells indispensable for restraining Th17 responses in different tissues, while tTreg cells were uniquely required to contain Th2 responses. Additionally, we identified previously underappreciated subset of pTreg cells potentially specific for self-antigens in non-lymphoid tissues. Thus, pTreg cells exert immunomodulatory functions distinct from their thymic counterparts yet are capable of assuming at least some of tTreg cell functionality and counterbalancing their deficiency.

A TRANSCRIPTIONAL COORDINATOR OF DENDRITIC CELL MATURATION AND ACTIVATION STATES.

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Dendritic cells (DCs) are central to bridging innate and adaptive immunity. At steady state, DCs mature by upregulating immunoregulatory genes. Pathogen recognition further increases this process, leading to an activated state of DCs. In addition, a distinct DC state, termed mregDCs, DCs enriched in immunoregulatory markers, has also been identified across tissues, including tumors. These DC states of maturation, activation, and mregDC share overlapping immunoregulatory gene expression, yet a unifying regulatory mechanism remains undefined. Here, we identify a transcription-related factor as a key coordinator of immunoregulatory gene expression across DC states. This factor is known as a subunit of a regulatory complex of transcription and is essential for cell viability in cycling cells. Surprisingly, we found that it is dispensable for survival and differentiation in non-cycling monocyte-derived DCs (MDDCs). Instead, MDDCs lacking the factor maintained housekeeping gene expression and viability but showed reduced expression of immunoregulatory genes. Moreover, they exhibited impaired activation and diminished type I/III interferon responses in response to viral stimuli and pattern recognition receptor agonists. They also failed to fully upregulate mregDC-associated markers and processes. These findings reveal the novel concept that the various immunoregulatory states of DCs are coordinated by a common factor that regulates immunoregulatory transcription independently of vital gene expression, suggesting a decoupling of survival transcription from immunoregulatory responses in DCs.

S. AUREUS CELL WALL MODIFICATIONS REGULATE PHAGOSOMAL ESCAPE TO CONTROL DNA-TRIGGERED IMMUNITY DURING INTRACELLULAR INFECTION

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Central to immunity are pathogen-associated molecular patterns (PAMPs), microbe-derived molecules that trigger innate immune responses upon binding a cognate host receptor. To cause disease, some microbes shield their PAMPs from immune detection. One such microbe is the bacterium *Staphylococcus aureus* (*S. aureus*), a leading cause of infectious mortality. Although *S. aureus* survives within phagosomes of host cells, most research has focused on its extracellular lifestyle. Consequently, we have a limited understanding of how *S. aureus* evades immune detection during intracellular infection. To address this knowledge gap, we developed a bone marrow-derived macrophage (BMDM) intracellular infection model. Using this model, we performed a bacterial genetic screen and discovered that glycosylated wall teichoic acid (glycoWTA), an abundant cell wall modification, promotes release of the inflammatory cytokine IL-1β. Consistent with prior studies, we found the cell wall-modifying enzyme O-acetyltransferase (OatA) reduces IL-1β release. Strikingly, OatA-deficient *S. aureus* contained more WTA than WT strains, suggesting OatA blunts IL-1β release by reducing glycoWTA abundance.
IL-1β release is controlled by a cytosolic immune complex called the inflammasome. We infected BMDMs deficient in inflammasome components and found that *S. aureus* activates inflammasome-triggering DNA sensor AIM2, suggesting cytosolic DNA is the PAMP responsible for inflammasome activation. Consistent with this hypothesis, OatA reduced and glycoWTA increased activation of another cytosolic DNA-triggered immune sensor, cGAS. We hypothesized that bacterial DNA is released into host cells to trigger immunity. To determine if *S. aureus* cell wall modifications regulate bacterial DNA accessibility within host cells, we quantified DNA within infected BMDMs and found OatA reduces whereas glycoWTA increases free bacterial DNA abundance. PAMP availability can be regulated by movement of bacteria from the phagosome to the cytosol. To determine if this is the mechanism by which cell wall modifications control DNA-triggered immunity, we infected cells that develop fluorescent specks upon cytosolic translocation of *S. aureus* and found that OatA reduces whereas glycoWTA increases *S. aureus* cytosolic escape. Collectively, our data indicate that a competition between cell wall modifications controls *S. aureus* residence within phagosomes, thereby modulating immunity by acting as a meta-regulator of bacterial DNA accessibility during intracellular infection.

UNRAVELLING THE ROLE OF HOMEOBOX PROTEIN HHEX IN EARLY T CELL DEVELOPMENT

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The homeodomain-containing transcription factor Hhex has important functions in various developmental systems and cancer. In hematopoiesis, it is required for the maintenance of lymphoid potential in hematopoietic stem cells. While its later direct contributions to B cell development are relatively well understood, how Hhex fits into the T cell developmental program remains unclear. This is underscored by the paradox that, despite being required for T cell development in vivo, thymocytes repress Hhex as they commit to the T cell lineage, and Hhex remains silenced in mature T cells – sharply contrasting with most other hematopoietic cell types. Aberrant expression of Hhex during T cell development is a key driver of T-cell acute lymphoblastic leukemia (T-ALL). Therefore, we hypothesize that Hhex has stage-specific hit-and-run activity that is necessary to establish T-lineage competence in early hematopoietic progenitors, but that it antagonizes later T-lineage specification and commitment. To test this, we performed both gain- and loss-of-function studies by over-expressing and knocking-out Hhex at defined developmental stages of T-cell development and assessing the subsequent effects of T cell development. These experiments revealed that loss of Hhex accelerated T-lineage commitment, while its overexpression led to a reciprocal retardation. Furthermore, ectopic expression of Hhex in a “DN3-like” P2C2 cell line led to a collapse of the T-lineage program, despite maintenance of a high Notch signaling response, and unlocked access to alternative lineages. For deeper insight into the gene network context in which Hhex operates, we carried out complementary high-sensitivity mass spectrometry based proteomic profiling across early thymocyte stages. This analysis revealed that Hhex protein abundance closely tracks its transcript levels during pro-T cell development, indicating that Hhex regulation - unlike some of its known collaborators - occurs primarily at the transcriptional level. These findings present Hhex as a temporal regulator and gatekeeper of T-lineage commitment through its regulation of Bcl11b to fine-tune the timing of T-cell commitment.

STRESS-PRIMED MACROPHAGES EXHIBIT DISTINCT TRANSCRIPTIONAL RESPONSES TO INFLAMMATION THROUGH XBP1

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Macrophages are central to immune regulation and tissue homeostasis, but their function can be disrupted by cellular stress, contributing to chronic inflammation. Endoplasmic reticulum (ER) stress—often caused by oxidative damage or excessive accumulation of lipids—activates stress-response transcription factors to restore cellular proteostasis. Moreover, ER stress is known to amplify inflammatory cytokine production, yet how stress-responsive transcription factors regulate transcriptional networks beyond proteostasis remains poorly defined. To address this gap, we modeled acute inflammation (lipopolysaccharide, LPS) on pre-existing ER stress (tunicamycin) in bone marrow–derived macrophages. Our transcriptomic analysis revealed that 13.8% of genes were differentially expressed when acute inflammation occurred on a background of pre existing ER stress, compared to LPS alone. Notably, among these differentially expressed genes, ER stress alone minimally alters gene expression, but when combined with inflammatory stimulation, it dramatically expands and diversifies transcriptional responses. Indeed, our preliminary data found that 12.7% of chromatin accessible sites were differentially enriched in ER stressed macrophages compared to untreated controls. These findings underscore that ER stress substantially remodels chromatin architecture, priming macrophages for altered inflammatory responses.To uncover regulatory drivers of this remodeling, we focused on XBP1, a key ER stress–responsive transcription factor. Normal expression of XBP1 was required for elevated expression of *Il6* and *Tnf*, while XBP1 was not necessary to control *Relb* or *Areg*. The results indicate pathway bifurcation in inflammatory reprogramming of stressed macrophages and position XBP1 as one necessary arm in stress-driven transcriptional networks, integrating ER stress and inflammatory signals to orchestrate immune activation.

SYNAPSE-SEQ REVEALS CD4$^+$ T CELL CYTOTOXICITY SUPPRESSES HUMAN ANTIBODY RESPONSES

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Deciphering the molecular dialogue between cells is essential for understanding how immune responses are orchestrated. Here we applied **Synapse-seq, a new image-enabled cell interaction sorting and transcriptomic profiling method, to characterize CD4$^+$ T-B cell synaptic interactions in human spleen organoids**. We identified B cells synapsing with FOXP3$^+$ regulatory T cells (Tregs) expressing high levels of perforin and granzymes A and B, indicating that cytotoxicity is a major mechanism of CD4$^+$ T cell suppression of specific B cells. We also characterized antigen-specific T-B interactions using Hemagglutinin (HA) and Proteinase-3 (PRTN3) spheromers and found viral T-B pairs were enriched for T-GCB phenotypes whereas autoantigen T-B pairs were enriched for cytotoxic T-B phenotypes. T cell-specific knockouts of cytotoxic genes robustly increased antibody responses against both nuclear autoantigens and viral antigens. Consistent with these findings, patients with systemic lupus erythematosus (SLE) exhibited a significant enrichment of cytotoxic CD4$^+$ Tregs. Our findings support cytotoxicity as a dominant way CD4+ T cells regulate B cells and prevent autoimmunity. We also suggest Synapse-seq as a generalizable method to characterize immune cell interactions without requiring cell engineering.

OXYSTEROL-DEPENDENT B CELL TRAFFICKING SHAPES THE HUMORAL IMMUNE RESPONSE IN THE INFLAMED LUNG

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In situ humoral immune responses in the lung are critical for protection against pulmonary infections; however, the mechanisms that guide B cell recruitment to inflamed lung tissue remain poorly understood. Here, we identify an oxysterol ligand, 7a25HC, as a key regulator of the pulmonary humoral response following influenza infection. In contrast to lymph nodes, where CCR7 contributes to the entry of naïve B cells, CCR7 has been reported to be dispensable for B cell recruitment to the inflamed lung. Instead, EBI2 was required for B-cell entry into inflamed pulmonary tissue. Using an intranasal influenza model, we found that the accumulation of both naïve and memory B cells in the inflamed lung depends on expression of EBI2, the receptor for 7a25HC, in a B cell–intrinsic manner. We further demonstrate that the enzymatic machinery controlling EBI2 ligand availability is compartmentalized: Ch25h is required within the hematopoietic cell compartment, whereas Cyp7b1 is required in the stromal compartment, indicating coordinated ligand production within the lung microenvironment. Together, these findings establish oxysterol–EBI2 signaling as a previously unrecognized pathway governing B cell recruitment to inflamed lung tissue. Ongoing studies aim to define the cellular sources of the oxysterol ligand and to determine the functional consequences of impaired B cell recruitment on local antibody responses. This work provides a framework for modulating humoral immunity during pulmonary infection and inflammation.

A SCALABLE SCREENING PLATFORM FOR DISCOVERING PREDOMINANT miRNA TARGET GENES AND DRUGGABLE siRNA SEQUENCES.

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Animal microRNAs primarily regulate their targets through seed pairing. However, the contributions of non-seed regions and the identity of predominant targets in specific contexts remain poorly defined. We developed shRNAone, an shRNA design that produces highly homogeneous small RNAs and more closely mimics endogenous miRNA biogenesis. Using shRNAone, we built SynthoMir, a comprehensive human miRNA variant library enabling high-throughput dissection of miRNA features beyond the seed region. Applying this platform, we show that miR-494 regulates ISCA1 via 3′ supplementary pairing, and that ISCA1 is the predominant target mediating miR-494-associated sorafenib resistance in hepatocellular carcinoma. We further leveraged shRNAone's homogeneity in pooled tiling screens to map druggable RNAi target sites in TTR and PCSK9, two targets of FDA-approved siRNA therapeutics. Our findings indicate that the outputs from shRNAone screening can be readily translated into effective siRNA designs. Together, shRNAone provides a versatile platform for dissecting miRNA regulatory networks and guiding the design of therapeutic siRNA sequences.

A MULTIMODAL SIGNALING ATLAS FOR UNDERSTANDING CD8+ T CELL STATE AND FUNCTION

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Immune cells integrate myriad signals as they respond to challenges. T cells are an example: they sense a wide array of signals from other immune cells and their tissue milieu alongside antigen to discern potential threats and mount appropriate responses. To decode the rules by which T cells integrate and respond to these signal combinations, we developed a novel viral barcoding method, termed CellCode, that uniquely enables interrogation of effects of cell-extrinsic perturbations in a pooled, massively-parallel manner. Using CellCode, we measured the output responses of CD8+ T cells to 192 different signaling inputs, encompassing all 28 cytokines for which these cells expressed a receptor, along with T-cell receptor (TCR) and co-stimulatory inputs in varying combinations. Output responses analyzed included state changes measured using single-cell RNA sequencing, as well as cell proliferation both in vitro and in vivo, for signaling-perturbed CAR-T cells subsequently transferred into tumor-bearing mice. This signaling input-output atlas reveals a diverse range of CD8+ T cell differentiation states elicited by these signal combinations, including a hybrid Gata3-HI state with strong cytolytic and anti-tumor capability . Strikingly, while a large fraction of cytokines impact T cell state and function, many of these effects require coincident TCR signaling, underscoring the importance of antigen as a contextual factor in T cell cytokine responses. Our work provides a foundation for understanding T cell mediated immune responses and establishes a general approach for decoding signal integration other cell types.

DECIPHERING NOVEL REGULATORS INVOLVED IN iTreg DIFFERENTIATION AND FUNCTION

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Regulatory T cells (Tregs) are essential for maintaining immune homeostasis, sustaining peripheral tolerance, and preventing autoimmune disease. However, Tregs can also suppress anti-tumor immune responses, thereby promoting tumor progression. This dual role highlights the need to understand the molecular mechanisms governing Treg differentiation, stability, and function to advance immunotherapeutic strategies. Long intergenic noncoding RNAs (lincRNAs) are emerging regulators of immune cell gene programs and epigenetic landscapes, yet their roles in human Tregs remain poorly defined.

Here, we performed RNA sequencing on in vitro–induced human Tregs and TCR-activated controls to identify Treg-enriched lincRNAs. Several lincRNAs were upregulated in Tregs, and one candidate, validated by qPCR, was selected for functional analysis using locked nucleic acid (LNA)–mediated knockdown during Treg differentiation. Effects on Treg marker expression and function were assessed by qPCR, flow cytometry, and suppression assays, while global expression changes were analyzed by mass spectrometry and RNA sequencing. Silencing of the selected lincRNA reduced expression of key Treg markers, including FOXP3 and CTLA-4, and impaired Treg suppressive function. Pathway analyses revealed dysregulation of chromatin remodeling, transcriptional control, and cytokine signaling pathways, suggesting coordinated regulation of epigenetic and signaling networks during Treg differentiation.

Together, these findings identify a lincRNA as a new regulator of human Treg differentiation and function, highlighting its potential as a target for selectively modulating Treg activity.

GRAFT REGULATORY T CELLS INDUCED VIA IFN-γ PROMOTED ALLOGRAFT ACCEPTANCE IN A MOUSE CARDIAC TRANSPLANTATION MODEL

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Transplantation tolerance is a state in which donor alloantigen are selectively and persistently accepted by the host immune system without eliciting destructive immune responses. Although regulatory T (Treg) cells play a crucial role in transplantation tolerance, how graft-infiltrating Treg cells undergo functional adaptation to the allograft environment remains incompletely understood. Using a mouse cardiac transplantation model with costimulatory blockade, we investigated the role of Treg cells in transplantation tolerance with a particular focus on their adaptation within the allograft. Primary cardiac allografts in untreated mice were rejected within 10 days, whereas CD80/CD86-CD28 signal blockade prolonged graft survival for more than 100 days. Tolerant mice accepted secondary grafts from the same donor strain without immunosuppression but rejected third-party grafts, while removal of the primary graft 14 days before secondary transplantation resulted in secondary graft rejection. Depletion of Treg cells with diphtheria toxin abrogated both the induction and maintenance of transplantation tolerance. Notably, FCM analysis revealed a significantly increased proportion of Treg cells—particularly $CXCR3^+$ Treg cells—within tolerant grafts compared with the spleen. Graft-infiltrating Treg cells exhibited significantly elevated expression of *Foxp3*, *Batf*, *Tbx21*, and *Il10* relative to splenic Treg cells. During the induction phase, inhibition of IFN-γ signaling, which is involved in T-bet expression in Treg cells, abrogated the induction of transplantation tolerance. Finally, the prolonged survival of regrafted tolerant allografts was dependent on graft-infiltrating Treg cells. These findings indicate that graft-infiltrating Treg cells differentiate into type 1 Treg cells and adapt to the allograft environment via IFN-γ signals, thereby protecting the allograft from immune-mediated rejection.

HOW PHAGOCYTES KNOW WHAT THEY ARE EATING: A PROXIMITY LABELING APPROACH TO INNATE IMMUNE RECOGNITION

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Innate immune recognition is often studied using purified microbial ligands or synthetic agonists that activate individual pattern-recognition receptors in isolation. While these reductionist strategies have defined many receptor–ligand interactions, they do not capture how immune cells interpret the **complex, multi-ligand surfaces of intact microorganisms**. As a result, our understanding of how multiple receptors cooperate, reinforce, or counterbalance one another to generate context-appropriate immune responses remains incomplete. A major barrier to understanding innate immune signaling integration is that, at the most basic level, we do not yet know which receptors and signaling proteins are physically present and engaged in the face of a particular intact microbe. Establishing this **receptor landscape** is the first necessary step toward deciphering how immune signaling pathways are coordinated.

To address this gap, we took advantage of the **phagosome**, a signaling hub and immune recognition interface formed during phagocytosis, and applied **proximity labeling** technology to develop **PhagoPL**, a spatial proteomics platform designed to selectively label and identify proteins recruited to phagosomes containing intact microbial cargo. By allowing the immune cell to encounter microbes in their native biochemical and structural forms, **PhagoPL preserves the physiological receptor cooperation, spatial organization, and signal integration that are lost when using isolated ligands**.

Applying PhagoPL to phagosomes containing distinct microorganisms, we identified **programmed death-ligand 1 (PD-L1)** as a protein specifically enriched in phagosomes containing **fungi** (Li et al., Nature 2024). We found that PD-L1 binds processed fungal components and interacts directly with the fungal ribosomal protein Rpl20b, identified using a surface-display ligand screen. Acute depletion of Rpl20b demonstrated that PD-L1 recognition cross-regulates IL-10 production driven by other innate immune receptors, revealing a previously unrecognized mechanism of fungal immune tuning. These findings show that PhagoPL can uncover context-dependent receptor–microbe interactions.

Building from this foundation, **the Li laboratory is currently using PhagoPL to understand immune recognition of pathogenic bacteria and tissue damage**. We are also functionally characterizing additional phagosomal proteins identified in our initial proteomic screens to determine how they influence innate immune decision-making.

Together, this work reframes the phagosome as an information-integration organelle in which receptor cooperation encodes microbial identity. By establishing which receptors are actually engaged at the intact host–microbe interface, PhagoPL provides the critical foundation required to understand how innate immune signaling is coordinated during infection and in immune-mediated diseases.

HOW DO NASAL MICROBES SHAPE T CELL IMMUNITY?

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Commensal microbes account for the vast majority of microbial immune interactions and are key regulators of our immune system. Previous studies of the skin find that certain commensals can stimulate $CD4^+$ and $CD8^+$ T cells across an intact epithelial barrier. Despite large differences in tissue architecture, many microbes that colonize the skin are also common nasal commensals. The nares represent a critical but understudied barrier site, serving as an early point of contact for inhaled pathogens and allergens while lying in close proximity to the brain and lungs. In this study, we investigate if nasal colonists are able to modulate T cell immunity.
We develop a system for targeted colonization of murine nares and characterize T cell responses to microbial species that colonize both the skin and nose. We observe striking, site-specific differences in the ability of microbial strains to elicit robust $CD8^+$ T cell responses. Nasal colonists prime $CD8^+$ T cells in the absence of overt inflammation in a process that requires migratory dendritic cells, suggesting a regulated mechanism by which the immune system samples nasal colonists. Using co-colonization models of the skin and nose, we further find that nasal microbial identity shapes distal skin T cell responses. Together, these findings highlight the nasal microbiota as an important regulator of T cell immunity with potential systemic consequences.

MULTI-FACTORIAL DISSECTION OF KEY PATHWAYS UNDERLYING TISSUE TREG GENE REGULATION AND FUNCTION

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Regulatory T cells (Tregs) are central regulators of tissue immune homeostasis and inflammatory circuits, yet the signals that specify tissue adapted Treg programs remain poorly defined. To dissect how canonical activation cues shape pan-tissue versus tissue-restricted transcriptional states, blood-derived Tregs were stimulated with a combination of TCR ligation, co-stimulation and cytokines and analyzed by RNAseq. We found that canonical TCR/CD28/IL2 signaling drives a large proportion of the pan-tissue Treg gene signature; however, additional tissue-associated cues polarized Tregs specifically towards skin or gut associated gene signatures. Having established key drivers of tissue Treg identity, we sought to understand the individual and combined contributions of core Treg activating signals including TCR, CD28, TNFR2-agonist and IL2. We observed non-linear patterns of gene regulation including additive, synergistic and antagonistic effects. We found distinct patterns of overlapping and unique gene regulation between canonical signals and even within the different modulators of costimulation: CD28 and TNFR2 agonism. This work provides novel insights into the signaling logic supporting tissue-immune regulation and offers a mechanistic framework for the design of Treg-augmenting immunotherapies.

INTERMEDIATE DOMAINS REGULATE TRIF OLIGOMERIZATION TO PROPAGATE TOLL-LIKE RECEPTOR SIGNALING

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Inducible oligomerization is a common activity of proteins that regulate signal transduction, yet the mechanisms that transition small, non-signaling protein complexes to signaling-competent oligomers are obscure. In this study, we explored this question in the context of TRIF, a regulator of Toll-like receptor (TLR) signaling. Studies of TRIF functions during TLR signaling led to biological models that depict TRIF as a scaffold for signaling protein activation. However, these models are largely speculative, as we have more insight into the activities of proteins that operate in the TRIF pathway than we do of TRIF itself. Herein, we report that the Toll/interleukin-1 receptor/Resistance protein (TIR) domain of TRIF is not sufficient to form oligomeric signaling complexes after TLR activation. We identified a functional region adjacent to the TIR, consisting of an AlphaFold-predicted extended helix and a proline-rich flexible domain. Ultrastructural and functional analyses demonstrated that this region does not impact the ability of isolated TIRs to form small filaments *in vitro*, but it is required for oligomerization and signal transduction of full-length TRIF in macrophages. In contrast, the RHIM and ARIES domains within TRIF are required for downstream signaling but not for oligomerization. These findings demonstrate that oligomer formation is not a necessary consequence of TIR interactions. Instead, a conserved pattern of domain organization ensures proper signal propagation following TLR activation, through a regulated dimer-to-oligomer formation process.

RESTRICTION OF INNATE Tγδ17 CELL PLASTICITY BY AN AP-1 REGULATORY AXIS

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Type 3 lymphocytes are defined by the transcription factor RORγt and include Th17 cells, ILC3s, and IL-17–producing γδ T cells (Tγδ17). A hallmark of this lineage is its high degree of effector plasticity, contributing to immunity against infection but also autoimmunity when dysregulated. While several studies have reported the emergence of IFNγ$^+$ IL-17A$^+$ Tγδ17 cells in inflammatory settings, the nature, context-dependence, and regulatory control of Tγδ17 cell plasticity remain to be defined. To address this, we combined type 3 fate mapping with single-cell RNA+ATAC multiome profiling to track Tγδ17 state transitions *in vivo*. At homeostasis, Tγδ17 cells maintained stable type 3 identity across tissues, including intestinal T-bet^{hi} Tγδ17 cells that restrained IFNγ production. In contrast, oral *Salmonella typhimurium* infection induced selective type 1 conversion in innate-like Vγ6$^+$ Tγδ17 cells, generating IFNγ single-producers with loss of IL-17A, intermediate RORγt downregulation, and a global shift toward type 1 effector expression program. Following attenuated *aroA*$^-$ *S. typhimurium* infection, fate-mapped Vγ6$^+$ ex-Tγδ17 cells persisted long after bacterial clearance, indicating durable effector remodeling. Converted cells were selectively marked by TIM-3 and displayed enhanced type 1 functionality. Integrative analysis of gene expression, chromatin accessibility, motif activity, and gene regulatory networks along the Vγ6$^+$ conversion trajectory identified a dominant AP-1 regulatory axis constraining plasticity. Genetic perturbation revealed JunB and Fosl2 restrict intestinal Tγδ17 conversion by stabilizing type 3 effectors (RORγt, IL-17A) and repressing type 1 effectors (T-bet, IFNγ). Moreover, genetic downmodulation of RORγt expression was sufficient to de-repress IFNγ. Conditional Fosl2 deletion in *Il17a*$^+$ fate-mapped cells reduced *S. typhimurium* burden, coincident with enhanced type 1 conversion of Vγ6$^+$ Tγδ17 and Th17 cells. CUT&RUN further showed extensive JunB–Fosl2 co-occupancy and direct JunB binding at type 3 (*Rorc(t), Il17a*) and type 1 (*Tbx21, Ifng*) regulatory elements, supporting a model in which AP-1 factors preserve type 3 identity while directly restraining type 1 reprogramming in Tγδ17 cells.

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TWO CONSERVED E-BOX MOTIFS IN THE *RAG1* PROMOTER SERVE AS A MOLECULAR SWITCH FOR V(D)J RECOMBINATION OF *TCR* AND *IgH* GENES.

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The vast diversity of antigen-specific receptors, T cell receptors (TCRs) and immunoglobulin (Ig), is a defining feature of adaptive immunity and is generated through V(D)J recombination mediated by the Rag1/2 complex. Because Rag1/2 expression is strictly restricted to developing T and B lymphocytes, its precise transcriptional control is fundamental to adaptive lymphoid lineage commitment.
We previously identified E2A-dependent, T- and B-cell–specific enhancers within the *Rag1/2* locus that are essential for chromatin accessibility, long-range chromatin interactions, and three-dimensional (3D) chromatin organization (Sci Immunol, 2020). However, how the *Rag1* promoter (*R1pro*) itself is regulated remained unknown. To address this, we generated a mutant mouse in which all seven E-box motifs within *R1pro* were disrupted to abolish E2A binding (*R1pro-Mut*). *R1pro-Mut* mice exhibited severe developmental arrest at the pro-B and DN3 stages in the bone marrow and thymus, respectively, accompanied by a profound loss of *Rag1* expression. In contrast to *Rag* enhancer–deficient mice, chromatin accessibility changes in *R1pro-Mut* cells were confined to the promoter region, and 3C assay revealed intact 3D chromatin architecture across the *Rag* locus. These results indicate that *Rag* enhancers and promoter control *Rag1* expression through mechanistically distinct pathways: enhancers regulate chromatin accessibility and long-range interactions, whereas the promoter governs transcriptional initiation.
Among the seven E-box motifs, two were evolutionarily conserved. Strikingly, reintroduction of only these two conserved E-boxes into the *R1pro-Mut* background (*R1pro-Res*) restored *Rag1* expression and normal T and B cell development. Integrated analyses of chromatin accessibility and histone modifications, and identification of E2A-associated novel transcription factors further underscored the critical role of conserved E-box motifs in *Rag1* transcription.
Together, our findings reveal distinct yet E2A-dependent roles of the *Rag1* enhancer and promoter and identify evolutionarily conserved E-box motifs in the *Rag1* promoter as a molecular switch that enables V(D)J recombination in adaptive lymphocytes.

HARNESSING ZBTB20-MEDIATED REPROGRAMMING OF CD4 T CELLS FOR CELL IMMUNOTHERAPY IN INTESTINAL INFLAMMATION

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Inflammatory bowel disease (IBD) poses significant health challenges that diminish the quality of life for patients; its increasing prevalence necessitates the generation of innovative therapeutic approaches. Our published data revealed that the expression of the master regulator transcription factor, Zbtb20, in endogenous regulatory T cells (Tregs), effectively protects mice against experimentally induced IBD. We hypothesize that genetically engineered Zbtb20-expressing conventional CD4 T-cells could serve as a novel therapy to treat and prevent IBD. To investigate this question, conventional mouse CD4 T cells were isolated and transduced by either Zbtb20-containing or empty vector (EV) retrovirus using the pMSCV-IRES-GFP construct. To determine the therapeutic potential, we adoptively co-transferred 300k T cells (Zbtb20 or EV transduced control) with 400k CD45RBhi CD4 T cells into the RAG1-/- mouse model of IBD (n=9). Our studies of Zbtb20-transduced cells in vitro indicated the significant upregulation of IL-10, a key immunomodulatory cytokine in intestinal inflammation. Strikingly, the adoptive transfers with the Zbtb20-transduced T cells into the T cell-induced RAG1-/- model led to protection from the disease phenotype. These data indicate that Zbtb20-transduced T cells produced in vitro have the potential to serve as a cellular therapy for IBD. Mechanistically, it also may suggest that Zbtb20 plays a role in modulating effector and regulatory programming of CD4 T cells in inflammatory environments, allowing for remediation of intestinal inflammation. Our studies propose a new approach to cellular immunotherapy in IBD, taking advantage of immunoplasticity by manipulation of transcriptional programming.

DYSREGULATED KERATINOCYTE RESPONSE TO CANDIDALYSIN DISRUPTS EPITHELIAL–IMMUNE CROSSTALK AND DRIVES CHRONIC CUTANEOUS CANDIDIASIS IN KID SYNDROME

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The role of immune cells in the skin's immune response to *Candida* has been extensively studied; yet, definitive in vivo evidence of keratinocytes' crucial role is lacking. Chronic cutaneous candidiasis (CCC) complicates Keratitis-Ichthyosis-Deafness (KID) syndrome, caused by mutations in the GJB2 gene. Since GJB2 is expressed in epithelial cells, we hypothesize that a keratinocyte dysfunction due to the KID mutations has impaired the skin's immune response to Candida.

To evaluate this hypothesis, we generated mice with keratinocyte-specific expression of Cx26 S17F, a common KID-associated mutation (epiKID). Upon *C. albicans* application, control mice eradicated the fungi, with significant accumulation of neutrophils in the stratum corneum. In contrast, epiKID mice developed progressive hyperkeratosis, with numerous *Candida* yeasts in the stratum corneum and hyphae invading the epidermis, accompanied by minimal neutrophil infiltration into the epidermis and stratum corneum. These phenotypic and histological features closely resembled those observed in CCC in KID patients. Bulk RNA sequencing showed that epiKID epidermis had blunted transcriptional responses to *Candida*, including reduced upregulation of pattern recognition receptors, chemokines for neutrophils, and antimicrobial peptides genes. This impaired response to *Candida* was independent of IL-17, as comparable infiltration of IL-17-producing γδ and αβ T cells was observed in both epiKID and control mice. On the other hand, the impaired epidermis response was pathogen-specific as epiKID mice eradicated *M. pachydermatis*, similar to control mice. Candidalysin-deficient *C. albicans* (ece1Δ) failed to induce CCC-like phenotype in epiKID mice, indicating the essential role of this toxin in *Candida* chronicity in epiKID mice. 3D structures from primary keratinocytes isolated from control mice showed upregulation of chemokine and AMPs genes upon stimulation with candidalysin. On the other hand, epiKID 3D stimulated with candidalysin showed aberrant keratinization and impaired expression of chemokine and AMP genes.

In conclusion, this study provides the first in vivo evidence that keratinocytes are indispensable for antifungal immunity. The KID-associated mutation in keratinocytes is sufficient to compromise anti-*Candida* defense, at least in part, by impairing epidermal response to candidalysin, attenuating chemokine-mediated neutrophil recruitment.

ORIGINS AND FUNCTIONAL DETERMINANTS OF LY49+ CD8 T CELLS

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Ly49 refers to a family of surface receptors that has been most carefully studied in natural killer cells, but that is also expressed by a subset of CD8 T cells. Ly49+ CD8 T cells have been termed CD8 regulatory T cells (CD8 Tregs) owing to their ability to control immune responses in various experimental settings. It is still not known how Ly49+ CD8 T cells arise, but the transcription factor Helios has been implicated in the stability of CD8 Tregs. We set out to better understand the development of Ly49+ T cells under homeostatic conditions. We find that, despite being called CD8 Tregs, many splenic Ly49+ T cells lack expression of both CD4 and CD8 co-receptors. A population of Ly49+ T cells expresses only the CD8α homodimer, and lacks CD8β expression. Furthermore, CD8 co-receptor expression can change dynamically upon stimulation with IL-15. These features are true primarily for the Helios-expressing sub-population of Ly49+ T cells. These properties suggested a possible connection between splenic Ly49+ T cells and CD8αα+ intraepithelial lymphocytes (IELs) of the small intestine, and we have investigated this connection using integrin blockade and splenocyte transfer experiments. Given that Helios has been shown to be important for CD8 Treg function, we examined the effect of Helios deletion on small intestinal CD8αα+ IELs. Finally, we identified T cell receptor (TCR) sequences among splenic Ly49+ T cells that have been previously associated with small intestinal CD8αα+ IELs. We hypothesize that T cells expressing TCRs that receive agonist selection signals in the thymus can give rise to both splenic Ly49+ T cells and small intestinal CD8αα+ IELs. We are testing this model experimentally using the TCRs we sequenced from splenic Ly49+ T cells.

UPREGULATION OF CD90 EXPRESSION PINPOINTS T LINEAGE COMMITMENT WITHIN DN2A THYMOCYTES.

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Early thymic progenitors (ETPs) retain access to alternative fates but are known to commit to the T cell lineage as they transition through the $CD4^{-}CD8^{-}$ double-negative (DN) stages of T cell development in the thymus. ETPs are c-$Kit^{+}CD44^{+}$ DN thymocytes, and upregulation of CD25 marks their progression to the DN2a stage. Our current understanding is that T lineage commitment occurs with the DN2a-to-DN2b transition, marked by downregulation of c-Kit and CD44 and activation of *Bcl11b*. However, *Bcl11b* reporter mice revealed heterogeneity within DN2a thymocytes, with *Bcl11b*$^{-}$ and *Bcl11b*$^{+}$ subsets differing in the expression of various genes, including *Thy1* and *Cd34*, and in alternative lineage potential. Despite these findings, prior studies have primarily examined DN2a thymocytes at the population level. Thus, the developmental relationships among potential DN2 subsets remain unclear.

To more precisely address this T lineage commitment checkpoint, we profiled the DN stages in the mouse thymus using single-cell multiomics. This revealed two distinct DN2a subpopulations: DN2a-1 and DN2a-2. DN2a-1 cells expressed high levels of CD34 but low levels of CD90 and *Bcl11b* compared with DN2a-2 cells. DN2a-1 thymocytes maintained expression of multipotency-associated markers, including *Spi1* and *Bcl11a*, at levels similar to ETPs, whereas DN2a-2 cells displayed features of committed T-lineage cells. Recapitulation of T cell development in vitro revealed that DN2a-1 thymocytes were more developmentally advanced than ETPs, but still multipotent, whereas DN2a-2 thymocytes were entirely restricted to the T lineage. Consistent with this, *in vivo* clonal lineage tracing demonstrated that both subsets belong to the same developmental trajectory. We showed that ETPs can give rise to B, NK, and myeloid cells *in vitro* and *in vivo*, consistent with previous studies, but B lineage potential was lost in DN2a-1 thymocytes.

Together, these findings show that what was previously defined as the DN2a stage is, in fact, a heterogeneous population that can be resolved into pre- and post-T lineage-committed cells based on CD34 and CD90 expression. DN2a-1 and DN2a-2 thymocytes align with previously defined *Bcl11b*-YFP^{-} and *Bcl11b*-YFP^{+} DN2a thymocytes, respectively. We have thus identified the precise timing of T lineage commitment during T cell development in the mouse thymus.

CHROMOSOME STRUCTURE REMODELING IN INNATE IMMUNE TRAINING AND GENE REGULATION

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Macrophages exhibit the capacity for long-lasting trained immune memory after prior stimuli, but the underlying mechanisms that maintain this state are not well understood. Previous studies have largely focused on histone modifications that define the enhancer landscape. Although these marks have been shown to be highly durable, they are, in principle, reversible through the action of eraser enzymes. Two primary mechanisms have been proposed to explain the maintenance of the enhancer landscape: sustained activity of signal-dependent transcription factors (SDTFs) or the engagement of lineage determining transcription factors (LDTFs). Here, we show that immune training also induces changes in chromosome organization and nuclear localization, suggesting a novel memory mechanism for maintaining the trained state of stimulus-exposed macrophages. Specifically, we report that immune training induces large-scale chromosome structural rearrangements that are associated with potentiated gene expression responses. Using high-depth Hi-C sequencing analysis and Integrative Genome Modeling (IGM), we reveal stimulus-specific changes in chromosome structure in human macrophages. By integrating Hi-C data with ATAC-seq and H3K4me1 CUT&Tag profiles, we found that these structural changes coincide with DNA accessibility and poised enhancer activity. Furthermore, by modeling the ensemble of single-cell 3D genome structures, we identified correlations between structural features, such as radial positioning, distance to the nuclear lamina, and proximity to nuclear speckles with stimulus specific gene expression. Finally, to mechanistically link these observations, we are developing a quantitative model that relates three-dimensional structural relationships, histone modifications and gene expression. Together, our findings suggest that innate immune training involves rearrangements of large-scale chromosome structure and nuclear positioning that contribute to the durability of innate immune memory.

FOXP3 M370I MUTATION ALLOWS THE ACTIVATION OF T EFFECTOR PROGRAMS IN REGULATORY T CELLS

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The transcription factor Foxp3 in mice (FOXP3 in human) dictates the development and function of regulatory T cells (Tregs) to maintain self-tolerance. The mutation of amino acid 370 (M to I) of FOXP3 protein was identified in IPEX patients with type-2 autoimmunity. To study the mechanism, we generated an M370I-knock in (KI) mice. The homozygous KI (Foxp3M370I/M370I) mice and the hemizygous KI (Foxp3M370I/Y) mice showed T cell activation with multi-organ inflammation. The KI Tregs can produce all effector T cell-related cytokines and Foxp3/CD25 was reduced. Heterozygous KI female (Foxp3M370I/GFP) mice which were healthy but KI Tregs still keep the intrinsic defects. The RNA-seq showed that KI Tregs from het have lower expression of genes related to Treg suppressive function. Ex vivo activation can restore Treg signatures. Importantly, in vitro suppression assay showed that activated KI Tregs can still suppress Tconv proliferation. IP-MS of Foxp3 showed an altered pattern of FOXP3 interacting proteins between WT and KI Tregs, including NFAT and RUNX1, two known transcription factors for regulating T effector and Treg programs. Structural modelling indicated that M370I may disrupt head-to-head dimerization of FOXP3. Another FOXP3 IPEX mutation F374C that may form an intra-domain disulfide to favor swap-domain dimer, we further generated FOXP3-F374C mutant mice which had similar phenotypes. Thus, our results indicate that enhanced formation of swap dimer of FOXP3 may preferentially dampened T effector programs silencing. Our findings indicate that silencing of T effector-like program and acquiring of suppressive function mediated by Foxp3 in Tregs can be separated, and targeting specific sites of FOXP3 for immune modulation is possible.
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REGULATION OF PERIPHERAL T CELL POPULATION SIZE BY CORONIN 1-DEPENDENT CELL DENSITY SENSING

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The establishment and maintenance of the appropriate number of peripheral T cells is important for the proper functioning of the immune system. In mammals, T cells are produced in the thymus before migrating to peripheral tissues early in life. Despite progressive thymic involution and the consequent decline of T-cell output, total T-cell numbers in the periphery are largely maintained with age in healthy adults. This raises an important question: what mechanisms ensure that circulating T cell numbers remain stable at appropriate levels, preventing both deficiency and excess? We found that coronin 1, a member of the evolutionarily conserved coronin protein family, plays an essential cell-intrinsic role in enabling T cells to reach and maintain appropriate population sizes. We have analysed the signaling pathways and molecular mechanisms underlying coronin 1-dependent regulation of T cell population size. We found that the coronin 1 pathway is important not only for maintaining the naïve T cell pool but also for generating T cell responses upon auto- and alloimmune stimulation, yet dispensable for immunity against pathogenic microbes. Work will be presented suggesting that coronin 1 operates within a previously uncharacterized cell-intrinsic pathway that couples cell density information to pro-survival signaling, regulating both peripheral T cell numbers and T cell-mediated immunity in health and disease.

MARKER-BASED CRISPR SCREENINGS TO CHARACTERIZE KINASES CONTROLLING MACROPHAGE ACTIVATION

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Identifying the complete set of components governing cellular signalling circuitries is a critical yet remarkably complex task in mammalian immune cells. Pathways controlling innate immune activation in response to diverse stimuli have been extensively characterized; however, it remains unclear whether the full complement of regulators shaping signal transduction and downstream gene expression has been identified. Protein kinases form the backbone of most signalling cascades in immune cells and have therefore been attractive targets for therapeutic intervention.

To systematically define the repertoire of kinases controlling macrophage activation, we developed a kinase-focused CRISPR screening strategy using the production of pro- or anti-inflammatory molecules as functional readouts. Bone marrow–derived macrophages were stimulated with either lipopolysaccharide (LPS) or interleukin-4 (IL-4) to induce divergent signalling programs and gene expression states. Stimulus-specific inducible markers were used to quantify pathway output, enabling the isolation and sequencing of cells displaying high or low marker expression following kinase inactivation.

These screens robustly recapitulated the majority of known positive and negative regulators of LPS- and IL-4-induced signalling. In addition, they uncovered multiple previously unrecognized kinases that modulate macrophage activation and transcriptional responses. Follow-up analyses integrating genetic perturbation, signalling dynamics, and transcriptional profiling further revealed that selected kinases influence macrophage activation through coordinated interactions across multiple regulatory layers, including unexpected cross-talk with additional signalling networks. Together, this work expands the landscape of kinases governing innate immune cell activation and establishes a modular, scalable framework to dissect signalling–gene expression coupling in immune cells.

FROM GENE EXPRESSION TO THE ORGANIZATION OF DIFFERENTIATED THYMIC EPITHELIAL CELLS

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The thymus contains a multitude of epithelial cell types that work in concert to educate immature T cells called thymocytes to distinguish self from non-self. A subset of thymic epithelial cells (TECs) express lineage defining transcription factors and differentiate into cell types -- such as muscle, neuroendocrine, and tuft cells -- that are typically found in peripheral tissues. However, the function and organization of these differentiated TECs especially in the human thymus are poorly understood. Specifically, much remains unknown about the gene regulation that enable their function, and their structural morphology and spatial localization in the thymus to ultimately orchestrate thymocyte education. To resolve the diversity of TECs at a single cell resolution, we simultaneously profiled the gene expression and chromatin accessibility of human thymus samples, and integrated it with all available single cell datasets to establish concordant cell annotations across studies. We identified unique gene signatures in the differentiated thymic TECs compared to their peripheral counterparts in 31 tissues using the CELLxGENE database. Differentiated TECs share substantial celltype restricted genes, yet take on a celltype specific identity, a phenomenon not shared by their peripheral counterparts. Zooming out from the expression of single maturing thymic muscle cells to cellular morphology in 2D tissue sections, we show that they have distinct morphologies as they mature. Further zooming out to a 3D whole-lobule level, the thymic muscle cells occupy distinct micro-environmental niches as they progress along their developmental path. Overall, we use a combination of genomic and imaging techniques to show that differentiated thymic celltypes, while sharing similarity to their peripheral counterparts, take on a thymus specific identity.

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SINGLE-CELL MULTIOMICS REVEALS ARCHETYPAL REGULATORY PROGRAMS SHARED ACROSS CD4 AND CD8 T CELL SUBSETS IN VIRAL INFECTION

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T cells protect against pathogens and tumors and can differentiate into various functionally distinct subsets. While each subset exhibits a characteristic epigenomic and transcriptional profile, essential immune functions such as self-renewal, expansion, cytokine production, and cytotoxicity are common to multiple subsets and therefore must be programmed via shared regulatory mechanisms. To uncover these regulatory “archetypes”, we integrated a new single-cell multiomic (scATAC+RNA-seq) dataset for CD4 and CD8 T cells responding to acute and chronic viral infection with a comprehensive compendium of bulk ATAC-seq data. Our analysis revealed that T cell identity is governed by a modular architecture, where distinct transcription factors drive the reuse of regulatory archetypes across T cell subsets and lineages. Notably, translationally important progenitor Tcf1+ CD8 T cells, critical for sustaining CD8 T cell responses and present in both acute and chronic infection, exhibited a composite regulatory state combining a CD8 T cell exhaustion program and, unexpectedly, a follicular helper CD4 T cell program. In sum, this resource will aid mechanistic dissection of adaptive immunity and immunotherapy design, and the framework will be broadly applicable across biological systems.

REGULATORY ROLE OF PU.1 AND OCT-2 IN TRANSCRIPTION OF ACTIVATION-INDUCED CYTIDINE DEAMINASE

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Activation-induced cytidine deaminase (AID), encoded by *Aicda*, is essential for B-cell somatic hypermutation, class switch recombination, and central B-cell tolerance. Because of its potent mutagenic activity, *Aicda* expression in mature B cells is tightly controlled by multiple regulatory mechanisms acting through four major cis-regulatory regions. How *Aicda* expression is regulated during precursor and early B-cell development remains poorly understood. Dysregulated AID expression at these stages has been implicated in genomic instability and leukemogenesis. We previously found that in a double-knockout mouse model lacking the ETS family members Spi-B and PU.1, mice develop precursor B-cell acute lymphoblastic leukemia (pre-B-ALL) with a mutational signature of AID and increased *Aicda* expression, suggesting that PU.1 acts as a negative regulator of *Aicda*. Adjacent binding sites within a negative regulatory domain in intron 1 (Region R2-1) were identified for PU.1 and the POU family transcription factor Oct-2, a B-cell-restricted factor involved in germinal center and immunoglobulin gene regulation. Thus, we hypothesized that *Aicda* regulation at R2-1 is mediated by direct, developmentally stage-specific interactions of key transcription factors. Using CRISPR-mediated mutagenesis in a pre-B mouse cell line, a clone harboring heterozygous mutations in both PU.1 and Oct-2 motifs in R2-1 exhibited significantly increased *Aicda* expression under LPS stimulation, as well as reduced Oct-2 binding without significant changes in PU.1 occupancy. In contrast, a clone with complete deletion of the R2-1 Oct-2 motif and a heterozygous mutation in the PU.1 site displayed significantly decreased *Aicda* expression following LPS treatment. Interestingly, this clone showed increased binding of both PU.1 and Oct-2 at the mutated loci. Together, these findings suggest that R2-1 functions as a molecular switch capable of activating or repressing *Aicda* expression depending on the precise combination of mutations. Ongoing investigations aim to elucidate the mechanistic basis of this regulatory switch and its contribution to pre-B-ALL pathogenesis, with potential implications for therapeutic targeting of aberrant AID mutagenesis.

IMMUNOMODULATORY EFFECTS OF COMBINATION THERAPY OF PHOSPHOLIPID NANOPARTICLES AND KETONE BODY ESTER (VBI-O) IN TREATMENT OF SEPTIC SHOCK.

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Background: Systemic inflammatory response syndrome (SIRS) is a major cause of extensive tissue damage, multi-organ dysfunction, and death in septic shock patients. Antibiotic resistance and the lack of FDA approved drugs result in a persistently high mortality. There is a need for non-antibiotic dependent treatments for SIRS and MODS. Hence, Vivacelle Bio, is developing a novel combination therapy, VBI-Optimum (VBI-O), consisting of phospholipid nanoparticles (VBI-1) and the ketone body ethyl-3-hydroxybutyrate (EHB), for treatment of septic shock. VBI-O is shown to overcome sepsis in a cecal ligation and laceration (CLL) rat model. Intraperitoneal injection of VBI-O (100% VBI-1+125mM EHB) in CLL rats causes complete recovery in 50-88% of rats from lethal sepsis as compared to control rats that died within 24-36hr. As M1 macrophages primarily initiate the early inflammatory response, we hypothesized that VBI-O may aid in recovery of the CLL rats by affecting M1 macrophages. Hence, the present study initially evaluated the immunomodulatory potential of VBI-O on pro-inflammatory macrophage functions in an *in-vitro* culture model.
Methods: Human THP-1 monocytes were first differentiated to M0 macrophages with phorbol-myristate-acetate (PMA: 48h), followed by 48hr differentiation to M1 lineage with 100ng/ml lipopolysaccharide (LPS) and 20ng/ml interferon gamma (IFN-γ). M1 macrophages were treated for 24hr with different combinations of VBI-1 (1%, 0.1%, 0.01%, 0.01%), EHB (67,5mM, 40mM, 20mM; 10mM) or VBI-O (0.1% VBI-1+ 20mM EHB) in presence of LPS and IFN-γ. Control included M1 cells cultured in serum containing RPMI-1640 media with or without the aqueous phase. Immunomodulatory effects were assessed by studying expression of pro-inflammatory genes (CXCL10, CCR7; IL-1β) by quantitative RT-PCR. Cytotoxicity was assessed by MTT assay and Trypan blue staining. The data was analyzed by Brown-Forsythe and Welsch ANOVA, nonparametric t-test and Mann Whitney U test.
Results: Statistically significant decline ($P<0.05$) in expression of pro-inflammatory CXCL10, CCR7 and IL-1β genes was observed in M1 macrophages with VBI-1 (1%, 0.1%, 0.01%, 0.001%), and EHB (67.5mM, 40mM and 20mM). However, VBI-1 was found to be cytotoxic at 1% concentration while EHB was cytotoxic at 67.5 mM and 40mM concentration. Also, the non-toxic combination of VBI-1 (0.1%) and EHB (20mM), further reduced the expression of these genes in M1 macrophages, as compared to individual treatments (VBI-1:1-2%; EHB:13-24%)
Conclusion: The combination of VBI-1 and EHB is immunomodulatory, as it decreases the expression of pro-inflammatory genes in M1 macrophages. Thus, we anticipate that this may be the major mechanism by which VBI-O aids in recovery of the CLL rats. Ongoing research will further explore the mechanistic potential and study the role of VBI-O on macrophage modulation in CLL rats.

NFkB cRel PROTEIN AMOUNT DETERMINES THE SELECTION OF GERMINAL CENTER B CELLS

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Germinal Centers (GCs) are the site of B cell proliferation coupled with somatic hypermutation of the B cell receptors (BCRs) to produce high-affinity antibodies. GC B cells circulate between two distinct zones: the light zone (LZ) and the dark zone (DZ). Proliferation and somatic hypermutation occur in the DZ and transit to the LZ to test for BCR affinity. GC B cells are selected based on their BCR affinity to differentiate into plasma cells in LZ. Following selection in the LZ, GC B cells can also reenter the DZ for additional rounds of division and somatic hypermutation. A higher expression of the cell cycle regulator cMyc characterizes the positively selected GC B cells. High cMyc-expressing LZ GC B cells show enhanced expression of the NFkB gene signatures, and importantly, cMyc is a cRel target gene. It has been demonstrated that the transcription factor NFkB cRel is required for B cell proliferation and GC B cell maintenance. cRel-deleted GC B cells show reduced somatic hypermutation and affinity maturation. Surprisingly, transgenic overexpression of cRel in GC B cells does not affect somatic hypermutation and reduce, if any, affinity maturation. Hence, the function of cRel in regulating GC B cell selection remains unknown.

To understand the function of cRel in GC B cell selection, we have generated cRel fluorescence reporter mice (mTFP1-cRel) and measured the kinetics of cRel expression at the protein level in GC B cells. We found that cRel expression is higher in GC B cells compared to non-GC B cells. Analysis of cRel expression in DZ and LZ B cells showed that about 10% of LZ B cells increased cRel expression compared to DZ B cells. We discovered that high cRel-expressing LZ B cells have higher expression of Myc, and cell cycle regulator CyclinD2. Surprisingly, cRel expression at the mRNA level remains similar in low and high cRel protein-expressing GC B cells. Higher somatic hypermutation leads to higher affinity maturation. We examined somatic hypermutation in the low and high cRel expressing LZ B cells by sequencing IgH JH4-intronic enhancer downstream of the rearranged VJ558DJH4 element. We found that high cRel-expressing LZ B cells have a higher somatic hypermutation than low cRel-expressing LZ B cells. Single cell study reveals high cRel LZ B cells are enriched for precursor plasma cells. Overall, our results suggest that cRel protein but not transcript levels regulate GC B cell selection. Our studies resolve conflicting findings about the function of cRel in GC B cells somatic hypermutation and affinity maturation.

IL-10–STAT3–DEPENDENT BILE ACID INACTIVATION LIMITS TGR5-MEDIATED TRAINED IMMUNITY

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Trained immunity (TI) induces antigen-agnostic enhancement of host defense against viral infections through transcriptional and epigenetic reprogramming of innate immune cells, promoting interferon (IFN) responses and MHC class II antigen presentation. In contrast, interleukin-10 (IL-10), a prototypical anti-inflammatory cytokine, inhibits TI by inducing STAT3-dependent tolerance programs. Notably, STAT3 has been shown to suppress bile acid (BA) biosynthesis, linking immune regulation to metabolic control. In parallel, BA have emerged as modulators of innate antiviral interferon responses. Whether IL-10–STAT3 signaling regulates BA metabolism as a mechanism to constrain the establishment of TI and antiviral defense remains unexplored. We performed integrated analyses of plasma metabolomics, bulk RNA-seq, and single-cell multi-omic profiling (scRNA-seq and scATAC-seq) from participants in the NIH IMPACC study to define transcriptional, metabolic, and chromatin programs associated with TI and IL-10 signaling. Chromatin accessibility at differentially accessible regions was analyzed together with transcription factor motif enrichment. Regulation of BA metabolism was assessed through STAT3-associated transcriptional programs. To validate the generalizability of these regulatory programs in an independent viral context, we analyzed DOGMA-seq data—including scRNA-seq, scATAC-seq, surface protein profiling, and quantification of HIV viral DNA and RNA—from memory $CD4^+$ T cells of antiretroviral therapy (ART)-suppressed people with HIV. Integrated multi-omic analyses identified the BA receptor TGR5 as a key mediator of TI induced by the unconjugated BA UDCA and DCA, associated with enrichment of STAT1- and CREB1-binding regulatory elements at interferon-stimulated gene (ISG) and MHC-II antigen-presentation loci, and accompanied by suppression of IL-10/STAT3 signaling at both transcriptional and chromatin-accessibility levels. We further found that STAT3 activation regulates enhancer activity at BA-modifying enzymes, including SULT2A1 and UGT2B7, leading to BA sulfation and glucuronidation. Functionally, sulfated and glucuronidated BA failed to activate TGR5, whereas unconjugated BA robustly engaged TGR5 signaling. Together, these findings indicate that IL-10 attenuates trained immunity by promoting BA conjugation and functional inactivation of TGR5 signaling. Notably, a similar IL-10–STAT3–associated metabolic program was observed in an independent viral context, where $vDNA^+$ memory $CD4^+$ T cells from people with HIV exhibited elevated IL-10 signaling compared with $vDNA^-$ counterparts, accompanied by altered expression of BA metabolic pathways. These findings identify IL-10–controlled BA remodeling as a metabolic checkpoint that constrains innate immune memory and antiviral immunity across viral infections.

DEFINING THE ROLL OF HDAC3 ENZYMATIC VS. ADAPTOR FUNCTION IN T CELLS

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T cells are an essential component of the adaptive immune system and are critical for an effective response against pathogens and cancer. Generation of T cells requires dynamic epigenetic alteration of histones to control gene expression at each developmental stage. The Class I histone deacetylases HDAC1, HDAC2, and HDAC3 have been shown to have 3 functions: adaptor, deacetylase (KAc), and decrotonylase (KCr) activity. The relative importance of each function in T cells is not known. CD2icre HDAC3 cKO mice develop severe colitis and demonstrate that HDAC3 is required during thymocyte development for positive selection, $CD4^+CD8^+$ (DP) survival, and $CD4^+$ lineage commitment. We generated a novel mouse model with Cre-mediated deletion of endogenous HDAC3 and linked re-expression of HDAC3 VRPP, which maintained adaptor function but lacks enzymatic activity. Like CD2icre HDAC3 cKO mice, HDAC3 VRPP mice have a block in positive selection due to failure to downregulate RORγt and decreased DP cell survival due to increased expression of P2RX7, demonstrating that HDAC3 enzymatic activity is required for these functions. Although fewer T cells are produced in HDAC3 VRPP mice, when the positive selection defect is bypassed, $CD4^+$ lineage commitment is restored. Strikingly, this mouse does not develop spontaneous colitis even up to one year of age, although T cell development is still impaired. Thus, there are different roles for HDAC3 enzymatic vs. adaptor function during T cell development which are made evident in HDAC3 VRPP mice.

PD-L1 IS AN INTRINSIC SWITCH FOR NATURAL KILLER CELL MEDIATED TRAIL DEPENDENT ANTIVIRAL FUNCTION.

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Each year, influenza A virus (IAV) infection of the lung causes half a million deaths worldwide. Patients with compromised immunity experience distinct influenza pathogenesis; however, most IAV-related research is done with wildtype mice or people who are otherwise healthy. We utilize a model of immunocompromised recombination-activating gene 1 (Rag1)-knockout mice to discover NK cell activation and regulation mechanisms during IAV infection. Treatment of IAV-challenged Rag1-KO mice with a monoclonal antibody (mAb) targeting Programmed Death Ligand 1 (PD-L1) triggers NK cell-intrinsic signaling of PD-L1 and significantly delays lethality. This treatment upregulates tumor necrosis factor related apoptosis-inducing ligand (TRAIL) on NK cells downstream of PD-L1 signaling and is required for the benefits of PD-L1 mAb treatment in IAV-challenged Rag1-KO mice. These results present a paradigm shift for understanding the innate immune response to respiratory virus infections, offering an alternative approach for therapeutic treatment of IAV infections in patients with compromised immunity.

A NON-REDUNDANT ROLE FOR CCL19 IN T CELL HOMING AND POSITIONING IN STEADY STATE AND INFLAMMATION

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CCL19 and CCL21 are chemokines constitutively expressed by T-zone fibroblastic reticular cells in secondary lymphoid organs during homeostasis. Because CCL19-deficient mice display minimal overt phenotypes under homeostatic conditions in previous studies, CCL19 has largely been considered functionally redundant with CCL21, leaving its in vivo roles incompletely understood. Here, we investigate the functions of CCL19 across homeostatic, acute inflammation, and chronic viral infection settings. At steady state, CCL19 deficiency impairs the homing of naïve T cells to the splenic white pulp, and intravascular labeling reveals an increased proportion of blood-exposed naïve CD8+ T cells. During acute inflammation and lymphocytic choriomeningitis virus Armstrong (LCMV-Arm) infection – conditions in which CCL21 is markedly downregulated – CCL19 is required for efficient recruitment of naïve T cells into the splenic white pulp, maintenance of the splenic T-zone, and lymphocyte recruitment into inflamed lymph nodes. CCL19-deficient mice chronically infected with LCMV-Clone 13 exhibit increased accumulation of CD8+ T cells in the splenic red pulp and a higher proportion of exhausted CD8+ T cells. In summary, our findings substantiate that CCL19 is non-redundantly required for efficient T cell homing and proper positioning in both steady and inflammatory contexts.

MATURE CD8 T CELL FATE ALLOCATION IS EXQUISITELY CONTROLLED BY DOSE OF BCL11B

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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 and adipose systems among others, and increases risk of some cancers. Of particular interest to us is its role in T lymphocytes, a collection of lineages that are dependent upon Bcl11b expression to establish and maintain the T cell gene expression program.

Complete Bcl11b loss is incompatible with the normal development, function and underlying lineage integrity of mature thymus-derived lineages. As a result, homozygous Bcl11b deficient cells are often absent, or are so altered as to have 'buried the evidence' of how Bcl11b maintained the lineage while it was still present. However, systems of partial or inducible Bcl11b loss can be used to interrogate the protein's function in the mature T cell lineages.

Using multiple complementary genetic models of Bcl11b gene dosage reduction, we have revealed that a <2-fold reduction of Bcl11b protein level redirects CD8 T cell differentiation preferentially to virtual memory fate, cell-autonomously, at the expense of the production of naïve T cells. RNA-seq and functional criteria clearly define the emergent phenotype, despite minimal change in chromatin accessibility at Bcl11b binding sites genome-wide in Bcl11b dose-reduced T cells. Timed conditional knockouts and single-cell RNA-seq narrow the developmental window involved and showed that Bcl11b levels determine diversion to virtual memory fate during intrathymic positive selection. Bcl11b haploinsufficiency biased CD8 T cell development in cohorts from the first days after birth through adult life and was not mimicked by post-thymic homeostatic processes. Thus, Bcl11b levels hypersensitively gate a unique intrathymic choice point at which cells interpret positive selection and maturation signal strength to determine a conventional naive or virtual memory biased fate.

INTERFERON GAMMA TRANSCRIPTIONAL MEMORY IS SEX-DEPENDENT, PRECISION-BASED, AND REGULATED BY A DUAL-FUNCTION TRANSCRIPTION FACTOR

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The innate immune system possesses a functional memory, known as trained immunity, where an initial stimulus induces stable cellular reprogramming for enhanced, non-specific hyper-responsiveness to subsequent challenges. This effect is achieved by priming, a preparatory process by which a cell is exposed to a cue that sensitizes it to a second, distinct signal (a trigger). Transcriptional memory, a specialized form of priming where the initial and recall signals are identical, remains mechanistically elusive, particularly regarding human variability. We utilize the interferon-gamma (IFN-γ) signaling as a model to investigate the regulatory mechanisms governing this phenomenon.

In the current study, using primary human macrophages, we unveil several novel principles of IFN-γ transcriptional memory. First, we establish that IFN-γ memory is a sex-selective process achieved by a reduction of variability in gene expression upon second stimulation, thereby defining IFN-γ recall as a function of transcriptional precision. We further characterize a novel family of genes displaying this memory and demonstrate that the effect is highly dependent on the cytokine concentration.

Mechanistically, we determined a dominant role of IFN-γ priming over other inflammatory signals. Moreover, our data shows that memory is critically dependent on the cellular phenotype, requiring differentiation for recall in some genes while for others differentiation being sufficient. Most significantly, we discovered a dual, opposing role for a key transcription factor in IFN-γ -mediated memory. It functions as an inhibitor of basal gene expression on one hand, but is simultaneously required for the maintenance of the memory state on the other.

Collectively, our findings redefine the IFN-γ transcriptional memory program. We establish this process as a sex-dependent phenomenon of gene expression precision, revealing a novel regulatory network governed by differentiation and a transcription factor's dual function. These insights provide critical, systems-level context for understanding immune heterogeneity and are crucial for the rational design of personalized therapies that safely harness innate immune memory.

A NOVEL SILICON-BASED PLATFORM FOR MULTIPLEXED INTRACELLULAR DELIVERY IN NEXT-GENERATION IMMUNE CELL ENGINEERING

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Immune cell engineering has transformative potential for cancer, autoimmunity, and infectious disease therapies, yet efficient delivery of modifying materials without compromising viability or phenotype remains challenging, especially in sensitive or unstimulated cells. We developed a silicon-based mechanoporation platform that transiently opens cell membranes by passing cells through pores on a thin silicon chip, enabling diffusion of CRISPR RNPs, mRNA, circRNA, siRNA, peptides, and viral vectors into the cytoplasm. This method is compatible with naive and activated T cells, B cells, NK cells, monocytes, neutrophils, iPSCs, and HSCs. Using the platform, we achieved multiplexed delivery in primary unstimulated T cells with a B2M-targeting CRISPR RNP, GFP mRNA, and fluorescent dextran, with 85% of live cells showing B2M knockout and co-expression of GFP and dextran. We additionally delivered GFP mRNA to PBMCs and neutrophils in whole blood with high viability and efficiency. The platform was used to enhance viral engineering by loading primary human B cells with a circRNA transduction enhancer, which increased GFP lentiviral expression. Demonstrating the potential for simplified manufacturing, T cells were engineered directly from whole-blood with CD19 and IL-2 cirRNAs with >50% double-positive T cells and >90% viability. The platform can engineer T cells at clinical scaleat over 1×10^9 cells per minute. In summary, this mechanoporation platform provides scalable, gentle, and versatile intracellular delivery, enabling multiplexed genetic and molecular engineering across diverse primary cells while preserving viability and phenotype, reducing manufacturing time and cost, and supporting next-generation cell therapies.

CIC IS A NEGATIVE REGULATOR OF MHCII EXPRESSION AND INFLAMMATORY RESPONSES IN ALVEOLAR MACROPHAGES

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Macrophages play a crucial role in surveying the host environment for invading pathogens and tumors. In the lungs, tissue-resident alveolar macrophages (AMs) sample the airspace and initiate the first line of defence against infection. One key function of macrophages at sites of infection is coordinating the activation and effector function of responding $CD4^+$ T cells. Regulation of T cell responses in the lungs is critically important, as prolonged T cell activation can lead to long-term lung damage and impaired gas exchange. However, how lung-specific macrophages regulate these responses remains unclear.

MHCII regulation in myeloid-derived macrophages is well studied, however our understanding of MHCII regulation in AMs remains limited. This gap in knowledge impedes our ability to effectively target lung immune responses and inflammation. Given the key differences in physiology and function between myeloid macrophages and AMs, which are fetal liver-derived, we hypothesized that the regulation of MHCII in AMs is unique compared to myeloid-derive macrophages. To test this hypothesis, we characterized the regulation of MHCII on AMs using an ex vivo model of AMs known as fetal liver–derived alveolar-like macrophages (FLAMs), which closely resemble murine AMs in morphology, gene expression, and functional mechanisms.

Using a CRISPR–Cas9 screen, we identified genes whose loss in FLAMs resulted in altered surface MHCII expression. This forward genetic screen identified known regulators of MHCII such as Irf8 and novel regulators including Capicua (CIC) and cullin ring ligases (CRLs). We confirmed that loss of CIC results in a robust increase in MHCII expression compared to wild-type controls on resting cells. Further characterization revealed that LPS stimulation of CIC-deficient FLAMs leads to hyper-inflammatory responses including over 100-fold increase in inflammatory cytokines. The loss of CIC in FLAMs directly alters T cell activation as resting CIC deficient FLAMs robustly activated naïve $CD4^+$ T cells while wild type cells did not, even after activation with interferon-gamma.

Together, these findings identify CIC as a negative regulator of antigen presentation and inflammation in AMs. Ongoing studies are dissecting the mechanisms by which CIC functions and are examining how CIC-deficient FLAMs alter $CD4^+$ T cell responses in vivo using adoptive transfer and genetically deficient mouse models. This work provides new insights into the molecular pathways governing MHCII regulation in AMs and their interactions with $CD4^+$ T cells, with the long-term goal of informing therapeutic strategies to prevent pulmonary disease.

IKZF3 COOPERATES WITH PU.1 AND SPI-B AS A TUMOR SUPPRESSOR IN A MOUSE MODEL OF B CELL LEUKEMIA

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Leukemia is a group of hematologic malignancies that arise from the dysfunctional proliferation of developing lymphocytes. Precursor B-cell acute lymphoblastic leukemia (pre-B-ALL) is driven by genetic mutations that disrupt the regulatory mechanisms of transcription factors proteins such as the zinc-finger transcription factor IKZF3. Our lab has previously described H195Y mutation of *Ikzf3* in a pre-B-ALL mouse model, termed *Mb1-CreΔPB*, these are germ-line null for Spi-B, and in which PU.1 is conditionally deleted in B cells by Cre recombinase under the control of Mb1. 100% of *Mb1-CreΔPB* mice develop B-ALL by a median age of 18 weeks. The goal of this research is to understand the role of IKZF3 in B cell development and leukemia. Igll1 encodes λ5, a component of the pre-B receptor, and has previously been shown to be directly repressed by IKZF3 during B cell development. WT or H195Y IKZF3 was introduced in the 38B9 pre-B mouse cell line. Anti-IKZF3 ChIP-seq analysis showed that both WT and H195Y IKZF3 interacted directly with the Igll1 promoter in 38B9 cells. Luciferase assay analysis showed that the *Igll1* promoter was active in 38B9 cells and WT IKZF3 was able to repress *Igll1* promoter activity, while H195Y IKZF3 failed to repress Igll1. To test the hypothesis that IKZF3 cooperates with PU.1 and Spi-B as a tumor suppressor in pre-B-ALL, triple knockout mice were generated in which *Mb1-CreΔPB* mice were crossed with *Ikzf3f/f* mice to delete IKZF3 in B cells. Triple knockout mice had accelerated leukemia development and earlier mortality at 9 weeks compared to the 18 weeks in *Mb1-CreΔPB* mice. Flow cytometry analysis showed an accumulation of leukemic B cells in the thymus, spleen and bone marrow. The immunomodulatory drug lenalidomide can be used to induce specific degradation of IKZF3 in human cells. Lenalidomide treatment partially degraded WT and H195Y IKZF3 after 8 hours reaching a complete degradation at the 48-hour mark. These data suggest that IKZF3 cooperates with PU.1 and Spi-B to function as a tumor suppressor in pre-B-ALL.

BI-DIRECTIONAL CD200R–ISEC1 SIGNALING DRIVES PAIN RESOLUTION IN MICE

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In inflammatory diseases, pain is often a major problem and frequently persists despite resolution of inflammation. In a carrageenan-induced model of transient inflammatory pain, we previously showed that pain resolution is an active process that requires monocytes/macrophages expressing the inhibitory CD200 receptor 1 (CD200R). While CD200 is dispensable, expression of the non-canonical CD200R ligand iSec1 by sensory neurons is required to resolve inflammatory pain. Here we studied whether signaling by CD200R is required to resolve pain, and whether iSec1 has the capacity to relay signals.

In mice depleted of monocytes/macrophages inflammatory pain fails to resolve. Resolution of thermal and mechanical hypersensitivity was rescued by adoptive transfer of *Cd200r1*$^{-/-}$ macrophages ectopically expressing wild type CD200R, but not after transfer of signaling-dead CD200R-expressing macrophages. In *iSec1*$^{-/-}$ mice resolution of thermal and mechanical hypersensitivity was rescued after reintroducing wildtype iSec1 in sensory neurons that innervate the inflamed paw. Of note, reintroduction of truncated iSec1 (iSec1Δ) that lacks the intracellular tail failed to rescue pain resolution. This shows that the intracellular tail of CD200R in macrophages and the intracellular tail of iSec1 in sensory neurons are both required to resolve pain, suggesting that signaling by both CD200R and iSec1 is needed for pain resolution.

To assess what kinases are potential targets of iSec1 signaling we performed PamGENE analysis. Neuroblast Neuro2A cells expressing iSec1 up-regulated activity of Proto-oncogene tyrosine-protein kinase Src (SRC) after stimulation with CD200R-Fc. In MED17.11 cells differentiated to sensory neurons, exposure to CD200R-Fc selectively up-regulated phosphorylation of SRC in cells expressing wildtype iSec1 but not in cells expressing iSec1Δ. Altered mitochondrial function is associated with chronic pain. Exposure of primary dorsal root ganglion cultures to CD200R-Fc reduced mitochondrial reactive oxygen species production. In addition, exposure to CD200R-Fc increased the refractory time of neurons, potentially limiting their maximal firing frequency.

Together these data show that iSec1 is a receptor with signaling capacity. In addition, the analgesic effect of the CD200R1-iSec1 interaction relies on signaling by both molecules, possibly through iSec1-mediated activation of SRC affecting neuronal function.

INDUCING A TYPE-I INTERFERON RESPONSE VIA REPLICATION-DEFICIENT ADENOVIRUSES TO ENHANCE *MYCOBACTERIUM BOVIS* BCG VACCINE EFFICACY

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Tuberculosis is the leading cause of death from a single infectious agent worldwide despite a WHO-approved and widely used vaccine, *Mycobacterium bovis* BCG. Although effective against severe, disseminated *M. tuberculosis* (Mtb) infections such as TB meningitis in children, BCG has limited efficacy against the pulmonary form of the disease particularly in adults due to a weak elicited memory response. Previous studies have noted that administering IFN-β or stimulating type-I IFN-mediated signaling during BCG vaccination can bolster protection against Mtb infection. However, these studies utilized approaches that have limited clinical application potential. Therefore, there is a need to develop a more clinically-feasible strategy that enhances the type-I IFN response during BCG vaccination. To do this, we infect bone marrow-derived macrophages with BCG and replication-deficient adenoviruses (RdAds), which have robust safety profiles in various vaccine clinical trials. This novel vaccine strategy leverages the ability of BCG to develop antigen-specific responses while relying on the ability of RdAds to boost type-I IFN signaling to promote the development of a more protective response. Findings indicate these adenoviruses can infect primary murine macrophages and elicit a strong type-I IFN response via the cGAS/STING signaling pathway both alone and when co-administered with BCG. Time course experiments analyzing proinflammatory cytokine dynamics revealed that the adenoviruses induce higher, more sustained IFN-β compared to BCG alone but do not induce TNF-α expression. Notably, the modified vaccine elicits late, synergistic iNOS production, a critical antimicrobial effector involved in protection against Mtb. We hypothesize that this effect results from the convergence of proinflammatory pathways initiated by BCG and paracrine type-I IFN signaling driven by the adenovirus via JAK-STAT1/2 signaling. Using a mouse Mtb infection model, ongoing and future work will evaluate whether the combination of BCG and adenovirus vaccinations reduces bacterial burden and improves mouse survival following Mtb challenge. Overall, the use of adenovirus shows exciting potential to enhance BCG-induced protection through targeted modulation of innate immunity.

Decoding the Fate and Function of Skin Commensal-Specific Immune Memory

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Commensal bacterial colonists comprise the vast majority of immune exposures during human life. These exposures are critical for healthy immune function in both humans and mouse models. *Staphylococcus epidermidis* (*S.epi*) is a ubiquitous human skin commensal that primes and recruits *S. epi*-specific $CD8^+$ T cells to the skin. Once activated, these T cells can protect against cutaneous opportunistic pathogens and distal challenges such as gut bacterial infections and cancer. Despite their known protective functions during active *S. epi* colonization, it is unknown whether *S.epi*-reactive memory T cells continue to support homeostatic health and counter disease long after initial microbial colonization. This question about *S. epi*-induced immune memory provides a unique and highly tractable window into understanding a fundamental aspect of immunity: how does any previous commensal exposure influence later immune function and human health?

Existing mouse models have been useful to evaluate skin commensal-specific T cells generated within 2-3 weeks of colonization, but cannot easily track long-lived memory T cells since these exist at far lower numbers than memory T cells generated by an inflammatory stimulus. Thus, to study the persistence and function of *S.epi*-specific tissue resident memory (T_{RM}) and central memory T cells (T_{CM}), we have developed new methods that allow us to faithfully identify only those T cells that are both *S.epi*-specific and primed at the time of initial colonization. By adoptively transferring polyclonal T cells which were activated by *S. epi* colonization in vivo into congenically marked recipient mice ('primed transfer'), we can clearly identify *S.epi*-specific T cells in the skin and secondary lymphoid organs months after initial commensal colonization. We also use an orthogonal inducible fate-mapping mouse model to label T cells recruited to the skin during initial *S. epi* colonization, to study endogenous *S.epi*-specific T_{RM}. We are now using these systems to evaluate the long-term persistence and recall potential of *S. epi*-specific T_{RM} and T_{CM}. We are interested in understanding both the ability of *S.epi*-specific memory T cells to be reactivated in response to the same commensal, as well as potential cross-reactivity to heterologous commensals. Additionally, we are probing how the presence of *S.epi*-specific memory T cells can alter the host response to inflammatory immune insults. We anticipate these lines of inquiry will shed light on how skin commensal-specific memory populations support host immunity and modulate the immune response to future challenges.

TRAJECTORY OF INNATE IMMUNE SIGNALING IN MOUSE CNS AUTO-IMMUNITY

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Auto-immune demyelinating diseases such as multiple sclerosis are characterised by the emergence of auto-reactive lymphocytes, reprogramming of tissue-resident macrophages, recruitment of peripheral myeloid cells, and the subsequent induction of neuroinflammation and neuronal cell death. Here we use high-sensitivity LC-MS/MS to map the trajectory of innate immune cell and astrocyte protein expression throughout the EAE model of neuroinflammation, providing a valuable resource for the community, beyond transcriptomics, and identifying novel stage- and tissue-specific signaling changes. Our data reveals that while CNS-resident macrophages engage in early and transient pro-inflammatory signaling, peripherally-derived myeloid cells are principally responsible for long-term maintenance of inflammatory signaling. Finally, we identify the retention of myelin as well as lipid- and cholesterol-associated proteins in macrophages during EAE, and the acquisition of a lipid-laden macrophage proteome, suggesting a potential novel therapeutic target for multiple sclerosis.

WHY DOES TCR SIGNALING HAVE TO BE SO EXHAUSTING? THE T CELL ADAPTOR GADS SKEWS CD8 T CELLS TOWARDS EXHAUSTION

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Introduction: Cytotoxic T lymphocytes (CTL) may limit tumor growth by responding to neoantigens; yet within the tumor, chronic antigen recognition triggers a hyporesponsive state (exhaustion), in which multiple immune checkpoints repress CTL activity. Downstream to the TCR, a cascade of tyrosine kinases triggers the assembly of a signalosome, nucleated by three adaptor proteins, SLP-76, LAT and Gads. Whereas LAT and SLP-76 are absolutely required for TCR responsiveness, Gads performs a modulatory role, encompassing both positive and negative regulatory functions.

Rationale: TCR signaling is a key driver of tumor immunity; yet also induces exhaustion. We propose to uncouple these responses, by moderate perturbation of the TCR signaling pathway.

Methods: We inducibly deleted Gads in developmentally normal OT-1 T cells. Gads-deficient OT-1 T cells were clearly marked by the expression of tdTomato. OT-1 T cells were then expanded and differentiated *ex vivo*, under conditions of acute or chronic exposure to their cognate antigen.

Results: Gads was largely dispensable for the antigen-induced proliferation and differentiation of OT-1 T cells into perforin- and granzyme-expressing CTL. Upon restimulation, Gads-deficient OT-1 CTL matched or exceeded wild type cells in antigen induced degranulation, production of cytokines, and killing of OVA-expressing target cells. Remarkably, deletion of Gads appeared to render the cells exhaustion resistant. Following eight days of chronic antigen stimulation, Gads-deficient cells retained the capacity to degranulate and produce cytokines in response to antigen restimulation. Moreover, the frequency of PD-1^{hi}TIGIT^{hi} cells was markedly reduced in chronically-stimulated Gads-deficient OT-1 CTL whereas the PD-1^{lo}TIGIT^{lo} population markedly increased.

Conclusions: Our data strongly support the possibility of uncoupling antigen-induced effector functions from antigen-induced exhaustion.

INTERSECTION OF STEROID HORMONE AND IL-23 SIGNALING IN THE REGULATION OF TH17 CELL STATE

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$CD4^+$ IL-17-producing T (Th17) cells are heterogenous; homeostatic Th17 cells ($Th17_{Hom}$) maintain tissue homeostasis and pro-inflammatory Th17 cells ($Th17_{Inf}$) can drive autoimmune tissue damage. While interleukin-23 (IL-23) is a well-established driver of pro-inflammatory Th17 cells,the pathways that preserve Th17 cells in a homeostatic state and the mechanisms by which IL-23 disrupts this homeostasis to promote inflammation remain poorly understood. Through transcriptomic analyses of homeostatic versus proinflammatory Th17, we uncovered that endogenous glucocorticoid (GC) production due to differential expression of *Cyp11a1*, a key enzyme for steroid hormone production, maintains Th17 cells in homeostatic state. Homeostatic Th17 further have higher glucocorticoid receptor (GR) expression and thus heightened GC sensing compared to proinflammatory Th17. We show that the endogenous GC production and sensing circuit is critical for maintaining homeostatic Th17 state and limiting Th17 pathogenicity during autoimmune central nervous system (CNS) inflammation. Moreover, by integrating transcriptome and proteome data downstream of IL-12R and IL-23R signaling, we identify that IL-23R signaling disables the activation of the GR, thereby disrupting the GC signaling circuit and releasing Th17 from homeostasis. In parallel, IL-23R signaling promotes the interaction between STAT3 and CHD1 and their co-occupancy at the *Rorc* locus, thereby reinforcing RORγt expression and enforcing the pro-inflammatory Th17 program. Finally, we show that TGFβ1 rescues impaired GC sensing in murine and human proinflammatory Th17, including proinflammatory Th17 from patients with Multiple Sclerosis (MS), suggesting therapeutic potential for treatment of steroid-resistance during autoimmune exacerbations. Together, our findings establish a cell-intrinsic GC circuit that maintains Th17 homeostasis and reveal two complementary routes by which IL-23 drives pathogenic Th17 reprogramming: disabling GR-dependent restraint while engaging the STAT3-CHD1-RORγt axis; highlighting therapeutic entry points to restore tissue homeostasis in Th17-mediated autoimmune diseases.

LIVE FLUOROSPOT: A REAL-TIME SECRETION IMAGING PLATFORM FOR DECODING IMMUNE CELL COMMUNICATION

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Understanding cell-cell communication in the immune system requires spatiotemporal visualization and quantification of humoral factor secretion dynamics. Heterogeneity in secretion timing, magnitude, and duration at the single-cell level plays a crucial role in orchestrating immune responses and maintaining tissue homeostasis.

To address this need, we developed Live FluoroSpot, a real-time imaging platform that combines waveguide-based total internal reflection fluorescence microscopy (TIRFM) with a wash-free immunoassay. This enables non-invasive, wide-field visualization of cytokine secretion from thousands of live cells. Since sandwich immunoassays yield temporally blurred signals due to cumulative binding and kinetic delays, we implemented a computational pipeline incorporating pixel-wise differentiation and deconvolution to reconstruct secretion rate maps at minute-scale resolution. By integrating brightfield imaging, we simultaneously tracked secretion dynamics, cell motility, and morphology in real time.

We applied this platform to three immune models using primary mouse cells: (1) IL-33–stimulated group 2 innate lymphoid cells (ILC2s) as an allergy model, (2) alveolar macrophages exposed to yellow dust or silica as an inflammation model, and (3) cytotoxic T lymphocytes (CTLs) co-cultured with murine gastric cancer cells (YTN16) as a cancer immunity model.

In all cases, Live FluoroSpot enabled robust visualization and quantification of cytokine or granzyme B secretion, revealing substantial heterogeneity in timing, magnitude, and cell behavior. Notably, in the CTL model, we captured the moment of granzyme B release upon cancer recognition, with cumulative secretion correlating with apoptosis onset in target cells.

Live FluoroSpot provides a powerful tool for decoding immune cell communication with unprecedented spatiotemporal resolution, offering new insights into the heterogeneity and regulation of immune responses at the single-cell level.

A LONG NONCODING RNA GENETIC RISK FACTOR OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Genome-wide association studies have identified numerous lupus risk single-nucleotide polymorphisms (SNPs), majority of which reside within the noncoding genome. The growing appreciation of noncoding RNAs however have raised the possibility that some noncoding SNPs may in fact operate through RNA-mediated mechanisms.

Using a three-tiered screen to identify disease-imposed long noncoding RNA (lncRNA) regulators of Toll-like receptor (TLR) responses, we have discovered a previously uncharacterized, conserved long noncoding RNA implicated by lupus SNPs. We find that the expression of this lncRNA is decreased in lupus patients and in vivo ablation of this lncRNA results in spontaneous development of lupus-like disease.

Mechanistically, we show that this lncRNA acts downstream of TLR7 signaling and is necessary to prevent exacerbated TLR7-drive lupus. The expression of this lncRNA is striking enriched in activated B cells and conditional deletion of this lncRNA in B cells confirms its B cell-intrinsic role in preventing autoimmunity. Accordingly, loss of this lncRNA drives aberrant expansion of both follicular and extrafollicular activated B cells. We find that this lncRNA prevents the expression of IKZF1, encoding the Ikaros transcription factor that is upregulated in lupus patients. Pharmacologic inhibition of Ikaros using an FDA-approved compound rescues lupus pathology.

Together, these findings uncover a novel genetic risk factor of SLE. More broadly, this work validates a generalizable framework for discovering RNA-mediated mechanisms by which noncoding genetic risk loci rewire immune tolerance and drive systemic autoimmunity.

REWIRING CHIMERIC ANTIGEN RECEPTOR (CAR) SIGNALING TO OVERCOME DEVELOPMENTAL SKEWING IN IPSC-DERIVED CAR T CELLS

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Chimeric antigen receptor (CAR) T cell therapies have achieved notable clinical success, yet their efficacy and scalability remain constrained by reliance on autologous cells and poorly controlled receptor signaling. Induced pluripotent stem cell (iPSC)–derived CAR T cells offer an attractive off-the-shelf alternative, but differentiation from pluripotent progenitors is frequently disrupted by aberrant intracellular signaling. In particular, tonic signaling initiated by CAR intracellular domains (ICDs) during early lymphoid development drives skewing toward innate-like lineages at the expense of conventional T cell fates.

Here, we altered CAR intracellular signaling properties to test how tonic signaling influences T cell developmental trajectories. Using a pool of engineered ICD variants, we quantitatively assessed signaling strength and its effects on iPSC-derived hematopoietic stem and progenitor cell (HSPC) differentiation. We identified a novel ICD configuration that reduced tonic signaling by approximately 2.5-fold relative to a canonical CD28-based CAR. This attenuation of basal signaling was associated with a fivefold increase in $CD4^{+}CD8^{+}$ double-positive cells, a key developmental checkpoint in T cell maturation, indicating restoration of canonical T lineage signaling programs.

Importantly, progenitors expressing this optimized ICD progressed to both functional $CD8^{+}$ cytotoxic T cells and $CD4^{+}$ helper T cells, demonstrating that early modulation of intracellular signaling cascades can durably shape downstream lineage commitment and effector differentiation. Together, these findings establish CAR intracellular domains as tunable signaling modules that control tonic signaling thresholds during human T cell development and highlight how precise manipulation of intracellular signaling networks can overcome developmental bottlenecks in iPSC-derived CAR T cell generation.

TISSUE RESERVOIRS OF HUMAN B CELL MEMORY SUSTAIN VACCINE IMMUNITY

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Vaccines and infections can induce immunological memory, though the duration and protective efficacy varies widely between vaccine and infection types. In humans, immune protection is associated with pathogen-specific antibodies detected in blood, which are produced by antigen-specific B cell populations; however, a comprehensive understanding of how immune memory is stored and maintained in humans is lacking as the cellular source of human B cell memory largely reside in tissues. Using a human tissue resource where we obtain multiple lymphoid and mucosal sites from human organ donors, we investigated immune memory to different childhood vaccines and seasonal infections to identify associations with functional antibody responses and the role of tissue and antigen in the establishment of B cell memory. We analyzed the tissue distribution, phenotype, transcriptional profile, and clonal relationship of seven antigen-specific memory B cell (MBC) populations across blood and tissues obtained from 106 donors ranging from childhood to late adulthood. Childhood vaccine and seasonal infection-specific MBCs were detectable across lymphoid organs including bone marrow, lung-associated lymph nodes (LN), and spleen, with lower numbers detected in blood, lungs, and gut-associated LN. Different antigen-specific MBC varied by isotype composition, and the proportion of IgG-expressing MBC in the LN and spleen correlated with the antibody level in plasma for each donor, establishing functional reservoirs of human MBC in tissues. We further found that MBC subset composition including class-switched and non-switched classical ($CD27^+$) MBC, atypical ($CD27^-CD21^-$) (aMBC) and tissue resident ($CD69^+$) MBC were features of the site; LNs were enriched in classical and tissue resident MBC while bone marrow was enriched in aMBC. Over age we observed an increase in aMBCs specific for SARS-CoV-2 and influenza in the bone marrow and spleen. Single cell transcriptional profiling revealed an additional MBC subset comprising marginal zone-like MBC in the spleen along with classical and atypical MBC. Notably, individual expanded clones of antigen-specific MBC were shared across sites, though the subset composition was determined by the tissue. Our findings reveal distinct tissue reservoirs of vaccine and infection-induced memory and the important role of tissues in MBC maintenance and sustaining humoral immunity across the lifespan.

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CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

Cold Spring Harbor Laboratory (CSHL or the 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.

Consistent with the Laboratory's missions, commitments and policies, the purpose of this Code is to set forth expectations for the professional conduct of all individuals participating in the Laboratory's meetings program, both in person and virtually, including organizers, session chairs, invited speakers, presenters, attendees and sponsors. This Code's prohibition against discrimination and harassment is consistent with the Laboratory's internal policies governing conduct by its own faculty, trainees, students and employees.

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 (*see below)*.

Similarly, meeting participants agree to refrain from:

1. Harassment and discrimination, either in person or online, in violation of Laboratory policy based on actual or perceived sex, pregnancy status, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, creed, nationality or national origin, immigration or citizenship status, mental or physical disability status, veteran status, military status, marital or partnership status, marital or partnership status, familial status, caregiver status, age, genetic information, status as a victim of domestic violence, sexual violence, or stalking, sexual reproductive health decisions, 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.

BREACHES OR VIOLATIONS OF THE CODE OF CONDUCT

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.

If you have questions or concerns, you can contact the meeting organizers, CSHL staff.

For meetings and courses funded by NIH awards:

Participants may 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.

For meetings and courses funded by NSF awards:

Participants may file a complaint with the NSF. See this page for information on how to file a complaint with the NSF.

Law Enforcement Reporting:

- For on-campus incidents, reports to law enforcement can be made to the Security Department at 516-367-5555 or x5555 from a campus phone.
- For off-campus incidents, report to the local department where the incident occurred.

In an emergency, dial 911.

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 is any unwelcome verbal, visual, written, or physical conduct that occurs with the purpose or effect of creating an intimidating, hostile, degrading, humiliating, or offensive environment or unreasonably interferes with an individual's work performance. Harassment 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 Harassment includes harassment on the basis of sex, sexual orientation, self-identified or perceived sex, gender expression, gender identity, and the status of being transgender. Sexual harassment is not limited to sexual contact, touching, or expressions of a sexually suggestive nature. Sexual harassment includes all forms of gender discrimination including gender role stereotyping and treating employees differently because of their gender. *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

MEETING ALCOHOL POLICY

Consumption of alcoholic beverages is not permitted in CSHL's public areas other than at designated social events (wine and cheese reception, picnic, banquet, etc.), in the Blackford Bar, or under the supervision of a licensed CSHL bartender.

No provision of alcohol by meeting sponsors is permitted unless arranged through CSHL.

Meeting participants consuming alcohol are expected to drink only in moderation at all times during the meeting.

Excessive promotion of a drinking culture at any meeting is not acceptable or tolerated by the Laboratory. No meeting participant should feel pressured or obliged to consume alcohol at any meeting-related event or activity.

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 ***(call for appointment)*** 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: M-F 9am – 9pm, Sat 8:30am – 1pm
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#: **(On your registration envelope)**

Laundry Machines
Dolan Hall, lower level

Photocopiers, Journals, Periodicals, Books
CSHL Main Library
Open 24 hours (with PIN# or CSHL ID)
Staff Hours: 9:00 am – 9:00 pm
Use PIN# (On your registration envelope) to enter Library
See Library staff for photocopier code.
Library room reservations (hourly) available on request between 9:00 am – 9:00 pm

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

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
Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681
Executive Limo Service	516-826-8172
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

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.

Cold Spring Harbor Laboratory Bookstore & Gift Shop

Main campus, lower level of Grace Auditorium

Store Hours

Did you miss your chance to shop at the CSHL Bookstore and Gift Shop during the conference?

No problem! Visit our Online Bookstore and Gift Shop.

It's a great way to bring home a piece of the experience!

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cshlvirtualstore.com

CSHL Campus Map

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