Abstracts of papers presented
at the 2023 meeting on

SYSTEMS IMMUNOLOGY

April 18–April 22, 2023

CSH Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2023 meeting on

SYSTEMS IMMUNOLOGY

April 18–April 22, 2023

Arranged by

Menna Clatworthy, *University of Cambridge, UK*
Kathryn Miller-Jensen, *Yale University*
Harinder Singh, *University of Pittsburgh*
John Tsang, *Yale University and*
National Institute of Allergy and Infectious Diseases

CSH Cold Spring Harbor Laboratory
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SYSTEMS IMMUNOLOGY
Tuesday, April 18 – Saturday, April 22, 2023

Tuesday	7:30 pm – 10:30 pm	**1** Single Cell Multi-Ome Analysis
Wednesday	9:00 am – 12:00 pm	**2** Human Systems Immunology I
Wednesday	2:00 pm – 5:00 pm	**3** Modeling of Immune Signaling and Gene Regulatory Networks
Wednesday	5:00 pm	*Wine & Cheese Party*
Wednesday	7:30 pm – 10:30 pm	**Poster Session I**
Thursday	9:00 am – 12:00 pm	**4** Cellular Dynamics, Interactions and Communication
Thursday	2:00 pm – 5:00 pm	**5** Engineered Cells and Systems
Thursday	7:30 pm – 10:30 pm	**Poster Session II**
Friday	9:00 am – 12:00 pm	**6** Tissue Systems Immunology
Friday	2:00 pm – 5:00 pm	**7** Immunoreceptors: Specificities and Signaling
Friday	6:00 pm	*Cocktails and Banquet*
Saturday	9:00 am – 12:00 pm	**8** Human Systems Immunology II

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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TUESDAY, April 18—7:30 PM

SESSION 1 SINGLE CELL MULTI-OME ANALYSIS

Chairpersons: **Christina Leslie,** Memorial Sloan Kettering Cancer Center, New York, New York
Harinder Singh, University of Pittsburgh, Pennsylvania

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WEDNESDAY, April 19—9:00 AM

SESSION 2 HUMAN SYSTEMS IMMUNOLOGY I

Chairperson: **John Tsang,** Yale University, New Haven, Connecticut / National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland
Shai Shen-Orr, Technion- Israel Institute of Technology, Haifa, Israel

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SESSION 3 MODELING OF IMMUNE SIGNALING AND GENE REGULATORY NETWORKS

Chairpersons: **Alexander Hoffmann,** University of California, Los Angeles
Gregoire Altan-Bonnet, National Cancer Institute, NIH, Bethesda, Maryland

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Non-coding fragility within interleukin-2 feedback circuitry shapes autoimmune disease risk
Dimitre R. Simeonov, Kyemyung Park, Jessica T. Cortez, Arabella Young, Zhongmei Li, Vinh Nguyen, Jennifer Umhoefer, Alyssa C. Indart, Jonathan M. Woo, Mark S. Anderson, John S. Tsang, Ronald N. Germain, Harikesh S. Wong, Alexander Marson.
Presenter affiliation: Massachusetts Institute of Technology, Cambridge, Massachusetts; Ragon Institute of MIT and Harvard, Cambridge, Massachusetts. 21

WEDNESDAY, April 19—5:00 PM

Wine and Cheese Party

WEDNESDAY, April 19—7:30 PM

POSTER SESSION I

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THURSDAY, April 20—9:00 AM

SESSION 4 CELLULAR DYNAMICS, INTERACTIONS AND COMMUNICATION

Chairpersons: **Ronald Germain,** National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland
Kathryn Miller-Jensen, Yale University, New Haven, Connecticut

Tipping points in biology—Quantitative and temporal control of responses from the cell level to organismal survival upon infection
Ronald N. Germain, Meng Lou, Naeha Subramanian, Pedro Milanez-Almeida, John Tsang, Federica La Russa, Tibor Veres, Emily Speranza, Qiaoshi Lian, Jyh Liang Hor, Kartika Padham, Anita Gola, Colin Chu, Andrea Radtke, Harikesh Wong.
Presenter affiliation: National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland. 22

SESSION 5 ENGINEERED CELLS AND SYSTEMS

Chairpersons: **Rebecca Pompano,** University of Virginia, Charlottesville
Mark Davis, HHMI, Stanford University School of Medicine, California

THURSDAY, April 20—7:30 PM

POSTER SESSION II

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FRIDAY, April 21—9:00 AM

SESSION 6 TISSUE SYSTEMS IMMUNOLOGY

Chairpersons: **Deborah Fowell,** Cornell University, Ithaca, New York
Menna Clatworthy, University of Cambridge, United Kingdom

Spatiotemporal regulation of T cell activation niches—Integrating intravital multiphoton imaging with spatial and temporal scRNAseq
Noor Bala, Alexander McGurk, Anastasia Rup, Evan Carter, Scott Leddon, Deborah J. Fowell.
Presenter affiliation: Cornell University, Ithaca, New York. 35

The immune checkpoint molecule Tim-3 regulates microglial function and Alzheimer's disease pathology
Kimitoshi Kimura, Ayshwarya Subramanian, Zhuoran Yin, Yufan Wu, Danyang He, Karen Dixon, Udbhav Kasyap Chitta, Xiaoming Zhang, Ruihan Tang, Thomas Pertel, Aastha Aastha, Masashi Nomura, Vasundhara Singh, Dennis Selkoe, Aviv Regev, Mario Suvà, Oleg Butovsky, Vijay Kumar Kuchroo.
Presenter affiliation: Brigham Women's Hospital, Boston, Massachusetts; Broad Institute, Cambridge, Massachusetts. 36

Lymphatic migration of unconventional T cells promotes site-specific immunity in distinct lymph nodes
Paulina Cruz de Casas, Marco A. Ataide, Konrad Knöpper, Milas Ugur, Sarah Eickhoff, Mangge Zou, Haroon Shaikh, Apurwa Trivedi, Anika Grafen, Tao Yang, Immo Prinz, Knut Ohlsen, Mercedes Gomez de Agüero, Andreas Beilhack, Jochen Huehn, Mauro Gaya, Antoine-Emmanuel Saliba, Georg Gasteiger.
Presenter affiliation: Würzburg Institute of Systems Immunology, Würzburg, Germany. 37

EMBO Young Investigator Lecture

SESSION 7 IMMUNORECEPTORS: SPECIFICITIES AND SIGNALING

Chairperson: **Jenny Jiang,** University of Pennsylvania, Philadelphia

FRIDAY, April 21—6:00 PM

COCKTAILS and BANQUET

SATURDAY, April 22—9:00 AM

SESSION 8 HUMAN SYSTEMS IMMUNOLOGY II

Chairpersons: **Eoin McKinney,** University of Cambridge, United Kingdom
Donna Farber, Columbia University, New York, New York

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DECODING IMMUNE CELL STATES IN SINGLE CELL AND SPATIAL DATA

Christina S Leslie

Memorial Sloan Kettering Cancer Center, Computational and Systems Biology Program, New York, NY

We will present recent machine learning from our group for the analysis of single-cell transcriptomic (scRNA-seq) and accessibility (scATAC-seq) data as well as spatial transcriptomic (ST) data with a focus on resolving immune cell types and states. These methods include conventional and neural network topic models for inferring gene expression programs in scRNA-seq and for deconvolution of ST data; a semi-supervised annotation model for scRNA-seq analyses of the tumor microenvironment that can accurately classify cells while disentangling batch effects; and a new sequence-informed embedding algorithm for scATAC-seq called CellSpace that maps DNA k-mers and cells to the same space to learn latent structure while exhibiting strong batch mitigating properties.

SINGLE CELL MOLECULAR READOUTS COUPLED WITH MIGRATION ASSAYS TO TEST UNIFYING CORE TISSUE RESIDENT MEMORY CD8+ T CELL IDENTITY

Milcah C Scott[1,2], J. Michael Stolley[1,2], Stephen D O'flanagan[1,2], Mark J Pierson[1,2], Sathi Wijeyesinghe[1,2], Marco Kuenzil[1,2], Lalit K Beura[1,2,3], Brandon J Burbach[2,4], Clare F Quarnstrom[1,2], David Masopust[1,2]

[1]University of Minnesota Medical School, Department of Microbiology and Immunology, Minneapolis, MN, [2]University of Minnesota Medical School, Center for Immunology, Minneapolis, MN, [3]Brown University, Department of Molecular Microbiology and Immunology, Providence, RI, [4]University of Minnesota Medical School, Department of Laboratory Medicine and Pathology, Minneapolis, MN

Tissue resident memory CD8 T cells (Trm) are best defined by their migration properties; they occupy many tissues of the body without circulating and persist long term. Cell surface markers are used as proxies for residence due to impediments of performing migration assays (e.g. parabiosis, grafting) that most accurately define Trm. But Trm are heterogeneous, and the absence of rigorously authenticated markers is a major barrier. We integrated migration assays, multi-dimensional flow cytometry, single cell transcriptomics, tissue environment, and stimulation history and performed a systematic interrogation of the correlation between flow cytometric staining profiles and the property of durable residence. Through these analyses we detail the limitations of low dimensional profiling and yet we hope to share a phenotyping strategy that reproducibly predicts residency across 12-16 tissues and that will be validated in parabionts with normalized microbial experience. These findings highlight that Trm phenotype and transcriptomic programs are a constellation of many variables and could inform our understanding of Trm and studies that do not incorporate cell migration.

QUANTIFYING HOW TCR SEQUENCE VARIATION AFFECTS T CELL FATE AT SINGLE-CELL RESOLUTION

Kaitlyn A Lagattuta[1,2,3], Mujin Kwun[2,3,4], Soumya Raychaudhuri[1,2,3,5]

[1]Harvard Medical School, Biomedical Informatics, Boston, MA, [2]Brigham and Women's Hospital, Genetics and Rheumatology, Boston, MA, [3]Broad Institute, Medical and Population Genetics, Cambridge, MA, [4]Harvard College, Computer Science, Cambridge, MA, [5]University of Manchester, Centre for Genetics and Genomics Versus Arthritis, Manchester, United Kingdom

Mucosal-Associated Invariant T (MAIT) and Natural Killer T (NKT) cells demonstrate that some T cell transcriptional fates are driven by the T cell antigen receptor (TCR). Though activation through the TCR is integral to T cell differentiation, the contribution of TCR sequence features to other T cell transcriptional fates remains yet to be defined. In this study, we identify how αβ V(D)J recombination affects T cell differentiation at single-cell resolution. By applying regularized and cross-validated Canonical Correlation Analysis (CCA) to 340,557 TCR clones collected from 256 individuals, we define a set of TCR scoring functions that quantify transcriptional fate predispositions conferred by the TCR. This unsupervised analysis shows that the major effects of TCR sequence on T cell fate correspond to cognate peptide presentation molecules: MR1- or CD1d- restricted *PLZF*^{high} innate-like (MAIT/NKT) transcriptional fate (8.5-fold increase in odds for top percentile compared to bottom percentile TCRs in external validation data, $P = 1.6 \times 10^{-48}$) and MHC class I-restricted CD8 fate versus MHC class II-restricted CD4 fate (25.0-fold increase in odds for top decile compared to bottom decile TCRs in external validation data, $P < 1 \times 10^{-300}$). However, a previously unknown relationship between TCR sequence and T cell fate emerges from the same statistical model. We observe that TCR sequence features preferred by thymic positive selection during fetal development continue to promote memory formation in the adult periphery (1.48-fold increase in odds for top decile compared to bottom decile TCRs in external validation data, $P = 5.3 \times 10^{-25}$). This result provocatively suggests that not all TCRs are equally capable of mounting an effector response, even after surviving thymic selection. We apply this TCR scoring function to T cells linked with UMI counts for 44 pMHC Dextramers, and find that biophysical variation in the TCR sequence modulates fate outcomes even among T cells that recognize the same antigen. By integrating across four single cell sequencing modalities sampled from over 350 individuals, our work demonstrates the utility of large-scale multimodal datasets in understanding key immunological processes such as T cell differentiation.

SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS REVEAL ABERRANT LYMPHOID DEVELOPMENTAL PROGRAMS DRIVING GRANULOMA FORMATION

Thomas Krausgruber*[1,2], Anna Redl*[1,3], Daniele Barreca*[1], Konstantin Doberer[4], Daria Romanovskaia[1], Lina Dobnikar[1], Maria Guarini[1], Luisa Unterluggauer[3], Lisa Kleissl[3,5], Denise Atzmüller[5], Carolina Mayerhofer[3], Aglaja Kopf[1,3,5], Simona Saluzzo[3], Clarice X Lim[6], Praveen Rexie[6], Thomas Weichhart[6], Christoph Bock[1,2,5], Georg Stary[1,3,5]

[1]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, [2]Medical University of Vienna, Institute of Artificial Intelligence, Vienna, Austria, [3]Medical University of Vienna, Department of Dermatology, Vienna, Austria, [4]Medical University of Vienna, Department of Medicine III, Vienna, Austria, [5]Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria, [6]Medical University of Vienna, Institute of Medical Genetics, Vienna, Austria

Granulomas are lumps of immune cells that can form in various organs. Most granulomas appear unstructured, yet they have some resemblance to lymphoid organ formation. To better understand granuloma formation, we performed single-cell sequencing and spatial transcriptomics on granulomas from 12 patients with sarcoidosis and bioinformatically reconstructed the underlying gene-regulatory networks. We discovered an immune stimulatory environment in granulomas that repurposes transcriptional programs associated with lymphoid organ development. Granuloma formation followed characteristic spatial patterns and involved genes linked to immuno-metabolism, cytokine and chemokine signaling, and extracellular matrix remodeling. We identified three cell types as key players in granuloma formation: Metabolically reprogrammed macrophages, cytokine-producing Th17.1 cells, and fibroblasts with inflammatory and tissue remodeling phenotypes. We characterized cell-cell communication networks inside granulomas and highlight the interplay of innate, adaptive, and structural cells in the creation and maintenance of granulomas. We validated and extended our results to granulomas in other organs and showed that key mediators of granuloma formation were shared across organs. Pharmacological inhibition of one of the identified processes attenuated granuloma formation in a sarcoidosis mouse model. In summary, we show that human granulomas adopt characteristic aspects of normal lymphoid organ development in aberrant combinations, indicating that granulomas constitute aberrant lymphoid organs.

* These authors contributed equally

ASSEMBLY AND MODELING OF A DYNAMIC GENE REGULATORY NETWORK THAT REGULATES HUMAN B CELL FATE DYNAMICS

Nicholas Pease, Jingyu Fan, Trirupa Chakraborty, Swapnil Keshari, Jishnu Das, Harinder Singh
University of Pittsburgh, Center for Systems Immunology and the Department of Immunology, Pittsburgh, PA

The quality and magnitude of humoral immune responses is determined by the frequencies of activated B cells that bifurcate into antibody-secreting plasmablasts (PBs) or germinal center B cell precursors (GCBC). PBs are short-lived and provide a rapid and transient source of low-affinity antibodies. GCBCs undergo delayed differentiation in secondary lymphoid organs, where their immunoglobulin genes are initially subjected to somatic hypermutation and affinity-based selection, to ultimately generate precursors of long-lived plasma cells that secrete high-affinity antibodies. We are attempting to assemble a comprehensive and dynamic gene regulatory network (GRN) that regulates the bifurcation of activated human B cells into PBs or GCBCs. We and others have previously demonstrated that reciprocal and sequentially acting negative feedback loops between pairs of signaling-induced TFs, IRF4 and IRF8 and Blimp1 and Bcl-6 regulate the primary bifurcation of murine B cells. Strikingly, naive human B cells bifurcate into similarly distinctive regulatory states ($IRF4^{hi}/IRF8^{lo}/BLIMP1^{hi}/BCL6^{lo}$, PB) or $IRF4^{lo}/IRF8^{hi}/ BLIMP1^{lo}/BCL6^{hi,}$ pre-GCBC) upon activation and several days of rapid cell division. To analyze the signaling and TF networks that dynamically program human B cell fate specification, we are coupling structural and functional genomics, ensemble and single-cell measurements, with Cas9 mediated perturbations and predictive modeling approaches. Assembly of a draft GRN and its analysis will be presented.

SIMULTANEOUS snRNA&ATACseq CHARACTERIZES SPECIFIC INNATE IMMUNE MEMORY

Jason Ossart[1], Neda Feizi[1], Matthieu Heitz[2], Sarah Masri[2], Hehua Dai[1], Steven Sanders[1], Amanda Williams[1], Fadi Lakkis[1], Aravind Cherukuri[1], Geoffrey Schiebinger[2], Martin Oberbarnscheidt[1], Khodor Abou-Daya[1]

[1]University of Pittsburgh, Thomas E. Starzl Transplantation Institute, Pittsburgh, PA, [2]University of British Columbia, Department of Mathematics, Vancouver, Canada

Purpose: Murine monocytes require A-type paired immunoglobulin-like receptors (PIR-As) to specifically recognize and acquire memory to major histocompatibility complex I antigens. Beyond the need for PIR-As, little is known about the mechanisms of specific innate immune memory. In this study, we aim to explore possible mechanisms of monocyte memory by investigating the epigenetic and transcriptional changes that specifically occur after allogeneic stimulation in splenic monocytes.
Methods: B6.RAG$^{-/-}\gamma c^{-/-}$ (BRG) mice were immunized by intraperitoneal injection of 20 million Balb/c irradiated splenocytes (allo). Splenic monocytes were FACS sorted at 0, 3, 7 and 28 days post-immunization. Simultaneous snRNAseq and snATACseq was then performed. To identify the changes that are specific to allo-stimulation and related to monocyte memory, monocytes were also sorted and sequenced from BRG mice injected with irradiated B6 splenocytes (syn) and allo-immunized BRG PIR-A$^{-/-}$ mice at day 7 after injection.
Results: Weighted nearest neighbor UMAP represented 4 visually distinct cell neighborhoods (N). N1 and N2 increased in abundance after allo-stimulation. Differential gene expression analysis revealed that N1 highly expressed genes encoding cell cycle proteins, Ly6C, and PIR-A. Monocytes in N2 from syn vs allo immunized groups were segregated. Clustering mainly resulted in the division of N2 into 3 clusters (C1-C3). C1 and N1 were more abundant in allo-immunized BRG and had a 3.5 and 2.4 fold reduction in allo-immunized BRG PIR-A$^{-/-}$ mice respectively. Flow cytometric analysis with EDU pulse-chase confirmed the patterns seen in the sequencing data. Pseudotime multiomic trajectory inference using Multivelo revealed an influx of cells from N1 to N2 at D7. Real-time multiomic trajectory inference using Waddington OT supported that N1 is the starting state for memory formation in response to allogeneic non-self. Real time and pseudotime trajectory inference suggested that the differentiation pathway connects N1 to N2 then splits to N3 and N4. N3 and N4 had a profile consistent with differentiation to macrophages and mo-DCs respectively. Static and dynamic gene regulatory network inference using Dictys revealed key transcription factors in N1 for monocyte memory formation and response.
Conclusions: Splenic monocytes are heterogenous and contain a subset that responds specifically to allo-stimulation with unique transcriptional and epigenetic changes that are consistent with memory formation and response.

EPIGENETIC STATES OF GERMINAL CENTER B CELLS DETERMINED BY SINGLE CELL MULTIOMICS ANALYSIS CAN PREDICT B CELL FATE

Christopher R Chin[1,2,3], Dylan R McNally[1,3], Cem Meydan[3], Laurianne Scourzic[1], Alexia Martinez de Paz[1,4], Steve Z Josefowicz[1,4,5], Christopher E Mason[2,3], Ari M Melnick[1,6], Wendy Beguelin[1,6]

[1]Weill Cornell Medicine, Medicine, New York, NY, [2]Weill Cornell Medicine, Computational Biology and Medicine, New York, NY, [3]Weill Cornell Medicine, Physiology, Biophysics and Systems Biology, New York, NY, [4]Weill Cornell Medicine, Pathology, New York, NY, [5]Weill Cornell Medicine, Immunology, New York, NY, [6]Weill Cornell Medicine, Pharmacology, New York, NY

Characterization of germinal center (GC) B cells through conventional flow-cytometry and sequencing methods is difficult, especially when genetic perturbations can affect markers used for sorting and characterizing populations. GC B cells consist of a continuous spectrum of cells transitioning from dark zone (where they rapidly proliferate while undergoing somatic hypermutation [SHM] of their immunoglobulin genes) to light zone (where they are selected based on the affinity of their surface antibody for the inducing antigen), exiting the GC as plasma or memory B cells, re-entering the dark zone for further rounds of SHM and proliferation, or undergoing apoptosis due to death from neglect. Single cell profiling of the entire GC not only addresses the issues that arises from sorting or characterizing cells by markers, but allows for identification and profiling of more rare cell types. With the goal of characterizing transcriptional and epigenetic profiles of all types of GC B cells, we sorted YFP^+ cells from GC B cell-specific YFP^+ reporter mice ($Rosa26^{LSL.YFP}$;$C^{\gamma}1Cre$) that were immunized with sheep red blood cells for 10 days, and performed single cell (sc) RNA sequencing and scRNA combined with scATAC (multiome) sequencing. We included a fraction of follicular CD4 and CD8 T cells. Through large scale integration of the scRNA data sets, we characterized different transcriptional profiles found within the GC reaction to label cell typesWe identified different subtypes of cells re-entering the dark zone (in total ~2% of the GC), including pre-plasmablast population (4% of re-entering, or ~0.16% of total cells). Using cell identities defined by scRNA-seq, we interrogated the epigenetic profiles of these cells and determined their accessibility changes. We find sets of enhancers containing motifs whose accessibility determines cell fate. As an example, Chromvar analysis of the pre-plasmablast population revealed increased accessibility of peaks that contain motifs for plasma cell differentiation transcription factors (TF) XBP1, PRDM1 and IRF4 (which are not expressed at this stage), as compared to the other dark zone re-entering cell subpopulations. These findings suggest that TF motifs become accessible prior to TF expression. Moreover, pseudotime lineages derived from ATAC data show earlier bifurcation than transcriptional lineages, indicating that cells with the same transcriptional profile can be divided by accessibility differences. Our results suggest that the epigenetic state of GC B cells can predict their fate.

SYSTEMS IMMUNOLOGY OF HUMAN IMMUNE SET POINTS

John Tsang[1,2]

[1]Yale University, Center for Systems and Engineering Immunology and Depts. of Immunology and Biomedical Engineering, New Haven, CT, [2]National Institute of Allergy and Infectious Diseases, NIH, Laboratory of Immune Systems Biology, Bethesda, MD

A hallmark of the human immune system is its extensive person-to-person heterogeneity, both at homeostasis ("baseline" state) and in response to perturbations such as vaccines and infections. This immune variability can pose a barrier to the design of effective vaccines and therapeutics in the population. For example, hyporesponsiveness.

to vaccines has been reported in otherwise healthy individuals and this has been linked to factors including age, genetics, geography, vaccination history, and baseline immune status. Independent of age, sex, and pre-existing antigen-specific immunity in adults, we and others have revealed that baseline immune status or "set point signatures" before vaccination, as reflected by the circulating immune cell frequency and their transcriptional state can predict and potentially determine the quality and quantity of response to vaccination, infection, and disease activities in autoimmune patients. However, how baseline immune status is established and how they continue to evolve within an individual over time and in response to external perturbations remain poorly understood. Here I will discuss our recent effort in dissecting the origin of such personal immune set points over both short (months) and longer (years) timescales, including systems immunological dissection of set point development from early life to adulthood and how inflammatory exposures can shape baseline immune status to impact future immune responses in antigen agnostic manners.

NOTE: This talk will cover work from the lab over more than a decade with contributions from numerous collaborators and lab members, who will be credited and highlighted during the talk.

TESTOSTERONE-INDUCED CHANGES IN TRANSCRIPTIONAL RESPONSES TO STIMULATION DURING FEMALE-TO-MALE SEX-REASSIGNMENT

Rikard Forlin[1], Tadepally Lakshmikanth[1], Camila Consiglio[1], Fabian Sardh[2], Hugo Barcenilla[1], Jun Wang[1], Ziyang Tan[1], Laura Gonzalez[1], Constantin Mugabo[1], Peri Noori[2], Anette Johansson[1], Lucie Rodriguez[1], Yang Chen[1], Jaromir Mikes[1], Olle Kämpe[2], Nils Landegren[2,3], Petter Brodin[1,4]

[1]Karolinska Institutet, Dept. of Women's and Children's Health, Solna, Sweden, [2]Karolinska Institutet, Department of Medicine, Karolinska Institutet, Solna, Sweden, [3]Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden, [4]Imperial College London, Department of Immunology and Inflammation, London, United Kingdom

There are several significant differences in the immune system and its functions between males and females. Females develop cancer less frequently than men, have better outcomes when infected and elicit a stronger response to vaccines. However, they also experience more adverse reactions to vaccines and are more prone to develop autoimmune diseases. One major factor that can explain substantial parts of these observed differences are levels of sex hormones, but previous studies have not been able to distinguish effects of hormones from genes. A better understanding of how sex hormones influence immune cell functionality will allow disease processes and human immune responses to vaccines to become more predictable, and more precise modulatory treatment could be devised. In this study, the role of sex hormones in human immunology was investigated by longitudinal sampling of PBMCs from subjects undergoing female to male transition by testosterone treatment. Samples were taken prior to (V1) and after 3 months (V2) of testosterone treatment. Each sample was divided into 3 batches, 2 with in vitro stimulation by either LPS or R848 for 4 hours and one left untreated (NTC) and single cell mRNA-sequencing was performed. This allowed the assessment of baseline transcriptional changes in unstimulated cells, as well as differences in the responses to stimulation. Testosterone-mediated changes differed among cell types but was most pronounced in plasmacytoid dendritic cells (pDCs) showing a distinct downregulation of pathways involved in antigen presentation and a decrease in type I interferon (IFN-I) related pathways. Further, testosterone treatment enhanced TNFα responses to LPS stimulation, suggesting a global shift from IFN-I to TNFα mediated responses which in part can explain differences in disease severity following viral infection and contribute to the sex-dimorphism of Lupus, an IFN-I driven disease. It also suggests that pDCs, the only cell with high basal expression of the androgen receptor at baseline, is a sentinel cell for sex-hormones in humans.

A SPATIAL THYMUS HUMAN CELL ATLAS MAPPED TO A CONTINUOUS TISSUE AXIS

Nadav Yayon[1,2], Veronika Kedlian[1], Lena Boehme[3], Chenqu Suo[1], Brianna Wachter [4], Rebecca Beuschel[5], Elizabeth Tuck[1], Emma Dann[1], Minal Patel[1], Alexandra Kreins[6], Muzlifah Haniffa[7], Luigi Notarangelo[4], Virginie Uhlmann[2], Ronald Germain[5], Tom Taghon[3], Andrea Radtke[5], John Marioni[2,8,9], Sarah Teichmann*[1,10]

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* we also would like to acknowledge the following co-authors Krzysztof Polański, Shani Perera, Laura Richardson (Teichmann lab), Monika Dabrowska, Alexander Predeus (Sanger Support teams), Jolien Van Hulle and Tom Putteman (Taghon lab), Graham Davies (Great Ormond Street Hospital, London), Justin Engelbert and Emily Stephenson (Haniffa lab), Fabrizio de Rita and David Crossland (Freeman Hospital, Newcastle), Marita Bosticardo and Francesca Pala (Notarangelo lab).

T cells develop in the thymus via a combination of intrinsic processes such as TCR recombination and through interactions with other cells. However, the underlying mechanisms and causal chain of events of T cell development (positive and negative selection) are poorly understood in humans.
To unravel these highly complex processes we need both spatial localization and accurate cell type identification. Here, we generated the largest human thymus resource encompassing multiple spatial platforms (Visium spatial transcriptomics and IBEX multiplex protein imaging) and single-cell modalities (single-cell, single-nuclei, CITEseq and V(D)J sequencing). We further propose a morphologically derived continuous axis of the thymus (OrganAxis), which explains the largest degree of gene expression variability within each section and broadly follows the T cell maturation trajectory. OrganAxis allows alignment of observations from different sections and spatial technologies to compare zonation of different T cell stages and interacting cells between fetal and pediatric thymus.
Taken together, our approach represents a first step toward simultaneous qualitative, quantitative and spatial mapping of T cell development.
This research was supported in part by the Intramural Research Program of NIAID, NIH.

MEDICINE AS A CONTINUUM – MONITORING IMMUNE-AGE AND DISEASE PROGRESSION

Shai S Shen-Orr

Technion-Israel Institute of Technology, Faculty of Medicine, Haifa, Israel

Individuals' show high inter-individual phenotypic and molecular variation, especially with age and disease conditions. This variation is often assumed to be due to genetic differences or disease subgroups, whereas longitudinal monitoring of individuals suggests that much of the observed molecular variation can be ascribed to lack of proper consideration of temporal changes in individual's state.

We studied the longitudinal dynamics of immune system alterations in older adults as well as longitudinal dynamics of disease progression in acute and chronic conditions. Analyses of such dataset with algorithms that explicitly take into account the dynamics of molecular state changes allows to identify 'progression scores', different from chronological age or disease onset which shows clear functional implications, effect treatment molecular responses and have prognostic clinical value.

Our findings shed light on the long-term dynamics of immune state variation and disease progression and provide a quantitative framework to bring time into the equation for precision medicine.

SYSTEMATIC DISCOVERY OF AUTOIMMUNE DISEASE-CAUSAL REGULATORY VARIANTS AND THEIR EFFECTS ON T CELL FUNCTION

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Genome-wide association studies have identified hundreds of thousands of variants associated with a broad number of complex traits, but the causal variants for >99% of these trait associations have yet to be identified and functionally validated. Thus, there continues to be a lack of understanding of the mechanisms that promote genetic risk for these complex traits. This is in part because >90% of common trait-associated variants are non-coding and trait-causal variants are often in tight linkage disequilibrium with 10s to 100s of non-causal variants. If we connected variants to their effects on disease-relevant cell types, we could better define disease-relevant variants and their mechanisms that drive disease. As part of our variant-to-function efforts, we recently defined an approach that enriches substantially for fine-mapped causal *cis*-regulatory variants for 6 autoimmune diseases using massively parallel reporter assays (MPRAs) in Jurkat cells and DNase Hypersensitivity I sites (DHS) in primary T cells, finding ~60-fold enrichment for statistically fine-mapped causal variants. Through combining MPRAs with DHS data in T cells, we identified 60 variants that likely cause autoimmune diseases, including a conserved variant, rs72928038 in the *BACH2* locus. We found deletion of this variant in mice to promote effector T cell differentiation, suggesting that this is a key mechanism for how the variant drives disease. To scale discoveries of variant effects on T cell function, we have additionally performed perturbational screens in primary T cells to identify variant *cis*-regulatory regions that alter T cell function. The resulting data connect variants and their regulatory regions to important T cell functions.
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IDENTIFICATION OF CD4+ T CELL ANTIGENS IN SJÖGREN'S DISEASE USING TSCAN-II

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CD4+ T cells play a fundamental role in orchestrating various aspects of immune responses and tissue homeostasis by recognizing a plethora of self and foreign antigens. However, our inability to unbiasedly associate various peptide HLA-II complexes with their cognate TCR has significantly hampered our understanding of CD4+ function and its role in the etiology of human disease. Therefore, we have recently developed TScan-II, a platform for unbiasedly discovering CD4+ antigens using genome-scale human and virome libraries. This platform simultaneously incorporates the endogenous HLA-II antigen processing machinery in APC cells and the endogenous T cell signaling machinery in T cells for antigen discovery. We illustrate the adaptability of the TScan-II for multiplexed HLA and TCR screens by leveraging the platform for de novo antigen discovery of clonally expanded CD4+ T cells in the salivary gland(SG) of Sjögren's disease(SjD) patients. We identified self-antigens expressed on multiple HLA alleles that can readily activate their cognate TCRs.
Furthermore, through spatial transcriptomics and single-cell sequencing of SG biopsies, we observed that the identified antigens are expressed within cells in the SG cells capable of processing and presenting HLA-II antigens. Collectively these observations implicate CD4+ in SjD pathogenesis. Future work on probing the antigenic landscape of expanded T cell clones across a large cohort of patients with SjD and multiple autoimmune diseases will further shed light on commonalities and differences in antigens governing pathogenesis.

MICROGLIAL-SPECIFIC miR-155 DELETION ENHANCES INTERFERON-DEPENDENT RESPONSE TO NEURODEGENERATION AND MITIGATES COGNITIVE IMPAIRMENT IN A MOUSE MODEL OF ALZHEIMER'S DISEASE.

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Background: Microglia, the resident brain immune cells, play a critical role in brain homeostasis and disease progression. In neurodegenerative conditions, microglia acquire the neurodegenerative phenotype (MGnD). We have previously identified that MicroRNA-155 (miR-155), a master regulator of inflammation, is involved in the transition of microglia to MGnD. However, the mechanism through which miR-155 impacts microglia during Alzheimer's disease (AD) remains unclear.

Methods: We established miR-155-cKO AD mice to elucidate the mechanism of miR-155 in microglia during AD. AD:miR155-cKO mice were crossed to Infgr1-KO mice to determine interferon-dependent signaling of miR-155. Our multi-omic approach included proteomic and transcriptomic analysis. We investigated the subtype changes of microglia upon miR-155 deletion using single-cell trajectory inference and modelling of gene-regulatory networks. Finally, we determined AD pathology hallmarks and behavior.

Results: We identify that microglial deletion of miR-155 induces a pre-MGnD activation state via INFg signaling. Blocking IFNg signaling attenuates pre-MGnD induction and microglial phagocytosis in miR-155 deletion background. Trajectory inference of microglia identified Stat1 and Clec2d as principal pre-MGnD markers. We determine that the pre-MGnD phenotypic transition enhances amyloid plaque compaction, reduces dystrophic neurites, attenuates plaque-associated synaptic degradation, and improves cognition.

Conclusion: Our study demonstrates a novel beneficial role of IFNg-responsive pre-MGnD in restricting neurodegenerative pathology and preserving cognitive function in an AD mouse model, highlighting miR-155 and IFNg as potential therapeutic targets for AD.

MACROPHAGE STATES DEFINED BY THE DYNAMIC RESPONSES TO STIMULI

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Single-cell RNA measurements have had tremendous impact on the study of cell populations as they have revealed heterogeneity and relationships between cell types and cell states that were previously not apparent. However, such measurements capture only the abundance of macromolecules, and not the flux or kinetic information of the molecular network, and may miss important information to describe the cell state. Here, I will describe my laboratory's ongoing studies to characterize cell states by measuring the temporal trajectories of macromolecules. We focus on macrophages, key immune sentinel cells capable of diverse stimulus-responsive immune functions, that occupy numerous functional cell states depending on microenvironmental cytokines. In one study, Apeksha Singh employed live cell imaging to reveal that the stimulus-responsive dynamics of the transcription factor NFκB are affected by microenvironmental cytokines, and thus, in turn, provide the basis for charting the functional state of macrophages. To do so, she developed and compared novel data analysis tools for dimensionality reduction of stimulus-response trajectories. Her analysis demonstrates how cytokine polarization specializes macrophage functional states. This is only revealed only by stimulation, with each functional state differing in degree of stimulus-response specificity. In a second study, Katherine Sheu developed a novel algorithm to reconstruct the single-cell trajectories of stimulus-response genes from scRNAseq timepoints. She found that dynamic features of gene expression trajectories hold substantially more information about stimulus and cell state than single timepoint measurements. This work is described in more detail in a separate abstract.

FLEXIBLE CONTROL OF T CELL MEMORY AND SELF RENEWAL BY A REVERSIBLE EPIGENETIC SWITCH

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The immune system encodes information about the severity of a pathogenic threat in the quantity and type of memory cell populations formed in response. This encoding emerges from the decisions of lymphocytes to maintain or lose self-renewal and memory potential during a challenge. By tracking responding CD8 T cells at the single-cell and clonal lineage level using time-resolved transcriptomics and quantitative live imaging, we identify a remarkably flexible decision-making strategy, whereby T cells initially choose whether to maintain or lose memory potential early after antigen recognition, but following pathogen clearance may regain memory potential if initially lost. Mechanistically, this flexibility is implemented by a cis-epigenetic switch that silences the memory regulator TCF1 in a stochastic and reversible manner in response to stimulatory inputs. Mathematical modeling shows how this strategy allows memory T cell numbers to scale robustly with pathogen virulence and immune response magnitudes. We propose that flexibility and stochasticity in cellular decision making ensures optimal immune responses against diverse threats.

Expanding our findings beyond acute challenge, we measure T cell self-renewal potential under chronic stimulation conditions characteristic of tumor challenge. Under these conditions, we find that cytotoxic T cells are initially plastic, able to reactivate TCF1 and return to a stem-like state upon rest, but lose plasticity and transition to terminal state upon prolonged stimulation. We further find evidence that this transition is mediated by epigenetic repression of gene loci associated with self-renewal. Elucidation of these mechanisms will shed light on the origin and maintenance of stem-like CD8 T cells in chronic settings, a population critical for anti-tumor immunity.

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

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Cells integrate extracellular cues through biochemical signaling pathways to induce the appropriate transcriptional programs. The signaling pathways that drive cytokine-induced gene expression are often depicted by a dozen or so landmark phosphorylation events related to STAT transcription factor (TF) activity. This evokes the perplexing problems of how cytokines utilize common STATs to induce distinct functional programs, and to what extent cooperating pathways support response diversity. We hypothesized that uncharacterized dynamic post-translational modifications orchestrate the activation or repression of cytokine-specific genetic programs. Our phosphoproteomic analysis of macrophage responses to IL-6 and IL-10, two cytokines that are dependent on the overlapping usage of STAT1 and STAT3, identified 1,944 cytokine-specific phosphosites and 250 cytokine-induced transcription factor phosphosites. To systematically identify STAT-cooperating pathways that support the expression of specific cytokine-regulated gene sets, we developed an interdisciplinary strategy that uses global, temporally-resolved phosphoproteomic and transcriptomic data, together with statistical modeling. We used three approaches to predict gene expression from phosphosite induction: L1-regularized linear regression (Lasso), partial least squares regression (PLSR), and a recently published version of N-way PLSR that encourages sparsity with the L1 penalty (sNPLS). Our analyses identified 23 highly predictive phosphosites using Lasso, 20 with PLSR, and 16 with sNPLS, out of which 5 sites were identified by all three methods. Since sNPLS retains the higher-order timeseries structure of our data, the phosphosites uniquely selected by this method may be more time-dependent in nature. We are experimentally validating candidate STAT-cooperating TFs whose phosphorylation was statistically associated with specific gene sets, using TF motif enrichment or differential expression of the TF mRNA to prioritize candidates. Our ability to identify causal links between phosphorylation events and cytokine-driven functional specificity betters our fundamental understanding of how extracellular cues are integrated and is a step towards identifying and manipulating the biochemical events required for healthy versus pathology-associated gene expression.

BUILDING MODELS OF CAR-T SIGNAL INTEGRATION, USING AUTOMATIZED/DYNAMIC HIGH-DIMENSIONAL EXPERIMENTAL PROFILING

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We present an experimental/theoretical pipeline to build quantitative models of leukocyte activation. We introduce a robotic platform to quantify the dynamics of cell differentiation and cytokine production/consumption by T cells ex vivo. These high-dimensional dynamics can be compressed into a 2D model using tools from machine learning. Our model highlights two modalities of T cell activation that enforce adaptive kinetic proofreading of antigen-TCR interactions, and that encode antigen discrimination. We test our model of antigen discrimination across varied immunological settings, including CAR-T and signaling-impaired T cells. To conclude, we highlight the power of lab automation, data integration, machine learning and theoretical modeling to usher new insights in systems immunology.

ORGANISM-WIDE ANALYSIS OF SEPSIS REVEALS MECHANISMS OF SYSTEMIC INFLAMMATION

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Sepsis is a systemic response to infection with life-threatening consequences. Our understanding of the impact of sepsis across organs of the body is rudimentary. Here, using mouse models of sepsis, we generate a dynamic, organism-wide map of the pathogenesis of the disease, revealing the spatiotemporal patterns of the effects of sepsis across tissues. These data revealed two interorgan mechanisms key in sepsis. First, we discover a simplifying principle in the systemic behavior of the cytokine network during sepsis, whereby a hierarchical cytokine circuit arising from the pairwise effects of TNF plus IL-18, IFN-γ, or IL-1β explains half of all the cellular effects of sepsis on 195 cell types across 9 organs. Second, we find that the secreted phospholipase PLA2G5 mediates hemolysis in blood, contributing to organ failure during sepsis. These results provide fundamental insights to help build a unifying mechanistic framework for the pathophysiological effects of sepsis on the body.

EXECUTABLE MODELS OF PATHWAYS BUILT USING SINGLE-CELL RNA SEQ DATA REVEAL IMMUNE SIGNALING DYSREGULATIONS IN PEOPLE LIVING WITH HIV AND ATHEROSCLEROSIS

Juilee Thakar

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Thakar Lab has made several advancements in recent years to identify molecular mechanisms underlying HIV associated comorbidies and signatures of HIV vaccine induced immune response. Thakar Lab uses systems level approaches of combining large-scale high throughput data with novel computational tools rooted in dynamic systems. Here I will present novel computational tools developed to investigate dysregulated pathways in people living with HIV (PLWH).
Atherosclerosis (AS)-associated cardiovascular disease is an emerging cause of mortality in an aging population of PLWH. To investigate underlying dysregulated immune signaling processes, we performed single-cell RNA-sequencing of peripheral blood mononuclear cells (PBMCs) from PLWH >=50 years of age. Further, this data is supplemented by a publicly available dataset of PBMCs from persons before and after HIV infection to investigate the effect of HIV infection. We developed the single-cell Boolean Omics Network Invariant Time Analysis (scBONITA) algorithm to infer and perturb executable dynamic pathway models of immune cell clusters to identify high-impact genes. We use these models to perform pathway analysis and to map sequenced cells to characteristic pathway-specific signaling states. scBONITA revealed that lipid signaling regulates cell migration into the vascular endothelium in AS+ PLWH. Moreover, pathways implicated included AGE-RAGE and PI3K-AKT signaling in CD8+ T cells, and glucagon and cAMP signaling pathways in monocytes. Attractor analysis with scBONITA facilitated the pathway-based characterization of cellular states in CD8+ T cells and monocytes. Thus, we identify critical cell-type specific molecular mechanisms underlying HIV-associated atherosclerosis (1).
scBONITA develops dynamic models for pathways with known network topologies. To expand the set of known pathway topologies for dynamics network modeling, we developed the WikiNetworks package to translate hand-curated biological pathways from WikiPathways database into networks (2).
In conclusion, I will present WikiPathways, scBONITA and cell-type specific dysregulated signaling in PLWH with AS.
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NON-CODING FRAGILITY WITHIN INTERLEUKIN-2 FEEDBACK CIRCUITRY SHAPES AUTOIMMUNE DISEASE RISK

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Genetic variants associated with human autoimmune diseases commonly map to non-coding control regions, particularly enhancers that function selectively in immune cells and fine-tune gene expression within a relatively narrow range of values. How such modest, cell-type-selective changes can meaningfully shape organismal disease risk remains unclear. To explore this issue, we experimentally manipulated species-conserved enhancers within the disease-associated *IL2RA* locus and studied accompanying changes in the progression of autoimmunity. Perturbing distinct enhancers with restricted activity in conventional T cells (Tconvs) or regulatory T cells (Tregs)—two functionally antagonistic T cell subsets—caused only modest, cell-type-selective decreases in *IL2ra* expression parameters. However, these same perturbations had striking and opposing effects in vivo, completely preventing or severely accelerating disease in a murine model of type 1 diabetes. Quantitative tissue imaging and computational modelling revealed that each enhancer manipulation impinged on distinct IL-2-dependent feedback circuits. These imbalances altered the intracellular signaling and intercellular communication dynamics of activated Tregs and Tconvs, producing opposing spatial domains that amplified or constrained ongoing autoimmune responses. These findings demonstrate how subtle changes in gene regulation stemming from non-coding variation can propagate across biological scales due to non-linearities within feedback circuitry, dramatically shaping disease risk at the organismal level.

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TIPPING POINTS IN BIOLOGY: QUANTITATIVE AND TEMPORAL CONTROL OF RESPONSES FROM THE CELL LEVEL TO ORGANISMAL SURVIVAL UPON INFECTION

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Many biological processes are highly non-linear, exhibiting functional behaviors that even with a small change in parameter value can take on one of two very dichotomous states at a ‘tipping point’ [bistability]. In this talk, I will review published studies exhibiting this phenomenon of bistability both at a cellular and organismal level, highlighting how small perturbation can lead to larger systemic effects. Beginning at the cell level, I will present findings showing how a modest change in the intracellular concentration of NOD1 can lead to ligand-independent activation of inflammatory responses in macrophages. I will then discuss the feedforward processes that lead to lethal influenza infection of mice. Both are examples of non-linear behaviors that translate small differences in initial conditions into dichotomous outcomes (inflammatory activation or death, respectively). I will then discuss unpublished studies on the quantitative aspects of CD8 T cell responses during lethal influenza infection, suggesting the possibility that small variations in antigen-specific T cell precursor frequency and the temporal pace of CD8 effector development can switch the outcome of infection from host protection to death. These studies will be placed in the larger context of how small changes in gene expression or cell number can influence propensities for autoimmunity or adverse responses to infectious disease and the strategies for post-infection therapy that emerge from these concepts.

This research was supported in part by the Intramural Research Program of NIAID, NIH

THE SPREAD OF INTERFERON-γ IN MELANOMAS IS HIGHLY SPATIALLY CONFINED, DRIVING NON-GENETIC VARIABILITY IN TUMOR CELLS

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Interferon-γ (IFNγ) is a critical anti-tumor cytokine that has varied effects on different cell types. The global effect of IFNγ in the tumor depends on which cells it acts upon and the spatial extent of its spread. Reported measurements of IFNγ spread vary dramatically in different contexts, ranging from nearest-neighbor signaling to perfusion throughout the entire tumor. We and others previously established that the spatial spread of many diffusible growth factors and cytokines away from their source is governed by simple diffusion and consumption dynamics. Accordingly, the length scale of cytokine spread, defined as the average distance traveled by a cytokine molecule, is determined entirely by three parameters: (1) the molecular diffusion rate, (2) the density of cells capable of consuming the cytokine, and (3) the number of receptors on cytokine consuming cells. We apply these concepts to experiments both in vitro and in vivo to study the spread of IFNγ in melanomas. We observe spatially confined niches of IFNγ signaling in 3-D mouse melanoma cultures and human tumors that generate cellular heterogeneity in gene expression and alter the susceptibility of affected cells to T-cell killing. Widespread IFNγ signaling only occurs when niches overlap due to high local densities of IFNγ-producing T cells. We measured length scales of ~30-40μm for IFNγ spread in B16 mouse melanoma cultures and human primary cutaneous melanoma. Our results are consistent with IFNγ spread being governed by a simple diffusion-consumption model, and offer insight into how the spatial organization of T cells contributes to intra-tumor heterogeneity in inflammatory signaling, gene expression, and immune-mediated clearance. Solid tumors are often viewed as collections of diverse cellular neighborhoods: our work provides a general explanation for such non-genetic cellular variability due to confinement in the spread of immune mediators. This conceptual and quantitative framework is a broadly useful and flexible approach to studying the spatial regulation of diffusible cytokines in densely-packed, three-dimensional (3-D) tissues.

MULTIPLEX IMAGING OF LOCALIZED PROSTATE TUMORS REVEALS CHANGES IN MAST CELL TYPE COMPOSITION AND SPATIAL ORGANIZATION OF AR-POSITIVE CELLS IN THE TUMOR MICROENVIRONMENT

Ece S Eksi[1,2], Cigdem Ak[1,2], Zeynep Sayar[1,2], Guillaume Thibault[1], Erik Burlingame[1,3], Young Hwan Chang[3]

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Mapping spatial cellular interactions of cancer, immune and stromal cells present novel opportunities for patient stratification and for advancing immunotherapy. In prostate cancer, single-cell studies revealed significant molecular heterogeneity in localized tumors, but we currently do not understand how this heterogeneity impacts cell type coordination and organization at large-scales. Here, we used cyclic immunofluorescent (cycIF) imaging to profile the spatial interactions of cancer cells with immune and non-immune stromal cells in low- and high-grade prostate tumors and tumor adjacent normal (TAN) tissues. Our results provide a whole-tissue spatial map of 699,461 single-cells from low-, intermediate- and high-risk patients. We delineated AR expression changes across 12 immune and 10 stromal cell subtypes in localized prostate tumors and show significant enrichment of immune infiltration in AR+ stroma in TAN samples. We identified fractions of mast cells with Granzyme B (GZMB), CD44, CD90 and AR expression and showed that GZMB+ vs. AR+ mast cells have distinct spatial interactions in high-grade prostate tumors, with M2 macrophages and with regulatory T cells (Tregs) respectively. We identified recurrent neighborhoods associated with clinical grade and loss of spatial heterogeneity in high-grade tumors. Our results uncover local and global changes in cellular organization that may inform disease progression and immunotherapy treatments for patients with prostate cancer.

EXPLORING THE REGULATION OF MACROPHAGE HETEROGENEITY ACROSS TISSUE CONTEXTS

Kathryn Miller-Jensen

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Macrophages are innate immune cells that fight infections, repair tissues, and contribute to maintaining tissue homeostasis. To accomplish these diverse functions, macrophages exhibit a high degree of plasticity and can polarize into different functional states. In this talk, I will discuss work in my lab that combines single-cell measurements with computational analyses to explore how macrophage heterogeneity enables diverse functional responses in vitro and in vivo. In one project, we are studying how the combination, order and duration of cues affects macrophage responses in vitro. I will also compare macrophage subsets in tissue contexts driven by macrophage plasticity—specifically, wound healing and tumor response to combinatorial immunotherapy.

KNOWLEDGE-BASED MACHINE LEARNING TO EXTRACT MOLECULAR MECHANISMS FROM SINGLE-CELL AND SPATIAL MULTI-OMICS

Julio Saez-Rodriguez

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Single-cell and spatially resolved omics technologies provide unique opportunities to the key study intra- and inter-cellular processes that drive immunological systems and their deregulation in disease. The use of prior biological knowledge allows us to reduce the dimensionality and increase the interpretability of the data, in particular by extracting from the data features describing the activity of molecular processes such as signaling pathways, gene regulatory networks, and cell-cell communication events. In this talk, I will present resources and methods from our group that combine multi-omic single cell and spatial data with biological knowledge and illustrate them on medically relevant cases.

MICROSCALE COMBINATORIAL STIMULATION OF HUMAN MYELOID CELLS REVEALS INFLAMMATORY PRIMING BY VIRAL LIGANDS

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Myeloid cells sense a wide variety of signals and respond by adopting complex transcriptional states. Experimentally exploring the state space of myeloid responses requires access to a large combinatorial perturbation space. Single-cell genomics coupled with multiplexing techniques provide a useful tool for characterizing cell states across several experimental conditions. However, current multiplexing strategies require programmatic handling of many samples in macroscale arrayed formats, precluding their application in large-scale combinatorial analysis. Here, we introduce StimDrop, a method that combines antibody-based cell barcoding with parallel droplet processing to automatically formulate cell population × stimulus combinations in a microfluidic device. We applied StimDrop to profile the effects of 512 sequential stimulation conditions on human dendritic cells. Our results demonstrate that priming with viral ligands potentiates hyperinflammatory responses to a second stimulus, and shows transcriptional signatures consistent with this phenomena in myeloid cells of patients with severe COVID-19.

DISSECTING THE CELLULAR EVENTS THAT CONTROL SWARMING DYNAMICS OF NEUTROPHIL POPULATIONS.

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Neutrophil swarming is a highly coordinated population response of many individual neutrophils to infection and inflammation. Sequential phases of chemotaxis are followed by neutrophil cluster formation, focalizing the microbicidal activity of a whole neutrophil population. The coordination of cells takes place on several levels, via environmental factors, cell-cell communication and cell-intrinsic mechanisms. By combining intravital microscopy and in vitro techniques, we could recently identify a cell-intrinsic mechanism that stops neutrophil swarming (Kienle et al., Science 2021). The limited accessibility of mammalian tissues has so far however complicated a detailed understanding of the cellular processes involved in neutrophil cluster formation. Utilizing a custom-build in vitro platform for high-quality live confocal fluorescence imaging of neutrophil crowding in response to bacterial stimuli, we are now able to tackle questions on single-cell and subcellular level and relate these information back to the swarming behavior of the whole neutrophil population. We are using this platform to address fundamental biological question, including the role of cytoskeletal coordination and metabolic requirements for neutrophil crowding dynamics. The experimental data, which we obtain from this platform, provides information on cellular dynamics, cell-cell interactions and communication. This has the potential to provide dynamic parameters as basis for computational analysis and mathematical modeling to further understand the self-organizing principles of neutrophil swarming dynamics.

MODELING ADAPTIVE IMMUNITY USING LYMPH NODES AND MICROFLUIDICS

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Predicting the response of the immune system to a new vaccine, immunotherapy, or growing tumor remains a grand challenge of biomedical science. Current models of immunity largely rely on in vivo animal studies that are difficult to control and analyze over time, or simple in vitro cultures that lack the spatial organization and cell-cell interactions of the body. Here, I will share our laboratory's development of technology to model immunity outside the body, by culturing intact, live cultures of ex vivo lymph node tissue with the environmental control afforded by microfluidic devices. Using this approach, we have developed tools to quantify the transport of cytokines through naïve and inflamed tissues, to test the impact of localized drug delivery, and to measure the response of the lymph node to co-culture with a tumor or a site of vaccination. Ultimately, we envision that these tools will be useful to visualize where, when, and how cells interact during immune responses, to inform the development of vaccines and immunotherapies.

SYNTHETIC CYTOKINE CIRCUITS DRIVE TARGETED INFILTRATION AND PROLIFERATION OF T CELLS IN IMMUNE-EXCLUDED TUMORS

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The promise of clinically active CAR T cells for solid tumors continues to be limited by the immunosuppressive tumor micro-environment (TME). In many of these tumors, CAR T cells like native effector T cells, are excluded from the TME. The limited set of anti-tumor T cells that do infiltrate into the tumor are typically functionally inhibited by host factors. Developing tools to overcome these barriers to T cell infiltration and activity in solid tumors remains a major challenge in immune cell engineering.

It has long been known that supplementing T cell activity with inflammatory cytokines (like IL-2) can promote anti-tumor function, but systemic IL-2 treatment has proven prohibitively toxic. Here we engineer CAR T cells with synthetic cytokine circuits in which a tumor-specific synthetic Notch (synNotch) receptor drives IL-2 production. This cell-autonomous mechanism for IL-2 production dramatically improves T cell infiltration into multiple challenging immune-excluded syngeneic tumor models. With the addition of a synthetic cytokine circuit producing IL-2, CAR T cells targeting the tumor-associated antigen mesothelin can completely clear orthotopic pancreatic tumors in fully immunocompetent mouse models. Unlike systemically delivered IL-2, this local cell-based IL-2 circuit does not show toxicity.

Tumors suppress T cell activity by both inhibiting T cell receptor (TCR) signaling and consuming inflammatory cytokines like IL-2. Within these constraints, we found that the exact circuit architecture used to produce IL-2 is critical to the activity of these synthetic cytokine circuits. Constitutive production of IL-2 led to cytokine production outside of the tumor, which had a negative effect on T cell fitness and exacerbated off-tumor toxicity. T cell activation induced IL-2 production was inhibited by the onset of T cell exhaustion. Only by producing IL-2 in a tumor-targeted manner that bypasses T cell activation was effective CAR T cell infiltration and tumor control seen. Furthermore, we found that autocrine production of IL-2 – where cytotoxic T cells produce their own IL-2 – proved to be critical.

Thus, to improve CAR T cell activity IL-2 must be produced at the right time, in the right location, and by the right cell. By bypassing requirements for T cell activation and by providing IL-2 in a preferred autocrine configuration these local IL-2 induction circuits overcome the main modes of tumor immune suppression and provide a potential general solution for driving effective T cell activity against immune-suppressed solid tumors.

ENGINEERING RNA EXPORT FOR MEASUREMENT AND CONTROL OF LIVING CELLS

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A system for programmable export of RNA molecules from living cells would enable both non-destructive monitoring of cell dynamics and engineering of cells capable of delivering executable RNA programs to other cells. We developed genetically encoded cellular RNA exporters, inspired by viruses, that efficiently and selectively package and secrete target RNA molecules from mammalian cells within protective nanoparticles. Exporting and sequencing RNA barcodes enabled non-destructive monitoring of cell population dynamics with clonal resolution. By incorporating fusogens into the nanoparticles, we demonstrated delivery, expression, and functional activity of mRNA in recipient cells. These engineered RNA export systems thus enable measurement of cell dynamics and cell-to-cell transfer of mRNA for applications in systems immunology and cell therapy.

REGULATORY T CELL SUBSETS IN AUTOIMMUNITY, IMMUNE ORGANOIDS, AND ANALYZING T CELL RESPONSES AT SCALE

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Previously we described the concerted mobilization of $CD4^+$, CD^8+, and γδ T cells in Following exploratory work on celiac disease (Han et al, *PNAS* 2013) and in a mouse model of autoimmunity (Saligrama et al., *Nature* 2019), and recently in humans with autoimmune diseases, we have found that a subset of $CD8^+$ T cells are able to kill pathogenic $CD4^+$ T cells, especially in the context of infection (Li et al., *Science* 2022). These are Kir+$CD8^+$ T cells in humans and $Ly49^+$ $CD8^+$ T cells in mice (as described earlier in the context of Qa-1 restriction by Cantor and colleagues). and are particularly active during an infection, presumably to control self-reactive T cells. Consistent with this proposed function, a conditional knock out of $Ly49^+$ $CD8^+$'s results in clear signs of autoimmunity in mice infected with either LCMV or influenza (Li et al., *Science* 2022). $CD4^+$Tregs do not seem to have an active role during an acute infection, and so we think these $CD8^+$ regulatory T cells may be more important in controlling autoreactivity arising from an infection. To explore this further, we have taken advantage of our recently developed immune organoid methodology using human tonsils or spleens (Wagar et al., *Nature Medicine* 2021) which reproduce key features of adaptive immunity in vitro. The malleability of this system, especially with the ability to perform gene editing using CRISPR-Cas9, is allowing us to test specific hypotheses and create models of autoimmunity in an entirely human system. Here we have asked what the division of labor is between $CD4^+$ and $CD8^+$ regulatory T cells? Since FoxP3 is a key driver of $CD4^+$ T reg function in $CD4^+$ but not $CD8^+$ regulatory cells T cells, we KO'd that gene in tonsil T cells and found that now the B cells are able to express self reactive antibodies, whereas disabling $CD8^+$ regulatory cells has only a minor effect this type of autoreactivity, but a unique effect on autoreactive $CD4^+$ T cells. Thus we can use these organoids and gene editing and other manipulations to rapidly investigate important mechanisms and interactions in human immunity. Lastly, a major problem in analyzing T cell responses in infectious diseases has been how to identify key specificities that are protective. Currently there is no accepted way to do this, and thus there are no T cell asays required for vaccine approval. Here we have developed a series of algorithms, GLIPH and GLIPH2 that are able to derive T cell specificity groups of related TCR sequences from very large data sets (Glanville, Huang et al., *Nature* 2017; Huang, Wang et al., *Nat. Biotech* 2020). This has enabled us to survey T cell specificities "at scale" and identify those that correlate with control of the pathogen, which in the case of TB, constitute less than 1% of the total (Musvosi, Huang et al., *Nat. Med* 2023). This is a general method that promises to help us quickly identify T cell correlates of protection, and should result in more effective vaccines.

ANTIBODY-LECTIN CHIMERAS FOR GLYCO-IMMUNE CHECKPOINT BLOCKADE

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Despite the curative potential of checkpoint blockade immunotherapy, a majority of patients remain unresponsive to existing treatments. Glyco-immune checkpoints – interactions of cell-surface glycans with lectin, or glycan binding, immunoreceptors – have emerged as prominent mechanisms of immune evasion and therapeutic resistance in cancer. Here, we describe antibody-lectin chimeras (AbLecs), a modular platform for glyco-immune checkpoint blockade. AbLecs are bispecific antibody-like molecules comprising a tumor-targeting arm as well as a lectin “decoy receptor” domain that directly binds tumor glycans and blocks their ability to engage lectin receptors on immune cells. AbLecs elicited tumor killing in vitro via macrophage phagocytosis and NK cell and granulocyte cytotoxicity, matching or outperforming combinations of monospecific antibodies with lectin-blocking or glycan-disrupting therapies. Furthermore, AbLecs synergized with blockade of the “don’t eat me” signal CD47 for enhanced tumor killing. AbLecs can be readily designed to target numerous tumor-associated antigens and glyco-immune checkpoint ligands, and therefore represent a new modality for cancer immune therapy.

A LYMPH NODE SLICE CULTURE MODEL TO CHARACTERIZE T CELL ACTIVATION DYNAMICS AND ANTI-VIRAL RESPONSES IN HUMAN TISSUE

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T cell activation in the lymph node (LN) is critical to initiating adaptive immune responses to vaccination, tumors, and infection. We previously demonstrated that T cells from human tissue sites (blood, LNs, bone marrow, lung) undergo conserved cell state transitions during activation. However, these studies were performed in suspension culture from dissociated tissue, depleting the spatial and signaling contributions of the tissue microenvironment. To address this, we developed techniques for culture and single-cell analysis of human LN slices, which preserve tissue architecture, local signaling, and physiological cell frequencies. Single-cell RNA-seq (scRNA-seq) of matched LN suspension and slice cultures stimulated with anti-CD3/28 antibody tetramers revealed distinct activation patterns. While anti-CD3/28 stimulation is commonly used to study T cell activation, this reagent circumvents antigen presentation and may not fully recapitulate antigen-mediated signaling. Conversely, antigen-dependent stimulation with superantigen mimics require MHCII/TCR binding and may benefit from the intact microenvironment in slice culture. We discovered a distinct metabolic intermediate through scRNA-seq analysis of superantigen-stimulated LN suspension and slice cultures that is not observed with anti-CD3/28 stimulation. Overall, these studies demonstrate the utility of analyzing T cell responses in intact tissue and comparative analysis of distinct mechanisms of activation.

SPATIOTEMPORAL REGULATION OF T CELL ACTIVATION NICHES: INTEGRATING INTRAVITAL MULTIPHOTON IMAGING WITH SPATIAL AND TEMPORAL scRNAseq

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Rapid mobilization and activation of T cells at sites of infection and malignancy is critical for effective immune-mediated protection and the ultimate goal of successful T cell immunotherapy. Upon entry into inflamed target tissues, effector T cells are met with a tissue landscape remodeled by innate cells, inflammatory mediators and the insult itself. How these environmental signals shape the position and function of newly recruited effector T cells is unclear. Chance encounter of T cells with antigen-bearing cells is enhanced by microanatomical clustering of antigen, antigen-presenting cells and a network of stromal and innate support cells. These dynamic activation niches appear to be positioned to maximize early T cell re-activation upon tissue entry and are often found perivascularly. Utilizing intravital multiphoton imaging and fluorescent photoactivation tools to mark T cells in time and space, we have employed bulk and single-cell RNAseq analysis to explore the impact of location and tissue-dwell time on the functional (re)programming of effector CD4+ T cells in inflamed peripheral tissues. Our NICHE-seq data has informed studies that identify critical immune players in the de novo assembly of the perivascular T cell activation niche. Early activation in the perivascular niche, sequestered from the modulatory effects of pathogen or tumor, may provide important signals that strengthen the effector T cell program and that can be leveraged for better anti-microbial or anti-tumor immune responses.

THE IMMUNE CHECKPOINT MOLECULE TIM-3 REGULATES MICROGLIAL FUNCTION AND ALZHEIMER'S DISEASE PATHOLOGY

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Microglia are the major resident immune cells of the central nervous system (CNS) playing essential roles in CNS homeostasis and disease. Although strongly implicated in Alzheimer's disease (AD) pathology, the regulatory mechanism of microglial activation remains to be fully elucidated. Furthermore, there is no consensus on whether microglial activation is beneficial or detrimental, raising the importance of identifying molecular players that dissociate different aspects of microglial activation and which may be finetuned to treat AD and other CNS diseases. By combining population and single-nucleus (snRNA-seq) transcriptomics, IP-MS, neuroinflammation assays, and behavioral studies, we investigated the role of the AD risk gene (Wightman et al., Nat Genet, 2021) and immune checkpoint molecule HAVCR2/TIM-3 in microglial homeostasis and activation. Tim-3 (Havcr2) is highly and specifically expressed in microglia compared to other cell types in both mouse and human brain. Gene expression profiles of Tim-3 deficient microglia resemble those of phagocytic microglia, and microglia in neurodegeneration (MGnD). Mechanistically, Tim-3 enhances TGFβ signaling by promoting phosphorylation of Smad2, thus contributing to the homeostasis of microglia, in a mechanism independent of its activity in T-cells. Importantly, we report that microglia-specific Tim-3 deficiency reduced Aβ plaque load and neuronal damage and resulted in improved cognitive function in a 5xFAD mouse model of AD. snRNA-seq identified a subpopulation among MGnD microglia in the Havcr2icKO:5xFAD mice, characterized by increased expression of phagocytic and anti-inflammatory genes, and concomitant decrease in inflammatory gene expression. Thus, Tim-3 may serve as a decoupling factor of phagocytotic and inflammatory functions of activated microglia in AD. Collectively, these results hold promise for a potential new therapeutic strategy targeting the checkpoint molecule Tim-3 in AD.

LYMPHATIC MIGRATION OF UNCONVENTIONAL T CELLS PROMOTES SITE-SPECIFIC IMMUNITY IN DISTINCT LYMPH NODES

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Lymph nodes (LN) function as immunological filters for lymph borne pathogens and are essential organs for initiating adaptive immunity after infection and vaccination. Here, we show that distinct lymph nodes mount characteristic immune responses and that a key determinant of such LN-specific immunity is the local spectrum of unconventional T cells (UTC). The UTC composition among lymph nodes differs in both, the TCR repertoire and the differentiation state, due to the fact that UTC are locally adapted to the LN that they populate. These differences within UTC in LN are a consequence of their migrational behavior from the tissue via the lymphatic route to the respective draining LN, similar to myeloid cells and molecules that continuously inform lymphocytes on changes in the drained tissues. Because each tissue harbors a distinct spectrum of UTC, every draining LN is thus populated by a distinctive tissue determined mix of these lymphocytes. Additionally, we show that UTC share the same homeostatic niche and functionally cooperate in interconnected units that generate and shape characteristic innate and adaptive immune responses differing between LN draining distinct tissues. The discovery that UTC functional units migrate from tissues to draining LN and influence the site specific immunity has potential implications for vaccination strategies and immunotherapeutic approaches.

THE GOOD, THE BAD AND THE BEAUTIFUL SIDE OF KUPFFER CELL ACTIVATION IN INFECTION.

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Macrophages have long been considered to be one of the most plastic cells of the body. They are thought to be able to change their phenotypes and functions at will to reflect their local microenvironment. However, many of the studies reaching this conclusion were performed either *in vitro* or before we had any idea of the heterogeneous nature of macrophages in any given (inflamed) tissue *in vivo*. Now that we better understand (1) that resident macrophages are generated before birth, are conditioned by other cells in their local homeostatic niche and are long-lived cells and (2) that in the non-homeostatic tissue many distinct subsets of macrophages exist, this has led us to question whether macrophages are really that plastic or if this heterogeneity contributes to the perceived plasticity. After all, if a macrophage is so exceptionally plastic, why would we then need to recruit new populations of macrophages in the context of infection and inflammation? We have taken a systems immunology approach to study hepatic macrophage heterogeneity and plasticity across infection and injury models using a combination of scRNA-seq, snRNA-seq and Spatial Transcriptomics approaches. Our data to date suggest that most plasticity indeed stems from the heterogenous populations of macrophages recruited to the tissue in these settings. Nevertheless, the resident macrophages of the liver, termed Kupffer cells (KCs) are capable of responding distinctly to different stimuli in an epigenetically pre-programmed manner. In infection, these responses are tightly regulated to prevent overt inflammation. However, as we will discuss, in certain settings, such overt inflammatory responses, actually provide a benefit to the organism, suggesting that manipulating KC activation in the clinic may be a useful strategy in the fight against infection.

TEMPORAL PROFILING OF HUMAN LYMPHOID TISSUES REVEALS COORDINATED DEFENCE TO VIRAL CHALLENGE

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Adaptive immunity is generated in lymphoid organs, but how these structures defend themselves during infection in humans is unknown. The nasal epithelium is a major site of viral entry, with adenoid nasal-associated lymphoid tissue (NALT) generating early adaptive responses. Here, using paired peripheral blood and nasopharyngeal adenoid biopsy samples, and applying single cell RNA sequencing, B cell and T cell receptor sequencing and high dimensional imaging, we examined longitudinal immune responses in NALT following viral challenge, using SARS-CoV-2 infection as a natural experimental model. We generated data on 162,738 cells from 23 subjects (10 healthy controls, 8 acute COVID-19 patients and 5 convalescent COVID-19 patients. There was a significant over-representation of germinal centre (GC) B cells and T follicular helper (Tfh) cells in NALT biopsies, and a greater magnitude of transcriptional change (both differentially expressed genes and pathways) compared to peripheral blood. In acute infection, infiltrating monocytes formed a subepithelial and peri-follicular shield, recruiting NET-forming neutrophils, whilst tissue macrophages expressed pro-repair molecules during convalescence to promote the restoration of tissue integrity. Germinal centre B cells expressed anti-viral transcripts that inversely correlated with fate-defining transcription factors. Among T cells, tissue-resident memory CD8 T cells alone showed clonal expansion and maintained cytotoxic transcriptional programmes into convalescence. Together our study provides a unique insight into how human nasal adaptive immune responses are generated and sustained in the face of viral challenge.

UNCOVERING THE SPATIOTEMPORAL DYNAMICS OF PLACENTAL IGG TRANSFER TOWARD PRECISION PRENATAL VACCINATION

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Neonates are born with a largely naïve immune system. Actively transferred maternal antibodies passively immunize newborns against pathogens previously encountered by the mother. Prenatal vaccination to boost pathogen-specific IgG transfer has been successfully used to reduce the incidence of neonatal tetanus, diphtheria, and acellular pertussis (Tdap) infections. Despite this, higher rates of infection among premature neonates signify a deficiency in current immunization programs. To identify maternal and placental regulators of IgG transfer and refine prenatal vaccine regimens, we used a novel *in silico* approach. To disentangle the role of placental Fc gamma receptors (FcγRs) in IgG transfer, we conducted multiplexed immunofluorescence (mIF) to measure FcγR expression in paraffin-embedded placental tissue. A mechanistic model of placental IgG transfer informed by single-cell RNA sequencing and the mIF data revealed FcγRIIb as a key driver of dynamic subclass-specific IgG transcytosis in placental endothelial cells (ECs). In model simulations and in vitro experiments with human umbilical vein ECs, we found that maternal IgG profile fine-tunes antigen- and subclass-specific IgG transfer dynamics. To identify immunization windows of opportunity for maximum IgG transfer, we simulated maternal vaccination by integrating a model of B cell activation and antibody production into the mechanistic model. This revealed first-trimester vaccination as a potential strategy to maximize Tdap-induced IgG transport to premature newborns. We conclude that optimal prenatal vaccine regimens depend on gestational length and placental features and novel prenatal vaccines can leverage these determinants of IgG transport to maximize neonatal immune protection.

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

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Autoimmune thyroid disease (AITD) affects 10% of Americans and is a valuable model to understand tissue immune tolerance in humans. In Hashimoto's thyroiditis, self-reactive T cells target hormone-producing thyroid epithelial cells (thyrocytes), leading to destruction of local tissue architecture. Intriguingly, 70% of Hashimoto's patients retain tissue function despite immune infiltration, suggesting additional tissue-specific mechanisms to constrain inflammation. To understand how cellular dynamics drive loss of tissue function in AITD, we applied multimodal single-cell genomic approaches to 23 thyroid surgical specimens from patients with and without AITD. We generated an unprecedented cellular atlas of paired single-cell RNA sequencing (scRNAseq) with TCR, BCR and 204 surface proteins (CITE-seq) comprising 375,000 parenchymal, stromal, and immune cells from tissue and 125,000 immune cells from matched blood samples.

Our results highlight several cellular and transcriptional programs underlying the clinical spectrum of AITD. We identify a novel population of thyrocytes in Hashimoto's patients that is strongly correlated with immune infiltration and displays interferon-gamma responsive transcriptional programs, including up-regulation of the MHCII antigen-presentation machinery. In addition, this thyrocyte population specifically upregulates several genes expected to inhibit T cell activity, including PD-L1. Our data suggest that these "immunothyrocytes" respond to and subsequently constrain thyroid immune infiltration, in turn preserving thyroid function. Cell-cell interaction analyses suggest that this immunothyrocyte phenotype is induced by activated tissue resident-like CD8+ T cells as well as GZMK+ CD8+ T cells. We postulate a homeostatic feedback loop, whereby CD8+ T cells induce the formation of immunothyrocytes that in turn downregulate the activity of those CD8+ T cells as well as thyroid-infiltrating CD4+ T cells.

We identify and characterize several disease-specific populations of ILC, B, CD4 T, and CD8 T lymphocytes as well as myeloid cells and fibroblasts. We will present analyses examining how these disease-specific cell populations may collectively interact to modulate immune infiltration. Our work will help decipher how immune-parenchymal-stromal interaction networks modulate chronic tissue infiltration and alter tissue function in organ-specific autoimmunity.

HIGH-THROUGHPUT AND HIGH-DIMENSIONAL SINGLE-CELL PROFILING OF T CELLS

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T cells are important to the initiation, prevention, and cure of many diseases. In the past several years, we have developed several tools to profile the T cell repertoire. In this talk, I will first introduce these tools and then give examples on how we use them to answer some of the fundamental questions in systems immunology, which in turn help us design new approaches in immune engineering.

QUANTITATIVE MODELING AND ANALYSIS OF TCR CROSS-REACTIVITY

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A central problem in immunology involves predicting TCR-pMHC specificity solely from their amino acid sequences. Screening of TCR libraries against individual peptides has generated datasets [1] and led to development of tools (e.g. [2]) to predict the specificity of a candidate TCR to a given peptide. However, progress on the inverse problem, i.e., predicting the binding affinity of different peptides to a particular TCR, has been hampered by lack of adequate training data. Predicting epitope specificity is important in multiple contexts: it would allow 1) to understand TCR cross-reactivity (i.e., ability of a TCR to bind to multiple peptides), which is believed to be a key component of negative selection of T cells [3,4], and 2) to investigate the effects of epitope mutations on peptide-TCR specificity, which have implications in pathogenic immune escape [5].

To address this problem, we combined peptide-TCR binding data for human HLA-A*02:01 MHC antigens from 15 experimental studies covering more than 10 different TCRs, and built a library of TCR-specific binding and non-binding epitopes, for testing hypotheses about TCR specificity and cross-reactivity. We evaluated different amino acid sequence distance functions (i.e. Hamming distance, r-contiguous distance, BLOSUM, PAM, and Atchley factors) to quantitatively characterize the "recognition space" of a TCR, based on its ability to separate TCR-specific strong-binding epitopes from non-binding epitopes. The results indicate that sequence distance functions based on amino acid substitution frequencies, e.g., BLOSUM and PAM, are better classifiers than other distance measures for our data, with a balanced accuracy in the range of 75-85%. We then visualize TCR-specific peptide recognition landscapes and predict the effects of point mutations at different positions of immunogenic index peptides. Furthermore, the clustering of binding and non-binding epitopes by the sequence distance functions allows quantification of specificity and cross-reactivity of individual TCRs, and a hierarchical classification of TCRs based on such properties, which is shown to be consistent with their structural evaluations. Our results challenge models of negative selection that use Hamming distance for TCR-peptide recognition, and call for a re-evaluation of self-non-self discrimination models.

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AN INTEGRATED ANALYSIS OF THE ANTIGEN-SPECIFIC T CELL LANDSCAPE IN AUTOIMMUNITY

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T cells exhibit unique specificity and plasticity through a highly diverse repertoire that is defined by their T cell receptors (TCRs). TCRs from CD4+ and CD8+ T cells recognize antigens presented in class I and II major histocompatibility complexes (MHC), respectively. Cognate peptide-MHC:TCR interactions initiates signaling that integrates with environmental signals to drive distinct transcriptional programs that shape their fate and function. Advanced sequencing technologies have allowed for unprecedented characterization of T cell states and repertoires. However, defining the antigen specificity of T cells remains a challenge. To that end, we developed an antigen discovery method that uses Signaling and Antigen-presenting Bifunctional Receptors (SABRs). SABR libraries presenting peptides in MHC class I or II can be used to identify TCR specificities de novo. Here we present a pipeline that leverages paired single cell RNA and TCR sequencing (scRNA/TCRseq) to match T cell states with TCRs of interest then experimental and computational antigen discovery based on SABR libraries. Using this pipeline, we sought to define the T cell repertoire that infiltrates pancreatic islets of non-obese diabetic mice and mediates destruction of insulin-secreting βcells in a model of Type 1 Diabetes. scRNA/TCRseq of islet-infiltrating T cells from 6-, 8-, and 10-week-old NOD mice revealed distinct cell states corresponding to naïve/naïve-like, activated, restrained, and regulatory T cells. Clonal expansion of T cells correlated with activated and restrained states. We expressed the top clonally expanded CD4+ TCRs and used a SABR library expressing 4,075 β-cell-derived epitopes in MHC class II to identify the cognate epitopes of 10 TCRs, followed by computational TCR clustering to deconvolute 5 additional TCRs. The top hierarchy of infiltrating CD4+ T cells was dominated by reactivity to Insulin-derived epitopes, including Hybrid Insulin Peptides. Using latent factor modeling, we showed that inhibitory receptors such as Lag3, and modulators of intracellular signaling such as Ndfip1, were key regulators of clonal expansion. Using regulatory network analysis, we observed that Notch signaling through Rbpj plays a key role in CD4+ T cell expansion within islets. We are extending these to determining the antigen specificities of CD8+ T cells from the same dataset, and combining antigen discovery with spatial transcriptomics to define the inter- and intra-islet T cell repertoire to uncover mechanisms underlying β cell destruction in mice and humans. We envision our integrated analysis approach as a blueprint to investigate T cell repertoires in distinct immune contexts.

DECODING AND REWIRING IMMUNE RECOGNITION AT THE SINGLE-CELL LEVEL

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The adaptive immune system, such as T and B cells, has evolved to recognize foreign antigens through a high diversity of immune receptors, such as TCR and BCR. To understand how antigen recognition influences T cell fate decisions and function, it is essential to decode antigen specificity of T cells and link the recognition to T cell state. We describe ENTER, a lentiviral-mediated cell entry platform engineered to display ligand proteins, deliver payloads, and record receptor specificity. This platform is optimized to decode interactions between TCR-MHC peptides, BCR-antigen, and other receptor-ligand pairs. We further engineer ENTER to deliver genetic payloads to antigen-specific T or B cells, allowing us to selectively rewire cellular behavior in mixed populations and reshape the immune repertoire. Single-cell readout of ENTER by RNA-sequencing (ENTER-seq) enables us to multiplex enumerate antigen specificities, TCR clonality, cell-type and states of individual T cells. ENTER-seq of CMV-seropositive patient blood samples reveals the viral epitopes that drive effector memory T cell differentiation and inter- vs intra-clonal phenotypic diversity targeting the same viral epitope.

ENGINEERING NEXT-GENERATION T CELLS FOR CANCER IMMUNOTHERAPY

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The adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has demonstrated clinical efficacy in the treatment of advanced cancers, with anti-CD19 CAR-T cells achieving up to 90% complete remission among patients with relapsed B-cell malignancies. However, challenges such as antigen escape and immunosuppression limit the long-term efficacy of adoptive T-cell therapy. Here, I will discuss the development of and clinical data on next-generation T cells that can target multiple cancer antigens and resist antigen escape. I will also present recent work on tuning CAR signaling activities via rational protein design to achieve greater in vivo anti-tumor efficacy. This presentation will highlight the potential of synthetic biology in generating novel mammalian cell systems with multifunctional outputs for therapeutic applications.

GERMLINE-ENCODED AMINO ACID-BINDING MOTIFS DRIVE PUBLIC ANTIBODY RESPONSES

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Despite the vast diversity of the antibody repertoire, infected individuals often mount antibody responses to precisely the same epitopes within antigens. The immunological mechanisms underpinning this phenomenon remain unknown. Here, by mapping 376 “public epitopes” at high resolution and characterizing several of their cognate antibodies, we show that germline-encoded binding motifs (“GRAB motifs”) in antibodies drive recurrent recognition. Furthermore, systematic analysis of antibody-antigen structures uncovered 18 human and 21 largely different mouse germline-encoded GRAB motifs within heavy and light V gene segments that bind specific amino acids and are critical for public epitope recognition. Thus, GRAB motifs represent a fundamental component of the architecture of the immune system that ensure antibody recognition of pathogens and promote species-specific reproducible responses that can exert selective pressure on pathogens.

MAPPING THE T CELL REPERTOIRE TO A COMPLEX DEFINED GUT BACTERIAL COMMUNITY

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Immune regulation has become central to the treatment of cancer, autoimmune disease, metabolic disease, and infectious disease. Certain bacterial strains from the gut microbiome modulate immune function potently and specifically. However previous reports have profiled strains from the microbiome under artificial conditions of mono-colonization. This approach identifies strains that has a potential to modulate immune cell function, but it is unknown how a strain behaves in the context of a complex microbial community. This knowledge gap has hindered a rational design of a therapeutic microbial community.
Here, we establish a “physiological” gut by colonizing germ-free mice with a complex defined gut community and profile T cell responses to each strain individually so that we can decode a strain-by-strain view of immune modulation by the gut microbiome. We generate a complex defined bacterial community (hCom) with the following features: containing >100 strains from the common taxa in the human gut microbiome, and manipulable – we can easily test the function of each strain on the background of the rest of the microbial community. We colonize germ-free mice with hCom and examine T cell responses to each strain. Unexpectedly, the pattern of T cell responses suggests that many T cells in the gut repertoire recognize multiple bacterial strains from the community. We perform scTCR-seq and construct T cell hybridomas from 92 T cell receptor (TCR) clonotypes; by screening every strain in the community against each hybridoma, we find that nearly all of the bacteria-specific TCRs exhibit a one-to-many TCR-to-strain relationship, including 13 abundant TCR clonotypes that are poly-specific for 18 Firmicutes in the community. By screening three pooled bacterial genomic libraries against 13 pooled hybridomas, we discover that they share a single target: a conserved substrate-binding protein (SBP) from an ABC transport system. Treg and Th17 cells specific for an epitope from this protein are abundant in community-colonized and specific-pathogen-free mice. Our work reveals that T cell recognition of Firmicutes is focused on a widely conserved cell-surface antigen, opening the door to new therapeutic strategies in which colonist-specific immune responses are rationally altered or redirected.

MULTI-OMIC ANALYSIS IDENTIFIES METABOLIC ENHANCEMENT OF IMMUNE MEMORY BY LYSINE DEACETYLASE INHIBITION DURING IMMUNISATION AND INFECTION

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An increased understanding of the mechanisms responsible for immune memory formation could facilitate more effective vaccination strategies. We screened transcriptional signatures of T cell memory against drug response profiles and validated predicted hits using an in vitro model of T cell memory differentiation. We identified a subclass effect of lysine deacetylase inhibitors (KDACi), promoting memory differentiation at low dose treatment. Using multi-omic analysis of in vitro stimulated, KDACi-treated human CD8+ T cells, we identified enhanced glutaminolysis as the mechanism responsible. We validated the ability of selected KDACi to enhance – and glutaminolysis inhibition to reverse – T cell memory expansion in vivo using murine models including SARS-CoV-2 spike protein immunisation and influenza infection. We confirmed the same effect in a human clinical study, demonstrating that low dose KDACi treatment can expand, maintain and increase the breadth (2-10 fold) of both cellular and neutralising antibody responses to seasonal influenza vaccination. Our data demonstrate that clinical repositioning of selected KDACi could enhance the efficacy of existing vaccination strategies and indicate a role for glutaminolysis in promoting immunological memory.

MULTI-DIMENSIONAL INTEGRATION OF PROTEIN INTERACTOMES WITH GENOMIC AND MOLECULAR DATA DISCOVERS DISTINCT RA ENDOTYPES

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Rheumatoid arthritis (RA) is a complex autoimmune disease with polyetiological genetic basis and dysregulation of a plethora of cellular and molecular phenotypes. The presence of rheumatoid factor (RF) and anti-citrullinated peptide (CCP) antibodies in serum represent two key diagnostic criteria for RA; however, it is unknown whether this difference merely represents a variability in biological markers or two distinct endotypes of RA. To address this, we first dissected differences across ~900 RA patients half of whom were serologically positive for both CCP and RF (i.e., double positive – DP), and half that were positive for RF alone (RF). Using a rigorous restricted maximum likelihood approach, we estimated the difference in heritability across the DP and RF groups. Surprisingly, there was a significant difference in heritability across these groups (~30%), suggesting that there are fundamental differences in the generic risk and bases of these two kinds of RA. Next, we carried out a genome wide association analysis (GWAS) and identified the HLA locus as explaining part of but not the entire difference in heritability between DP and RF RA. To delve into the bases of the missing heritability, we implemented a network-based GWAS approach. Our network approach first uses a creative adaptation of Linkage Disequilibrium Adjusted Kinships (LDAK) to aggregate the impact of multiple regulatory SNPs associated with a gene into a single score, taking into account the underlying LD structure. It then uses a random walk with restart algorithm to propagate this score across the reference human protein interactome network, and identify network modules that explain significant the differences in heritability across DP and RF. These modules include HLA genes, but also capture other cytokines, chemokines and immune regulators and almost completely capture the differences in heritability across these two endotypes of RA. We also developed a novel heritability partitioning approach that takes into account the structure and topology of the protein network and provides key insights into the relative importance of these modules in explaining the endotype-specific differences in heritability. We were also able to further validate these modules by recapitulating some of the corresponding differences at the transcriptomic and proteomic level. Together, our results suggest that DP and RF RA are different disease endotypes with distinct genetic bases and pathophysiology.

HUMAN INFANT AND ADULT T CELLS EXHIBIT DISTINCT PROGRAMS OF RESIDENCY, ACTIVATION, AND EFFECTOR FUNCTION ACROSS TISSUES

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The first years of life are essential for the development of adaptive immunity and lay the foundation for a lifetime of protection against invading pathogens. T cells coordinate virtually all aspects of the adaptive immune response and rapidly differentiate into memory cells that populate the body's diverse tissue sites during a critical window in infancy, under two years of age. However, the degree to which tissue localization shapes infant T cell responses remains poorly understood and whether the activation states of tissue T cells during infancy reflect those during adulthood is unclear. To dissect the diversity of T cell responses across tissues and age, we use single cell RNA-sequencing to profile resting and activated T cells from eight lymphoid and mucosal tissues obtained from eight individual human organ donors, ranging from 2-9 months and 40-65 years of age for each cohort. Using single cell Hierarchical Poisson Factorization (scHPF), we define key transcriptional states associated with T cell lineage, tissue localization, and activation throughout the body in infants and adults. We find that relative to adults, infant resident CD8+ T cells across tissues demonstrate a stem-like transcriptional profile with increased expression of transcription factors TCF1 and LEF1 associated with self-renewal, yet exhibit profoundly reduced activation capacity with limited production of effector cytokines and cytotoxic mediators. We further elucidate divergent transcriptional signatures associated with tissue residency between infant and adult T cells and leverage a gene regulatory network constructed by ARACNe to uncover transcription factors driving these unique T cell states. We identify Helios (*IKZF2*) as a critical transcriptional regulator of the infant-specific tissue program and interrogate its role in primary infant tissue T cells via CRISPR-Cas9 gene editing. Our results reveal key differences in infant versus adult T cell transcriptional programming and activation capacity across tissues, providing new insights to T cell function and regulation during the formative years of infancy.

DYNAMIC IMMUNE LANDSCAPES DURING MELANOMA PROGRESSION REVEAL A ROLE FOR ENDOGENOUS OPIOIDS IN DRIVING T CELL DYSFUNCTION

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Background Immune checkpoint blockade (ICB) therapies aimed at invigorating the anti-tumor immune response have achieved unprecedented responses in several tumor types, including melanoma. Despite this great success, approximately 50% of melanoma patients either fail to respond or develop resistance to ICB. As the immune response becomes progressively disabled as tumors advance, achieving a better understanding of the immunosuppressive mechanisms that take hold during tumor progression is needed to identify novel therapeutic targets and extend the benefit of immunotherapy to more patients.
Methods We performed single-cell RNA-sequencing to profile the immune infiltrate of B16F10 melanoma tumors harvested at different time points after implantation. We characterized the cell populations and investigated dynamic changes in the cell population compositions and in the gene expression within each population over the course of tumor progression.
Results We identified 23 tumor-infiltrating immune cell populations in 25,920 cells from 18 individual B16F10 melanoma tumors across 5 time points. We uncovered an unexpected role for endogenous opioid signaling in the development of CD8+ T cell dysfunction during melanoma progression, a population decreased in relative frequencies. The endogenous opioid-polypeptide hormone pro-enkephalin (Penk) was progressively up-regulated in CD8+ T cells that transitioned from the effector to a terminally exhausted T cell state with tumor progression. We validated the pro-exhaustion role of Penk in tumor antigen-specific responses using gain- and loss-of-function experiments.
Conclusions Our data reveal an unexpected role for endogenous opioids in driving T cell dysfunction, thereby linking analgesic pathways and the dampening of T cell functionality in cancer. Finally, our findings have high clinical relevance as patients with advanced tumors are often treated with opioids, which may ultimately limit anti-tumor CD8+ T cell responses and ICB efficacy.

DEVELOPMENT OF ADAPTIVE IMMUNITY IN HUMAN TISSUES

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Infancy and childhood are critical life stages for the generation of immune memory for protection against pathogens. This process has been challenging to elucidate in humans as most memory T cells reside in tissues. Here, we define the genesis of human T cell memory, its localization, and developmental pathways through examination of T cells in mucosal sites, lymphoid tissues, and blood from 90 pediatric donors aged 0-10 years. We reveal that memory T cells preferentially localize in mucosal sites during infancy and accumulate more rapidly over childhood compared to blood and lymphoid organs. Transcriptional analysis reveals that mucosal memory T cells in infancy lack pro-inflammatory function and exhibit stem-like transcriptional profiles, but progressively adopt effector capacity and signatures of mature tissue resident memory T cells over childhood. Single cell transcriptome profiling further reveals a distinct cytokine profile and activation trajectory of early life T cells compared to T cells in adults. Our results show that exposures during the formative years establish the landscape of immunity through tissue-specific generation and adaptation of memory T cells.

DISCOVERY OF NOVEL TREG MODULATORS BY INTEGRATING GENE EXPRESSION PROFILING WITH COMPUTATIONAL MODELING AND FUNCTIONAL ASSAYS

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Regulatory T cells (Tregs) control immunity in homeostasis and their dysregulation can result in inflammation and autoimmunity. However, their context-specific functions in tissue regulation are not well understood, and the molecular mechanisms underlying these processes appear remarkably complex. We have integrated RNA-seq analyses, computational modeling and a suite of functional assays to predict and validate key regulators of tissue Treg function. Sorted bulk and sc-RNA sequencing profiles from immune and stromal cells in a range of healthy and diseased human tissues, were analyzed and integrated using computational methods. Based on these learnings, we established *in vitro* culturing conditions that induce the expression of tissue pathways in primary human Treg. High-resolution maps of cell specific immune-regulatory pathways generated a prioritized set of molecular targets that were assessed in a scalable 7-dimensional *in vitro* screening cascade against a range of mechanistically and clinically relevant Treg functions. This combination of deep *in silico* analyses and complex functional interrogation in clinically relevant assays represents a foundation for informing the development of therapeutics for the treatment of autoimmune and inflammatory disorders.

SINGLE CELL PROFILING OF BLOOD IMMUNE CELL SUBSTATE KINETICS IN EARLY SEPSIS REVEALS PROGRESSIVE DECREASE IN MONOCYTIC MYELOID-DERIVED SUPPRESSOR CELLS

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Background: Dysregulation of the immune response to bacterial infection is a central, but poorly understood component of sepsis. Evaluating changes in immune cell states over time, especially in the early, critical stages of sepsis, can reveal key mechanistic insights into sepsis immunopathogenesis, yet these data are scant. Single-cell RNA sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs) from sepsis patients in our laboratory has demonstrated a novel monocyte transcriptional state (MS1) enriched in sepsis. The gene expression profile of MS1 cells is similar to that of myeloid-derived immune suppressor cells (MDSCs), which are immune regulatory cells best characterized in malignancies. In this study, we profiled blood samples obtained at different time points from patients with sepsis, and controls comprising patients with non-infectious critical illness (sterile inflammation), and healthy individuals. We evaluated the kinetics of MS1 and other immune cell substates, and plasma protein correlates of MS1.
Methods: PBMCs were obtained from patients with sepsis (n=37), sterile inflammation (n=18) and healthy controls (n=8) at hospital presentation (Day 0), Day 1 and Day 3. We analyzed ~1500 single cells per sample and identified transcriptional states by subclustering within immune cell lineages. Substate abundances were compared between patient phenotypes and across time using Wilcoxon rank sum testing with Benjamini-Hochberg correction. We measured plasma concentrations of MS1-derived plasma proteins based on the MS1 transcriptional profile.
Results: MS1 fractional abundance was higher in sepsis than both sterile inflammation and healthy control patients at all time points, and in sepsis patients, decreased from Day 0 to Day 3 (padj=0.027). Naïve and memory CD4+T and CD8+T fractions negatively correlated with MS1 at Day 0 for sepsis patients (significant Pearson's correlations < -0.4). Plasma concentrations of IL-6 and IL-10, cytokines that induce MDSC production, progressively decreased in sepsis patients. Resistin and calprotectin, proteins predicted to be highly expressed by MS1 cells, were both elevated in sepsis; resistin, but not calprotectin, paralleled MS1 kinetics.
Conclusion: This study demonstrates progressively decreasing abundance of monocytic MDSCs in early sepsis and corresponding kinetics in some plasma protein correlates. Negative correlations between MS1 and T cell fractions on presentation, but not later, suggest that these monocytes may facilitate systemic immunosuppressive activity very early in sepsis; the subsequent decrease in MS1 abundance may facilitate an adaptive microbicidal response to infection.

COMPUTATIONAL DETECTION OF ANTIGEN-SPECIFIC B-CELL RECEPTORS FOLLOWING IMMUNIZATION.

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B-cell receptors (BCR) play a crucial role in recognizing and fighting foreign antigens in our bodies. However, when dealing with such data, we are faced with an immense repertoire of different receptors, with only a few of them being effective against a specific pathogen due to their ability to bind to it.
In order to identify BCRs that recognize specific antigens, the authors propose a computational method that utilizes real BCR sequencing data. This approach involves identifying clusters of expanding BCR sequences at a single time point, the peak of infection, by combining information on nearest neighbors and frequencies. Nearest neighbors refer to sequences that are similar in terms of amino acids, thus sharing similar functionalities. By comparing these clusters to expected values in healthy patients using generative models, the method is able to detect antigen-specific BCRs. The effectiveness of this method is demonstrated using longitudinal data and experiments assessing the affinity of the BCRs to the antigen. The results are validated on two different sets of data: a public one with five humans vaccinated for flu and one provided by the Large Molecules Research platform at Sanofi with ten immunized mice. This new method offers a powerful tool for understanding the dynamics of B-cell immunity following vaccination and has potential for application in vaccine development, personalized medicine, and antibody-derived therapeutics.

DEVELOPMENT OF MASTER iPSCs WITH SWITCHABLE HLA GENES BY MEGA-BASE-SCALE GENOME ENGINEERING

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This presentation introduces **two types of master iPSCs** that we created to deepen our understanding of HLA-mediated cell-cell interactions between immune and target cells and to develop allogeneic therapeutic cells. The first master cell (HLA-I free master iPSCs) has **~400 kb deletion** of all the six HLA class I gene loci and integration of our landing pad called UKiS donors at both alleles (UKiS: Nature Comm. 13, 4219, 2022), whereas the second one (All HLA free master iPSCs) has **~1 Mb deletion** of all of the six HLA class I and all the 11 HLA class II gene loci and integration of UKiS donors at both alleles. The UKiS donor alleles can be replaced with any gene cassettes of your interest. Here, to obtain POCs, we created two different iPSC lines from HLA-I free master iPSCs by replacing both UKiS donor alleles with the following gene fragments: 1) anti-CD19 CAR & the entire gene territory of HLA-E*01:01:01 gene containing the signal sequence of HLA-A or 2) the entire gene territories of HLA-A*24:02:01, HLA-B*07:02:01, HLA-C*07:02:01 and HLA-E*01:01:01. In both cases, 10 kb-scale integration of the entire HLA gene territories including promoters and introns was effectively performed and all the integrated HLA genes were sufficiently expressed. These master iPSCs are useful for studying functional differences of each HLA type in interactions with molecules on various immune cells, which will provide us the HLA-mediated interaction rules between immune cells and their target cells as well as platform cells to develop cell modality for future cell therapy and regenerative medicine.

POST-COVID SYMPTOMS AND ENDOTYPES ARE ASSOCIATED WITH THE INABILITY TO MODULATE THE TRAJECTORY OF IMMUNE AND HEMOSTATIC PATHWAYS

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Background: Persistent symptoms after COVID-19 infection (long COVID; post-acute sequelae of SARS-CoV-2 infection), can affect almost half of COVID-19 survivors, leading to poor quality of life. Despite its prevalence, the pathophysiology of this phenomenon is poorly understood.
Objective: To identify differences in gene expression between patients with and without post-COVID symptoms in hospital, at follow-up, and longitudinally.
Methods: As part of the Banque Québécoise de la COVID-19 biobank, 24 adult patients who were hospitalized due to respiratory COVID-19 were enrolled. Whole blood was collected within the first 10 days in hospital and 4-12 weeks after discharge. Differentially expressed (DE) genes were identified between patients with and without post-COVID symptoms in hospital, at follow-up, and longitudinally. K-medoids clustering was performed to further separate patients into clusters for DE analysis and pathway enrichment.
Results: When comparing patients in hospital and at follow-up, very few DE genes were related to post-COVID symptoms. However, analyzing per-patient gene expression trajectories showed that patients with no post-COVID symptoms had large temporal gene expression changes from hospital to follow-up compared to symptomatic patients (5,533 vs 1,580 DE genes); these were particularly enriched for the downregulation of genes in inflammatory and hemostatic pathways. Clustering analysis revealed three unique patient endotypes that also differed in trajectories. Cluster 1, with the lowest proportion of post-COVID symptoms, had a robust immune and hemostatic response in hospital that resolved by follow-up. Conversely, Clusters 2 and 3, with higher proportions of symptomatic patients, had either a persistently dampened or activated immune and hemostatic responses, respectively. Clusters were defined by a specific 6-7 gene expression signature that could be used for classifying patients for diagnosis and guiding tailored treatment.
Conclusions: High rates of post-COVID symptoms in hospitalized patients were associated with an inability to mount, and subsequently resolve by 4-12 weeks after discharge, a robust early response involving immune and hemostatic pathways.

QUANTIFYING CELLULAR COOPERATIVITY AND INFERRING THE EXTRACELLULAR SIGNALS DRIVING IT

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We present an integrated experimental-computational approach aiming to determine, in a quantitative manner, the functional role played by different cell subsets and extracellular communication signals in shaping the behavior of intercellular networks. This approach utilizes a microwell-based experimental system, that allows isolating and culturing small groups (up to a few dozens) of cells over extended periods of time, while monitoring their interactions and states using fluorescence microscopy. We designed this system to specifically facilitate effective quantitative inference of cellular networks, relying on a few key properties: (i) Each experiment consists of more than 3,300 biological replicates, providing a large sample size; (ii) The biological variability associated with small numbers of interacting cells yields a relatively comprehensive sampling of possible behaviors; (iii) Ability to (probabilistically) control and measure the cellular composition of microwells; (iv) Multiplexed measurement of up to 14 extracellular communication signals in each microwell; (v) Non-invasive measurements, enabling to follow each microwell over time. In parallel, we present a simple mathematical framework for the analysis of the data generated by this system, which provides a semi-quantitative measure of cellular cooperativity, and which allows decoupling the degree of cooperativity associated with interdependent biological processes. We further present a statistical framework that enables to quantitatively infer the contribution of each measured extracellular signal in determining observed biological outcomes, such as cell fates.
We demonstrate the capabilities of this system studying the differentiation of mouse Th17 cells. We show that this is a collective process, that requires a relatively large number (> 10) of intermediate intercellular communication steps, in which information processing is carried out by a relatively small percentage (10-20%) of the interacting cells. We further derive two predictions, which we validate experimentally: First, we correctly predict a correlation between the time in which communication signals are expressed during differentiation, and the degree of intercellular cooperativity associated with their production. Furthermore, we generate an accurate quantitative prediction of cell-level behaviors. In addition, we infer the functional role played by different cytokines in Th17 differentiation, including both known and predicted novel relations, which we validate in a separate experimental system.
We propose that our approach can complement existing single-cell profiling technologies for the study of intercellular networks, shedding light on the quantitative role played by their components in governing their behavior.

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PROFILING EARLY LIFE IMMUNE-MICROBE INTERACTIONS TO DETERMINE VACCINE AND ALLERGEN RESPONSES

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The first months of life represent a critical period of immune maturation. The immune system is in part influenced by our genetics, however initial development may largely be driven in response to environmental exposures (Brodin et al., 2015). Systems level immune profiling during early development suggests gut colonization as an important source for the immune variation observed amongst infants (Olin et al., 2018). The gut microbiome, which is a reservoir of antigens and metabolic mediators, begins a sequential colonization from birth and is easily perturbed. Early life may therefore also be a period of extreme vulnerability. Epidemiologically, gut perturbations such as antibiotics have been linked to allergies and obesity amongst other diseases. We examine IgG and IgE phenotypes to childhood vaccine antigens and allergens during the first years of life to identify immune and gut microbial determinants of serological responses.

Using a longitudinal cohort of infants (n=259), parents (n=270) and cord blood (n=214) from Karolinska University Hospital in Sweden we collected whole blood to apply a bead-based assay to measure the relative titers of IgG specific to childhood vaccine antigens and IgE specific to known allergens. Immune cells from mass cytometry and plasma protein compositions were also acquired, and metagenomics libraries were generated from stool. Immune profiling data is currently being analyzed and will be important to reveal distinguishing immune correlates between clusters of subjects. Reconstructing the taxonomic and functional profiles from the gut will help us understand the contribution of microbial colonizers to allergy and vaccine responses. Furthermore, clinically verified diagnosis of atopy is available for a small subset of the cohort. Preliminary analyses reveal IgG vaccine response trajectories that stratify with gestational age for multiple antigens.

This provides the unique opportunity to study with high resolution how gut colonizers may influence immune populations and thus play a role serologic IgG and IgE responses. Identifying and understanding these determinants will be crucial for limiting perturbations to promote vaccine responses and immune tolerance.

ENGINEERING OF STEM CELL-DERIVED CAR-MACROPHAGES CARRYING PAYLOADS TO REMODEL THE TUMOR MICROENVIRONMENT

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Using chimeric antigen receptors (CAR) to direct macrophages against tumor antigens is a promising new approach to overcoming limited infiltration in tumors faced by current CAR-T-cell-based therapies. However, this approach is hampered by the availability of primary human macrophages and technical challenges in the number of genetic manipulations that can be introduced. Here, we present engineered stem cell-derived CAR-macrophages carrying payloads to infiltrate and remodel the immune-suppressive tumor microenvironment (TME). The use of human pluripotent stem cells allows genetic manipulations. We used human pluripotent stem cells (PSC) to generate macrophages to achieve this. Following 30 days of cytokine-based differentiation, we detected the expression of macrophage markers, such as CD11b and CD14. After 2 to 4 days of exposure to LPS+IFNg or IL-4, they polarized to M1- or M2-like state, respectively, which was sustained for 4 weeks in culture. In co-culture systems, stem cell-derived macrophages showed phagocytosis of RFP-labeled E.Coli and of FITC-labeled HepG2 liver cancer cells. We then confirmed that introducing a GPC3-targeting CAR into stem cell-derived macrophages and THP-1-derived macrophages enhanced the phagocytosis of GPC3+ HepG2 cells. Next, we constructed multiple GPC3-targeting CAR constructs to deliver our payloads. As payloads, we chose two pro-inflammatory chemokines, IL12 and IL18, as well as two single chain variable fragments (scFv) targeting PD-1 and TGFb. The first two will promote a tumor-killing milieu. Latter two will block the immune-suppressive milieu. We showed that after piggyback-based delivery, human PSCs showed doxycycline-inducible expression of the CAR and of the TGFb scFv. Using fluorescent reporter cell lines, we then confirmed the secretion of TGFb scFv, PD-1 scFV, or IL18 by our PSCs, and of IL12 by HEK293T cells carrying our CAR-construct. Validation of secretion of the payloads by the respective CAR-macrophages is ongoing. So far, we have established a platform to generate stem cell-derived CAR-macrophages carrying payloads with the potential to remodel the TME. Building on this, we will now contribute to the next generation of stem cell-derived cell-based cancer therapies.

APPLYING MACHINE LEARNING TO PREDICT CLINICAL RESPONSE FROM INTESTINAL MICROBIOTA IN MELANOMA PATIENTS UNDERGOING ANTI-PD1 TREATMENT

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Immunotherapy (such as anti-PD1 therapy) is a relatively new treatment for cancer that manipulates the patient's own immune system to better fight cancer. It has been shown to be particularly effective against immunologically "hot" cancers such as melanoma, but even there its effectiveness varies by patient for a variety of factors. One such factor is differences in the patients' intestinal microbiome. As part of a meta-analysis of our own melanoma cohort combined with four others, we have applied various supervised machine learning methods on batch corrected microbiome data in order to create models to predict the clinical response (responder or non-responder).

The machine learning methods used were generalized linear models, random forest, and polynomial support vector machines. We created models on individual cohorts (with a 70% training, 30% testing breakdown) as well as creating models trained on all cohorts but one and testing on the remaining cohort.

These models, even with the relatively small size of these cohorts, can make predictions that are statistically significant in some cases and their feature weights can be examined to determine which microbial taxa are most important to clinical response. We will improve these models by incorporating more (and larger cohorts) and by incorporating RNA-seq data where available to create models that combine gene expression and the microbiota for greater power.

STUDYING THE EVOLUTION OF TUMOR-ASSOCIATED MACROPHAGES IN A 3D SPHEROID MODEL OF THE EARLY MELANOMA TUMOR MICROENVIRONMENT

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Tumor-associated macrophages are a promising therapeutic target to combat resistance to immune checkpoint blockade (ICB) therapy. For example, patients with melanoma, a deadly form of skin cancer, often develop ICB resistance. While treatment with ICBs has had some success in melanoma patients, 50% of patients do not have objective responses. Tumors unresponsive to ICBs often display increased numbers of tumor-associated macrophages (TAMs), which are generally immunosuppressive and associated with a poorer prognosis, making them a promising target.

The immune activity of TAMs and other cells in the melanoma tumor microenvironment (TME) is determined by cell-cell interactions and a network of secreted signals. During tumor progression, homeostatic networks between macrophages, fibroblasts, and cancer cells are imbalanced and lead to immunosuppressive programming of TAMs. Understanding how TAM subpopulations are recruited and programmed within the tumor and their functional role in the immunosuppressive signaling network will support development of targeted therapies to reestablish an effective immune response, especially in cases of ICB resistance.

To model tumor-fibroblast-macrophage interactions, we constructed a 3D *in vitro* spheroid model of the melanoma TME to study interactions between an immunogenic murine melanoma tumor cell line (YUMMER1.7), bone marrow derived macrophages, and fibroblasts (3T3MEF). Spheroid models have been shown to best recapitulate the physical properties of tumors, including direct cell-cell interactions and spatial orientation of cells. Here, we demonstrate the ability to form spheroids with these three cell types embedded into a 1.5mg/ml Collagen I gel, which can be analyzed through several assays such as, live imaging, immunofluorescence staining, and phenotypic macrophage profiling which can be benchmarked against *in vivo* tumors.

This model can be applied to investigate the recruitment and programming of TAMs during tumor development, and the balance between pro and anti-inflammatory macrophages in the immunosuppressive TME. Additionally, potential for pro-inflammatory activation of TAM subpopulations can be studied in spheroids perturbed by macrophage-targeted therapies, such as agonistic CD40 (CD40ag). Together the development of this *in vitro* model of the melanoma tumor enables investigation of TAM function during the early establishment of the immunosuppressive TME and during rescue of TAM anti-tumor function with macrophage-targeted treatment.

CO-STIMULATORY SIGNALS VIA CD28 AND CD27 COLLABORATE TO REGULATE IL-2 PRODUCTION IN CD8$^+$ T CELLS

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CD8$^+$ T cells play a central role in the immune protection against infectious and malignant disease. Efficient effector responses and differentiation of CD8$^+$ T cells are regulated by the spatio-temporal availability of co-stimulatory and inhibitory molecules. However, it remains incompletely understood how different co-stimulatory pathways interact to induce efficacious T cell responses. By interrogating the costimulatory requirements for effective activation of CD8$^+$ T cells using co-stimulation-deficient systems, we found that cytokine production, in particular IL-2 secretion, by virus-specific CD8+ T cells was collectively dependent on both the CD27-CD70 and CD28-CD80/86 co-stimulatory pathways. This co-stimulation-dependent effect on the quality of antigen-specific CD8$^+$ T cells was programmed early after viral infection, and persisted long-term. Interestingly, the CD27-CD70 and CD28-CD80/86 co-stimulatory pathways differentially impacted the transcriptional program of CD8$^+$ T cells following T cell receptor engagement, and distinctly contributed to de novo transcription and post-transcriptional regulation of *Il2* mRNA. Moreover, expression and nuclear translocation of the transcription factor c-Rel, a NF-kB family member known to be critical for IL-2 transcription, was differently regulated by CD27- and CD28-mediated co-stimulatory signals, with full induction requiring collective signaling. In line with the autocrine role of IL-2, deficient co-stimulation via CD27 and CD28 severely impaired T cell expansion during primary and memory responses, which could be partially rescued by constitutive IL-2 expression. Collectively, our results demonstrate that the CD27-CD70 and CD28-CD80/86 co-stimulatory pathways act in a distinct, but collaborative manner to instruct effective cytokine and proliferative responses of CD8$^+$ T cells. These findings may inform the conception and development of effective immunotherapies harnessing the protective capacity of CD8$^+$ T cells, including novel vaccine strategies and adoptive cell therapies.

MAPPING COOPERATIVITY AND ANTAGONISM BETWEEN TRANSCRIPTION FACTORS AT IMMUNE RESPONSE GENE PROMOTERS

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Immune system responses to pathogenic infection are highly regulated at the transcriptional level. Upon infection, transcription factor proteins (TFs) modulate the expression of immune response genes by interacting with regulatory DNA regions. Detailed mapping of these interactions is essential for understanding immune responses, as their dysregulation can contribute to immunodeficiency and autoimmunity. Most experimental approaches to map these protein-DNA interactions (PDIs), such as genome-wide ChIP-seq and CUT&Run and TF-wide enhanced yeast one-hybrid assays, generally evaluate the DNA binding of individual TFs. However, TF-DNA binding can be modulated by the presence of other TFs within the nucleus. For example, many key TFs involved in immunity, such as NF-κB, AP-1, and nuclear receptors, are known to function as heterodimer complexes, while other TF-pairs have been shown to bind DNA cooperatively or antagonistically. Here, we developed paired yeast one-hybrid (pY1H) assays to detect cooperative binding and antagonism between TF-pairs at DNA regions of interest. Using pY1H assays, we screened 307 pairs of human TFs for their interactions with promoters of 20 cytokine genes, an important class of immune signaling genes highly regulated at the transcriptional level. We identified 168 cooperative TF binding events at 15 distinct cytokine gene promoters, expanding the existing cytokine gene regulatory network. This includes PDIs involving obligate heterodimers such as AP-1 dimers, RXRG/RARG, and MAX/MYC as hubs of cooperative binding. We have also identified 253 instances of DNA binding antagonism between TFs at 12 cytokine promoters. We observe that certain TFs such as MAX and FOS are commonly antagonized at cytokine promoters, while other TFs such as TBP and HIF1A act primarily as antagonists. Interestingly, we identified 19 TF-pairs that act cooperatively or antagonistically depending on the promoter, suggesting that the functional relationship between TFs is highly dependent on the target DNA sequence. Our findings illustrate the complex interplay between TFs in DNA binding and suggest potential mechanisms to achieve regulatory specificity for immune responses.

THE LIVER-INFILTRATING IMMUNE LANDSCAPE IN CHRONIC NORWAY RAT HEPACIVIRUS INFECTION AT SINGLE CELL RESOLUTION

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Chronic hepatitis C virus (HCV) infection afflicts over 58 million people worldwide and results in severe liver pathologies such as cirrhosis and hepatocellular carcinoma. Although it is now possible to cure chronic HCV infection with highly effective direct-acting antiviral drugs, an effective vaccine remains elusive, and significant knowledge gaps about the complex interplay of viral infection, adaptive immunity, and ensuant liver disease persist. One major limitation in studying the immune response to HCV is a lack of experimentally tractable, immunocompetent animal models. Initially identified in 2014 and adapted to mice in 2017, Norway rat hepacivirus (NrHV) is closely related to HCV, and transient antibody-mediated depletion of CD4 T cells immediately prior to NrHV challenge results in chronic infection in standard C57BL/6 mice, even after replenishment of the CD4 T cell compartment. Chronically infected mice exhibit many similarities to human patients chronically infected with HCV, including hepatitis, liver fibrosis, and CD8 T cell dysfunction. To characterize the immune response to chronic NrHV infection in this novel mouse model, we transiently depleted CD4 T cells and infected C57BL/6 mice with NrHV and analyzed liver-infiltrating leukocytes at 6-, 12-, and 18- months post infection using multimodal single cell transcriptomics methods (multiplex single cell RNA-Seq and scTCR-Seq). We observed diverse immune cell populations whose frequencies and subpopulation composition changed over the course of infection, including monocytes, macrophages, dendritic cells, and neutrophils. Within T cell populations of infected mice, we noted an increased frequency of regulatory CD4 T cells and a remarkable diversity of exhausted CD8 T cell subsets, including apparent progenitor, intermediate, and terminally exhausted populations. Further analysis indicated that exhausted CD8 T cell subsets were partitioned into two distinct lineages, each characterized by distinct gene expression programs and TCR clonotypes. Of note, in one of these lineages, we observed across multiple mice, convergent, “public” TCR clonotypes predicted to recognize common, putatively viral, antigens. These high-resolution transcriptomic studies of this novel, immunocompetent model of chronic viral hepatitis will help define the immunological mechanisms that hinder viral clearance and contribute to the pathogenesis of viral liver disease.

META-ANALYSIS ON INDEPEDANT SJÖGREN DISEASE COHORTS IDENTIFIES CONSENSUS GENE MODULES AIMED TO HELP STRATIFY PATIENTS AND PREDICT RESPONSE TO TREATMENT

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Primary Sjögren disease (pSD) is an autoimmune disease characterized by lymphoid infiltration of exocrine glands leading to dryness of the mucosal surfaces and by the production of various autoantibodies. The pathophysiology of pSS remains elusive and no treatment with demonstrated efficacy is available yet.

To better understand the biology underlying pSD heterogeneity, we aimed at identifying Consensus gene Modules (CMs) that summarize the high-dimensional transcriptomic data of whole blood samples in pSS patients. We performed unsupervised classification on four data sets and identified twelve CMs. We interpreted and annotated each of these CMs as corresponding to cell type abundances or biological functions by using gene set enrichment analyses and transcriptomic profiles of sorted blood subsets. Correlation with independently measured cell type abundances by flow cytometry confirmed these annotations. The Consensus modules may help the identification and translation of blood-based predictive biomarkers and the treatment of pSD.

MATHEMATICAL MODELING OF THE DYNAMIC STRUCTURE OF CIRCULATORY CD4+ MEMORY T CELL SUBSETS

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Healthy mice rapidly develop and maintain large populations of circulating memory phenotype (MP) CD4+ and CD8+ T cells, presumably in response to environmental, commensal, or self-antigens, which can also enhance new T cell responses to infectious challenge. By quantifying the dynamics of MP T cell maintenance and their rules of replacement, we can gain novel insights into how classical, pathogen-specific memory T cells are sustained. Previously, we showed that both effector (TEM) and central memory (TCM) CD4+ MP T cells each comprise at least two subpopulations with markedly different rates of turnover, and are continually supplemented by newly generated memory cells throughout life. However, the lineage relationships between fast and slow TCM and TEM once they enter the memory pool are not well understood. Further, we see distinct dynamics of both TCM and TEM populations within young and aged mice, suggesting that host and/or cell age impacts the persistence of memory T cell populations. Here we address these issues by combining DNA labeling with an established fate-mapping system, which allows us to follow cells and their offspring as they enter and reside in the CD4 TEM and TCM compartments. Using mathematical models, we characterize the lineage relationships between fast and slow memory cells and show that their population dynamics, and potentially their ability to be replaced by new memory cells, change as a function of their residence time. Our study reveals that CD4 MP T cell populations, and so potentially 'conventional' memory cells, have a rich dynamical structure with heterogeneity in self-renewal, loss, and susceptibility to displacement by newer, immigrant memory cells.

LARGE-SCALE DISCRETE EXECUTABLE MODELING OF ALVEOLAR MACROPHAGES

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Cytokine stimuli in the tissue microenvironment drive specialized homeostatic functions of resident macrophages. In the lung, steady-state signaling by the cytokine GM-CSF is particularly critical for supporting development and functions of alveolar macrophages (AMs). While role for GM-CSF in AM development and surfactant catabolism have been described, it is not well understood how ongoing exposure to GM-CSF shapes the inflammatory potential of these cells. To investigate GM-CSF's role in macrophage activation, we created a discrete executable model that encompassed a traditional mechanism of canonical macrophage M1 (pro-inflammatory) and M2 (anti-inflammatory) activation. We expanded the model to include the GM-CSF receptor and known downstream pathways, including peroxisome proliferator-activated receptors (PPARs) and interferon regulatory factors (IRFs). To validate the model, we recapitulated published experimental data of bone marrow-derived macrophages cultured in GM-CSF, with and without lipopolysaccharide (LPS) induction. We also demonstrate that the model is able to simulate canonical M1 and M2 activation states when given canonical M1 and M2 activation signals (LPS and Interferon gamma, or Interleukin 4 and 13, respectively) alone, and in complex co-induction experiments. With the validated model, we investigated how long-term exposure to GM-CSF alters regulation of macrophage responses to inflammatory stimuli. We show the most likely manner through which GM-CSF primes and modulates AM activation and response is via the NF-kB pathway. Altogether, our model recapitulates known macrophage activation states, as well as published experimental data of bone marrow derived macrophages cultured in GM-CSF. We will use this validated model to formulate testable hypotheses about lung-specific responses to clinically significant stimuli which we will validate with experimental results.

MULTIMODAL HIERARCHICAL CLASSIFICATION ALLOWS FOR EFFICIENT ANNOTATION OF CITE-SEQ DATA

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Single-cell RNA sequencing (scRNA-seq) is an invaluable tool for profiling cells in complex tissues and dissecting activation states that lack well-defined surface protein expression. For immune cells, the transcriptomic profile captured by scRNA-seq cannot always identify cell states and subsets defined by conventional flow cytometry. Emerging technologies have enabled multimodal sequencing of single cells, such as paired sequencing of the transcriptome and surface proteome by CITE-seq, but integrating these high dimensional modalities for accurate cell type annotation remains a challenge in the field. Here, we describe a machine learning tool called MultiModal Classifier Hierarchy (MMoCHi) for the cell-type annotation of CITE-seq data. Our classifier involves several steps: 1) we use landmark registration to remove batch-related staining artifacts in CITE-Seq protein expression, 2) the user defines a hierarchy of classifications based on cell type similarity and ontology and provides markers (protein or gene expression) for the identification of ground truth populations within the dataset by threshold gating, 3) progressing through this user-defined hierarchy, we train a random forest classifier using all available modalities (surface proteome and transcriptome data), and 4) we use these forests to predict cell types across the entire dataset. Applying MMoCHi to CITE-seq data of immune cells isolated from eight distinct tissue sites of two human organ donors yields high-purity cell type annotations encompassing the broad array of immune cell states in the dataset. This includes T and B cell memory subsets, macrophages and monocytes, and natural killer cells, as well as rare populations of plasmacytoid dendritic cells, innate T cells, and innate lymphoid cell subsets. We validate the use of feature importances extracted from the classifier hierarchy to select robust genes for improved identification of T cell memory subsets by scRNA-seq. Together, MMoCHi provides a comprehensive system of tools for the batch-correction and cell-type annotation of CITE-seq data. Moreover, this tool provides flexibility in classification hierarchy design allowing for cell type annotations to reflect a researcher's specific experimental design. This flexibility also renders MMoCHi readily extendable beyond immune cell annotation, and potentially adaptable to other sequencing modalities.

LONGITUDINAL SINGLE-CELL RNA SEQUENCING REVEALS B CELL REPERTOIRE DIFFERENCES INDUCED BY WILD-TYPE MEASLES VIRUS COMPARED TO LIVE-ATTENUATED MEASLES VACCINE

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Despite the availability of a safe and effective vaccine, measles still causes significant mortality and morbidity among children in developing countries. As a disease that induces immune amnesia with increased susceptibility to other infections but also life-long immunity to measles, little is known about the immunogenetics of B cells that elicit measles-specific antibodies (Abs) in natural infection vs vaccination. We therefore conducted single-cell RNA sequencing (scRNA-seq) of longitudinal peripheral blood mononuclear cells (PBMC) and lymph node (LN) samples from wild-type (WT) measles virus (MeV) and live-attenuated measles vaccine (LAMV)-infected rhesus macaques (RMs) and characterized their gene expression, B cell repertoires and clonotype changes via bioinformatic pipelines. Preliminary analysis of two RMs across four timepoints (one WT MeV- and one LAMV-infected RM) revealed similarities and differences in immune cell populations and B cell repertoires, providing important mechanistic insights into B cell clonal development in natural infection as compared to vaccination.

ORGANOID-MODELING OF SARS-COV-2 CYTOKINE STORM AND VASCULITIS

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Cytokine storm and vasculitis are the most concerning complications of SARS-CoV-2 infection. Human organoids are a tempting model to understand how innate immune cells damage vasculature. However, the lack of immune cells and vasculature in organoids hampers the attempt to model the activation of innate immune cells and vasculitis. Here we demonstrate the protocol to endow innate immune cells and vasculature in organoids. We previously established human embryonic organoids from pluripotent stem cells showing robust differentiation of hemato-endothelial lineages [1]. Consecutive-directed differentiation of the organoids induced maturation of both myeloid progenitors and lymphoid progenitors. However, the vascular network and its integrity were not solid. To overcome this limitation, we developed a new organoid by incorporating mesodermal progenitor and mesenchymal cells in a single aggregate. The resultant aggregate differentiated into vascular, hematopoietic, and stromal cells. Notably, we achieved apical-out differentiation of endothelium and hematopoiesis at the core of the organoid, suggesting the potential to model pathogen exposure. We propose that this model will advance the understanding of SARS-CoV-2 cytokine storm and vasculitis.

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MODELING AND MODULATING STAT ACTIVATION FEATURES THAT PREDICT EXPRESSION OF CYTOKINE-SPECIFIC GENE SETS

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Cytokines encode diverse and specialized cellular function by signaling via a limited set of janus kinases (JAKs) and signal transducers and activators of transcription (STATs). The combinations of JAK-STAT interactions alone cannot distinguish between cytokine-induced responses, and thus many open questions remain related to mechanisms regulating functional specificity. Previous studies have focused on understanding cytokine-induced signaling dynamics, while others have attempted to alter the JAK-STAT pathway and measure transcriptional outcomes. However, few have worked to link transcriptional factor activation dynamics to global gene expression patterns. In this study, we have created a computational workflow to make gene predictions from IL-6 and IL-10-induced STAT phosphorylation dynamics in macrophages, as these cytokines signal via STAT1 and STAT3, but induce distinct dynamic patterns and opposing inflammatory gene expression. We integrated mechanistic and machine learning models to identify distinct cytokine-induced gene sets that were predicted by early versus late STAT3 phosphorylation. A parameter sensitivity analysis of our mechanistic model predicted that JAK2 modulated IL-6-induced dynamic features in STAT-specific manners. We validated these predictions using the selective JAK2 inhibitor, Fedratinib, and demonstrated that inhibition of JAK2 results in large decreases in peak STAT1 phosphorylation and slightly shifts the timing of the STAT3 phosphorylation response. Finally, we used our full pipeline to predict gene sets that were sensitive or insensitive to JAK2 inhibition. This novel modeling and analysis workflow links STAT signaling dynamics to gene expression and serves as a first step towards developing models to predict pathology-associated genes driven by specific STAT-regulating signaling mechanisms.

CYTOKINES SURGE IN THE BLOOD YEARS BEFORE A CANCER DIAGNOSIS IN ELDERLY INDIVIDUALS

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How the human immune system interacts with a tumor before diagnosis is almost entirely unknown. Here we analyzed a longitudinal cohort of 133 healthy individuals (from 18 to over 90 years old), 28 of whom developed cancers over nine years. We found that a large group of cytokines surges 2-3 years before a cancer diagnosis, but not with other conditions (such as inflammatory or cardiovascular diseases). And these cytokine surges precede a cancer diagnosis only in those 80 years or older. In the The Cancer Genome Atlas (TCGA) we found that from 1,741 tissues across 8 types of cancers, elderly individuals elevate the transcription of a broad set of cytokines (including IFNG, IL1B, IL15 and others). Defects in cellular senescence (TP53 or CDKN2A/p16 mutations) sensitize the cells for the inflammation associated with advanced aging. Advanced aging elevates IRF1, a p53 and p16-independent transcription factor for apoptosis and inflammation. The rise of cytokine transcription in early-stage cancer tissues thresholds at the age of 80 years, similar to our serum findings. Noticeably, it has been reported that the cancer incidence rate decreases after 80 years old in the general population.

USING NETWORK MODELING OF TISSUE RESIDENT TREGS TO FACILITATE THE DEVELOPMENT OF NOVEL THERAPEUTICS IN AUTOIMMUNE DISEASES

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For inflammatory and autoimmune diseases, understanding the immune processes at the site of inflammation is critical in generating important insights into novel therapeutic strategies. To this end, we established an experimental and computational platform that allows a deep understanding of human tissue Treg behavior and is aimed at modulating gene regulatory circuits at the location where the disease manifests. Understanding the coordination of context specific signals and the complex physiological behaviors they produce is key to identifying high efficacy targets for the treatment of disease. To provide a systems-perspective model of these key processes, we have developed transcriptional regulatory networks derived directly from bulk RNAseq datasets of healthy and diseased human tissues as well as from primary Tregs cultured in vitro with controlled perturbations. These samples provide the biological foundation for consensus- and network-based methods to deconvolve the key inputs and outputs of immune stimulations, thereby generating a model that predicts gene regulatory hubs that cytokine stimulations and signaling cascades must be transmitted through in order to affect the function of Tregs. The model also offers a mechanism to fingerprint and de-convolve highly correlated, but distinct, pathways specific to Treg biology in different contexts including inflammatory and autoimmune diseases. Finally, the model enables differentiation of closely related drug targets and provides early signposts for indication selection and toxicity concerns during therapeutic development.

SYSTEMS-LEVEL ANALYSIS REVEALS BASELINE IMMUNE PROFILES ASSOCIATED WITH CANCER METASTASIS AND CLINICAL OUTCOMES IN CHILDREN WITH SOLID TUMORS

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Background
Children with solid tumors undergo multimodal treatments and survivors often suffer from significant side effects associated with treatments. To enable immunotherapies in children with solid tumors, a better understanding of the immune response elicited against different types of tumors is now needed.
Methods
We performed systems-level analyses of the immune system in blood from 94 patients aged 0-18 years with diagnoses of 13 different types of solid tumors at Karolinska University Hospital in Sweden. The baseline samples were collected before treatment and analyzed in a systems-level approach. Plasma proteins were measured using Olink assays (Olink AB). Single-cell phenotypes were analyzed using mass cytometry. We investigated the association between baseline immune variance and patient age, sex, and tumor type. We also studied the differences in immune components between patients who developed and did not develop neutropenic fever after chemotherapies. Furthermore, we compared the immune system of patients with and without metastasis at the time of diagnosis.
Results
In the baseline variance analysis, tumor type explained most of the baseline variance both in immune cell composition (about 43% of total variance) and plasma protein profiling (about 48%). Similar plasma protein profiles were observed among different types of brain tumors and bone tumors. Higher expression levels of proteins CD70, CD27, CXCL9/10 and IFNγ were found in malignant tumors including high-risk neuroblastoma and retinoblastoma. In the neutropenic fever analysis, a subtype of B cells was more abundant in patients without neutropenic fever which may play a role in preventing the body from infection. In the metastasis analysis, we observed a higher level of protein CD70, IFNγ, GZMA, GZMH, CXCL9 and CXCL10, and two subsets of cytotoxic CD8+ T cells in non-metastatic patients which possibly inhibited the tumor metastasis and indicates stronger antitumor responses.
Conclusions
Tumor type explained most baseline variance in immune cell composition and plasma protein profiles. Antitumor response-related cell and protein signatures were found in a subset of patients which can potentially be biomarkers for predicting metastasis status and clinical outcomes at the time of diagnosis.

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

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COVID-19, the illness caused by infection with SARS-CoV2, is characterized by a broad range of symptoms and severity and can result in a protracted course. However, the long-term effects of COVID-19 on the immune system are unclear. Durable changes in the immune system following COVID-19 could influence subsequent immune responses to pathogens, vaccines, or even contribute to long-term clinical symptoms. Despite clinical observations of long-term sequelae, the nature of persistent molecular and cellular changes following COVID-19 are poorly understood.

Recent studies have established that innate immune cells and their progenitors can maintain durable epigenetic memory of previous infectious or inflammatory encounters, thereby altering innate immune equilibrium and responses to subsequent challenges. This innate immune memory, has been attributed largely to persistent chromatin alterations that modify the type and scope of responsiveness of the cells that harbor them, including innate immune cells, and hematopoietic progenitor cells and their mature progeny cells.

We hypothesized that exposure of HSPC to inflammatory signaling events during COVID-19 may result in epigenetic memory and persisting altered phenotypes following COVID-19. The post-acute sequelae of SARS-CoV-2 (PASC), particularly among those admitted to the ICU, suggests that persistent changes or alterations in immune activity may play a role.

We have identified epigenetic innate immune memory that results from SARS-CoV-2 infection by characterizing the molecular features of the post-infection period. We focused on comprehensive analyses of alterations in chromatin and transcription at the single cell level in monocytes and their HSPC progenitor cells.

To study HSPC in-depth, at single-cell resolution, we developed a new workflow to enrich and profile rare HSPC from peripheral blood. This workflow addresses the challenge in access to HSPC by bypassing bone marrow biopsies. We paired our workflow with single-nuclei combined RNA-seq and ATAC-seq. This approach revealed a high-resolution transcriptomic and chromatin accessibility map of diverse HSPC subsets and PBMC in a unique cohort of convalescent COVID-19 study participants following acute SARS-CoV-2 infection from months to a year.

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

qMAST: A SINGLE CELL TRANSCRIPTOMIC PIPELINE FOR ANALYSES OF QUANTITATIVE PHENOTYPES

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Current single cell transcriptome analyses pipelines are restricted to phenotypes that are categorical in nature. This approach can be limiting when analyzing continuous or quantitative phenotypes. Commonly used methods for differential gene expression testing in single cell data, such as MAST, DESeq2 and the Wilcoxon test, all implemented in the Seurat package, do not take into account the quantitative nature of clinical data. In HIV research, many clinical parameters, including plasma viral load (VL), CD4 T cell counts and reservoir size are continuous. However, they are binned into categorical groupings usually based on average or median values for downstream analyses; for example, a high VL and a low VL group. This limitation impacts the analysis of single cell RNA-seq (scRNA-seq) experiments in systems immunology studies, where sample size is often small. The lack of a computational framework for quantitative phenotypes can dilute or bias single cell gene association analyses and miss true positive signals.

To address this problem, we created a modified version of MAST (Model-based Analysis of Single-cell Transcriptomics), a software pipeline for scRNA-seq analyses. MAST models scRNA-seq expression through a two-part generalized regression model with the rate of expression modeled using logistic regression and the positive expression mean modeled as Gaussian simultaneously (Finak et al., 2015). In our modification of MAST, qMAST (quantitative MAST), the response variable is continuous rather than categorical.

We used qMAST to analyze two continuous HIV phenotypes: viral load, which is participant-specific, and viral RNA (vRNA) levels measured in single donor cells. Plasma VL measurements were available for 21 individuals in an acute HIV infection cohort. scRNA-seq analysis was performed to assay host genes and vRNA levels in single cells from donors. We used VL or vRNA levels as a response variable and analyzed gene expression in all memory CD4+ T cells. As a continuous variable we could estimate the $\log_2$ fold change in target gene expression per unit change in VL per participant or vRNA levels in a cell. Specific host genes whose expression correlated with these features were identified and validated. The qMAST method was validated in a publicly available scRNA-seq dataset and was found to be robust. Associations between host genes and vRNA levels were significant when viral expression was either measured as UMI counts or log normalized values. The qMAST pipeline can also be applied to other single-cell modalities.

MODELING THE HETEROGENEOUS INTERFERON RESPONSE OF HUMAN AIRWAY EPITHELIUM AT SINGLE CELL RESOLUTION

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The human airway is continuously exposed to external pathogens, and epithelial cells are both a barrier against and a target for infection. In response to infection, epithelial cells produce interferons (**IFN**) that serve as signals to alert nearby cells to pathogen threats. Here, we explore the IFN response in a well-differentiated human airway epithelial (**HAE**) tissue model at air-liquid interface that recapitulates the major epithelial cell types of the human airway. HAE cultures derived from multiple donors were subjected to IFN-β treatment and harvested at 0, 2, or 6 hours for single-cell gene expression and chromatin accessibility sequencing (scRNA-seq and scATAC-seq). The single-cell measurements enabled resolution of transcriptional signatures from the heterogeneous cell types in the HAE model. In total, 8 epithelial cell populations were identified.

We identified cell-type-resolved interferon regulated genes (**IRGs**). We recovered groups of genes that display both unique and shared expression patterns. “Early” interferon stimulated genes (**ISGs**) were enriched for IFN signatures, while a later set of ISGs were enriched for genes with roles in antigen presentation. Interesting, similarity in chromatin accessibility profiles between epithelial cell populations was not a good predictor of similarity in interferon-induced transcriptional responses. To identify the regulatory mechanisms driving cell-type-shared and unique IFN responses, we built gene regulatory networks (GRNs) from our scRNA-seq and scATAC data. GRNs describe regulatory interactions between transcription factors (TFs) and genes. We used the Inferelator algorithm, which (1) assumes the GRN is sparse and (2) enables integration of TF binding site predicitons from the scATAC-seq, to guide GRN model construction from the scRNA-seq data.

From the GRN, we identified “core” subnetworks, predicted regulatory circuits that are specific to epithelial populations and conditions (e.g., 6h IFN-β response in basal cells). We first identified known and novel transcription factors (TFs) at baseline. For example, in ciliated cells, we identified well-established (e.g., FOXJ1) and novel regulators (e.g., KLF12). We next uncovered the unique and shared regulators of the IFN response in the major epithelial cell types. As expected, we recovered STAT and IRF regulation, as well as noncannonical TFs (e.g., RORB, BATF2) regulating cell-type shared expression patterns. For the cell-type unique gene expression patterns, cell-type-specific TFs at baseline were often predicted to drive cell-type specific IRGs. Having experimentally validated cell-type-specific IRGs using immunohistochemistry, future work will involve experimental testing of the predicted TF regulators. Long-term, we anticipate that our human airway models will inform new therapeutic strategies to improve host defense against infection.

SYSTEMATIC ANALYSIS OF SIMPLE AND COMPOSITE TRANSCRIPTIONAL ELEMENTS ENCODED IN IMMUNE CIS-REGULOMES USING MASSIVELY PARALLEL REPORTER ASSAYS

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Mammalian transcriptional regulatory sequences are comprised of complex combinations of simple transcription factor (TF) motifs. Stereospecific juxta-positioning of simple TF motifs generates composite elements (CEs), that increase combinatorial specificity of TF-DNA interactions. Although a small number of CEs have been characterized, a systematic analysis of CE diversity and properties has not been undertaken. We have developed a computational pipeline termed CEseek to discover approximately 4,000 novel CEs in open chromatin regions of diverse immune and hematopoietic cells and validated many of them using CAP-SELEX, ChIP-Seq, and STARR-seq datasets. This set of CEs together with 153 core simple TF motifs represent a comprehensive set of immune transcriptional regulatory motifs. To functionally screen these motifs for their regulatory activities in diverse immune cell types, we have designed a TF motif reporter library that can be used to perform massively parallel reporter assays (MPRAs). This TF motif MPRA library is being used to probe the regulatory states and networks of distinct immune cell types. The MPRA datasets will be used to train machine learning models that can predict the cell type specific regulatory activity of a given DNA sequence and the impact of variants.

NETWORK DYNAMICS OF THE IMMUNE SYSTEM FOR UNDERSTANDING ASTHMA HETEROGENEITY

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Immune system is a complex multi-scale network of cytokines, cells, tissues, and organs that work in unison to protect the body against foreign invaders. With asthma as our model disease, we aimed to understand the complex patterns of immune cell activation underlying diverse inflammatory responses in asthma subtypes. Currently, asthma severity (subtype) is determined based on patient symptoms and lung function phenotypes and assigned retrospectively by physicians using the Global Initiative for Asthma guidelines, often resulting in morbidities in patients. To understand asthma heterogeneity, we first constructed a comprehensive cell-cell interaction network model, where the nodes represent pathway entities, the directed edges represent the causal interaction from a source node to a target node, and the directionality of the edge (+ or -) represents the biochemical nature of regulation. We extracted 36 hallmark pathways involving transcription factors, cytokines, T cells from large-scale public interaction databases. To investigate nonlinear interplay of the system, we evolved the network as a dynamical system under different stimulants that activate different combinations of TH cell pathways and blood biomarkers. Through network dynamics, we gained two important insights about asthma immunity. First, the immune model demonstrates emergent behavior i.e. the context in which immune pathways are activated influences the severity of inflammatory response. Accordingly, asthma severity cannot be clearly defined by the mere presence or absence of TH cells or peripheral blood cells. One needs to know the immune mechanism activating these peripheral blood cells. Second, multiple TH cell mechanisms exhibit identical downstream peripheral blood cell responses, suggesting that the same clinical symptoms or lung function changes may arise through different immune mechanisms, and thus, the clinical phenomenon may not be the most effective way of characterizing immune system heterogeneity in asthma. Using this knowledge, we disentangle diverse immune mechanisms involved in complex crosstalk patterns and group them based on the severity of the peripheral blood inflammatory response (TH cell mechanisms) to propose a novel endotyping protocol for asthma subtyping. By testing our model in two independent patient cohorts, we demonstrate that our model characterizes disease severity and drug response more precisely than clinical protocols based on physician phenotyping. Overall, our systems immunology model offers an important and unique contribution that extends beyond asthma to other diseases.

GENE BUNDLING: DERIVING OPTIMAL CLUSTERINGS OF GENE-CORRELATION NETWORKS IN SEPSIS

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Sepsis, responsible for one in five deaths worldwide, is characterized by the body's response to infection-induced inflammation and can result in life-threatening tissue damage. Finding gene expression patterns depicting illness severity or type of infection can possibly improve patient diagnosis and treatment. The Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO) leads an international observational study with the goal of improving survival for patients with sepsis. Here, we develop a new parameter-free algorithm for gene correlation-based clustering centered on graph-theoretic principles and spectral clustering using blood samples collected at hospital admission from 505 subjects at ACESO sites in the United States, Ghana, and Cambodia. The resulting transcriptomic data was used to develop and test the new method of clustering.

Any given dataset can have multiple ways of forming clusters, each highlighting different aspects of the data. Our primary goal is to build the machinery needed to capture these aspects and derive optimal gene bundles composed of genes that are co-clustered in the most prominent clustering regimes utilizing spectral theory. This method is called gene bundling and its simplicity and versatility allow for different scales of clustering to be inspected to determine optimal gene clustering. Using our sepsis dataset, we found that gene bundles have significant correspondence to known biological pathways. Furthermore, by using 28-day mortality and a scoring method, we were able to find gene bundles that differentiate patients who survived or died from the whole population. This algorithm could help understand the pathology of biological pathway interaction networks in sepsis patients which should lead to improvements in sepsis diagnosis, prognosis, and therapy.

TOWARDS THE INCORPORATION OF AN IMMUNE-STATE METRIC INTO ROUTINE CLINICAL ASSESSMENT

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Genetics, the environment, and our lifestyle choices all shape the state of our immune system as we age, and contribute to its inevitable dysfunction and resulting susceptibility to disease. The state and strength of the immune system have a strong effect on the outcome of a wide range of medical conditions and life-saving treatments. However, existing tools do not provide us with a simple metric for measuring one's immune system strength - holding it from being integrated into the informed medical decision-making process.

Recent work from our lab tracked the longitudinal movement of ~140 healthy individuals across 9 years and identified a high-dimensional trajectory that shed light on the dynamics of immunosenescence. Critically, the position of a given patient at a given time along this trajectory defines a single-score metric we term immune-age (IMM-AGE), which outperforms current clinical criteria for predicting cardiovascular risk and all-cause mortality.

However, to date, IMM-AGE is assessed by whole transcriptome sequencing or by measuring the frequencies of 18 different bloodborne immune cells by CyTOF - expensive, technically challenging methods unavailable to the clinical world. Here, through the use of computational modeling and experimental validation, we detail a scalable, cost-effective IMM-AGE metric compatible with clinical flow cytometers. Notably, we significantly reduced the requirements for IMM-AGE estimation from 18 to 5 cells by identifying cell combinations that exceed the performance of the original IMM-AGE CyTOF panel. Moreover, we introduce an improved, robust IMM-AGE gene expression signature that is both technology- and batch-independent, which in-turn aids further investigation into immune aging. Finally, by leveraging publicly available datasets, and our new approaches, we show the predictiveness of IMM-AGE in various, additional disease conditions.

Overall, driven by the importance of answering one fundamental question – "what is the state of a patient's immune system, and how does it relate to clinical outcomes?" - our work helps to establish a future where IMM-AGE serves as a widely used predictive tool for disease emergence and outcome, taking us one step closer toward personalized health management.

TUMOR MICROENVIRONMENTAL SIGNALS RESHAPE CHROMATIN LANDSCAPES TO LIMIT THE FUNCTIONAL POTENTIAL OF EXHAUSTED T CELLS

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Immunotherapy has had major clinical successes, but response rates remain low due in part to increased dysfunctional exhausted CD8+ T cells in the tumor, characterized by decreased cytokine expression and increased expression of co-inhibitory receptors, such as PD-1 and Tim-3. CD8+ T cell differentiation in the tumor is a progressive process, wherein progenitor (PD1lo/mid) cells with some functional capacity differentiate to terminally exhausted cells (PD1hiTim3+), with limited to no function. To understand the mechanisms promoting this progression, we used CUT&RUN, a low-input ChIP-seq alternative, to profile four histone modifications in progenitor and terminally exhausted CD8+ T cells in murine melanoma. Unexpectedly, we identified two chromatin features unique to terminally exhausted cells, both with limited transcriptional potential at key genes associated with stemness and function. First, we found a set of genes characterized by terminal exhaustion-specific active histone modifications without corresponding increases in gene expression. These chromatin regions are enriched for AP-1 transcription factor motifs, despite low expression of most AP-1 family members in terminally exhausted cells. Inducing expression of AP-1 factors using a 4-1BB agonist restored expression of these anticorrelated genes, which include pathways in T cell activation and inflammation. Second, we found a substantial increase in the number of genes with bivalent promoters, defined by the presence of both activating H3K4me3 and repressive H3K27me3, as well as decreased gene expression. Bivalent promoters in terminally exhausted T cells were aberrantly hypermethylated in response to tumor hypoxia, as decreasing tumor hypoxia was sufficient to recover expression of these genes. Furthermore, overexpressing Kdm6b, an oxygen-insensitive histone demethylase for H3K27 was sufficient to recover tumor-infiltrating T cell function without reversing the differentiation state of terminal exhaustion. Our study has described a unique decoupling of gene transcription from active histone modifications, clarifying the signals promoting exhaustion in the tumor through epigenetic alterations. Modulating activity of chromatin modifiers, such as Kdm6b, can increase the effector function of terminally exhausted cells, providing new avenues for immunotherapeutic approaches that specifically target terminally exhausted T cells, rather than their progenitors.

EVOLUTION OF PERIPHERAL AND INTRATUMORAL T CELL DYNAMICS IN CHEMOIMMUNOTHERAPY-TREATED OVARIAN CANCER

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While tumor infiltrating lymphocytes have been shown to be prognostic in epithelial ovarian cancer (EOC), anti-PD-1/PD-L1 monotherapy has modest benefit. Here, we assessed a cohort of newly diagnosed EOC patients (n=20) treated with neoadjuvant chemotherapy (carboplatin and paclitaxel) and anti-PD-1 (nivolumab). We assessed the phenotypic state dynamics of immune cells after treatment, correlated with clinical response, and compared this cohort to a cohort of patients treated with chemotherapy alone. Using multi-modal single cell profiling (scRNA/CITE/TCR-Seq) we studied tumors and PBMCs collected before and on treatment, as well as bulk multi-site TCR-Seq of pre and post-treatment tumors. Using a clinical definition of response based on chemotherapy response scores (CRS), we stratified patients into responders (R) (CRS 3, n=7) or non-responders (NR) (CRS 1,2, n=13). First, we found that increased number of T cells in tumors was not associated with response. Focusing on T cell phenotypes, we found that naive CD8 and CD4 T cells increased in frequency in on-treatment tumors of NRs. Using bulk TCR-Seq, we identified T cell clones that expanded on-treatment. We found that a CD8 CD103+ CD39- T_{RM} cluster was enriched for expanded TCRs and was elevated on-treatment in NRs. Interestingly, scTCR analysis revealed that this CD8 T_{RM} cluster was enriched for Influenza-specific TCRs, suggesting these cells are unlikely to be tumor-specific. Next, considering PBMCs, CD8 T cells shifted from naive to effector and memory states after treatment, with a larger relative increase in effector/memory CD8s in Rs. We identified PBMC CD8s with TCRs that expanded in tumors (based on bulk TCR analysis) and found that expanded TCRs were enriched in CD8 T_{EMRA} cells in Rs. To assess whether T cell dynamics were similar in patients treated with chemotherapy alone, we analyzed T cell scRNA data from EOC patients pre (n=41, published) and post (n=4, unpublished) chemotherapy. In these patients, we did not observe an enrichment of naive CD4 or CD8 T cells post-chemotherapy. In summary, these results suggest that chemoimmunotherapy in EOC may induce non-tumor-specific bystander T cells, which may explain the modest benefit observed with PD-1 blockade in EOC.

COOPERATIVE REGULATION OF T HELPER CELL TOLERANCE BY TGF-β AND PD-1 IN IMMUNE-HOMEOST

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Immune-tolerance mechanisms are essential to prevent detrimental self-reactive or overt immune responses and maintain immune-homeostasis. The regulatory cytokine transforming growth factor beta (TGF-β) and immune checkpoint inhibitory receptor programmed cell death protein 1 (PD-1) regulate T helper (Th) cell peripheral tolerance programs at different levels, but their interaction has not been fully characterized. We found that the two pathways cooperatively maintain Th cell self-tolerance in immune homeostasis. Mice lack both inhibitory receptors on their Th cells develop lethal autoimmunity in the first month of age. Deficiency for both inhibitory pathways render Th cells to be more activated, differentiate into IFN-γ-producing effector cells and infiltrate into tissues to cause severe auto-inflammation. The lethal autoimmunity is not fully dependent on downstream effector cells such as CD8 T cells or B cells, but largely dependent on the Th cell CD40 effector pathway. Our finding uncovers that TGF-β and PD-L1 cooperatively maintains Th cell peripheral tolerance by blocking the CD40 pathway in homeostatic conditions.

JOINT INFERENCE OF HUMAN IMMUNE GENE EXPRESSION AND GUT MICROBIOME TOPICS AND THEIR INTERACTIONS

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Although interactions between some pathogenic bacteria and the human immune system have been extensively characterized, much less is known about how commensal bacteria interact with our immune systems. Challenges to studying these interactions at systems-scale include high inter-individual variability of the microbiome, and multi-modality, high-dimensionality, and compositionality of data. Here, we introduce a novel interpretable probabilistic deep-learning based method that simultaneously discovers sparse "topics" of expressed human genes or microbial taxa, and a directed network of sparse interactions between them. To provide scalability to large data sets, we developed an efficient variational inference method that leverages recent results on neural topic models, and relaxations of Bayesian Networks and discrete probability distributions. We applied our method to a new time-series dataset simultaneously measuring the gut microbiome (shotgun metagenomic sequencing) from stool samples and host gene expression (mRNA-seq) from blood samples collected over twenty months, in two longitudinal cohorts of participants with TB-infection undergoing treatment with HRZE or Bedaquiline, Linezolid, Levofloxacin, Clofazimine, and Pyrazinamide. Our method discovered host gene expression and microbiome topics significantly enriched for GO categories or taxonomic clades, respectively, and also found interactions between human gene expression and microbiome topics that suggest novel connections between innate or humoral immunity and commensal bacteria.

CX3CR1 IS A GRADED AND UNIVERSAL DIFFERENTIATION MARKER UNIFYING HUMAN AND MURINE T CELL DIFFERENTIATION

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T cells differentiate into a range of functionally distinct effector and memory states upon antigen-encounter. These states, or ‘subsets’, are delineated by different cell surface markers for murine and human T cells, which hampers cross-species translation of T cell properties. Using a combination of CITEseq, high-parameter flow cytometry, CyTOF data, and functional assays, we identified that fine-graded expression of CX3CR1 distinguished functionally distinct states of antigen-experienced CD8+ and CD4+ T cells in both species. CX3CR1 levels, refined with CD62L accurately captured the continuum of high-dimensional T cell differentiation states. Fine-graded stratification of CD8+ T cells by CX3CR1 level delineated states with comparable functional properties in humans and mice. This applied to CD8+ T cells in healthy humans and mice, and to virus-specific CD8+ T cells that were tracked longitudinally in both species. In summary, measuring CX3CR1 expression levels provide a simple and practical strategy to translate the behavior of functionally distinct T cell differentiation states across species.

PEDOT:PSS COATED ELECTRODES FOR ELECTROPORATION OF MONOCYTES

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The precise and efficient delivery of molecules into single cells is critical for investigating basic biology as well as for applications in cellular therapeutics, drug delivery, and personalized medicine. This can be achieved by using pulsed electric fields to position and electropermeabilize, or electroporate, cells, without the need for toxic chemical or viral transfection agents. In addition to permeabilizing the cell membrane with an electric field, cells can be precisely positioned using dielectrophoresis (DEP) that arise between particles and media in non-uniform fields. Conventional bulk electroporation devices face major challenges with cell viability and heterogeneity after stimulation due to variation in fields generated across cell membranes. This may be mitigated through precise cell positioning with DEP in conjunction with electroporation, however these approaches may be limited by material properties of the chip surface, as well as electrochemistry that further reduces cell viability and electroporation efficiency. The conducting polymer PEDOT:PSS has emerged as an attractive material for electroporation due to its biocompatibility and electrochemical properties. In particular, PEDOT:PSS coated electrodes exhibit ideal capacitive behavior at low voltages without inducing redox reactions, and as such, separate the delivery of electric fields from electrochemistry at the electrode surface. These favorable material properties strongly motivate this study to develop devices with PEDOT:PSS coated electrodes and integrated microfluidics for highly efficient electroporation. In this work, we explore the performance of PEDOT:PSS coated electrodes in permeabilizing monocyte cell lines with a range of stimulation conditions to determine the optimal operating condition.

MOLECULAR REDUNDANCIES OF TCF-1 WITH LEF-1 AND COOPERATION WITH HEB, IKAROS AND RUNX1 IN DP THYMOCYTES

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We have established that TCF-1 shares DNA binding sites with other factors critical for T cell development such as Ikaros, Runx1, and HEB. Our earlier studies focusing on how the co-binding of TCF-1 and HEB shapes DP thymocytes showed that TCF-1 and HEB cooperate to establish the epigenetic and transcription profiles of DP thymocytes. Moreover, they showed that TCF-1 has an epistatic role on HEB through its ability to regulate the HEB protein levels. Our current studies focus on determining the molecular redundancies of TCF-1 with its paralogue, LEF-1 establishing how TCF-1 and LEF-1 impact the binding of Ikaros and Runx1 to common chromatin sites. In DP thymocytes, 79% of the sites occupied by LEF-1 genome wide were also occupied by TCF-1, supporting that LEF-1 is redundant to TCF-1. We find that in TCF-1 deficient DP thymocytes, LEF-1 gains ~8000 new sites and ~4,500 of these occupy sites previously bound by TCF-1. In these cells, LEF-1 also has significant overlap with Ikaros and Runx1, supporting the postulate that LEF-1 molecularly compensates for TCF-1. Altogether these findings suggest that differentiation of DP thymocytes depends on the coordinated actions of TCF-1, with Ikaros, Runx1 and HEB and that LEF-1 plays a compensatory role for TCF-1. We are using these new findings to determine how TCF-1, Ikaros, Runx1, and HEB, coordinate to establish the chromatin landscape and expression profile of DP thymocytes, to and define its molecular redundancies with LEF-1.

INTEGRATION OF A STRUCTURALLY RESOLVED PROTEIN NETWORK WITH SIGNALING CASCADES UNCOVERS IMMUNOMODULATORY MOLECULAR PHENOTYPES IN INFECTIOUS DISEASE

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Past work by us and others has demonstrated the critical importance of incorporating corresponding structural information in the integration of Mendelian mutations with protein networks, to elucidate molecular phenotypes underlying the corresponding genetic disorders, with high sensitivity and specificity. However, while Mendelian disorders are typically monogenic and often attributable to a handful of high penetrance mutations, the role of genomic variation in infectious disease is far more nuanced, as specific pathogens cause these disorders and the genetic variants are immunomodulatory. Here, we present a novel pipeline that integrates a structurally resolved reference human protein interactome with signaling cascades inferred from expression and chromatin accessibility data to uncover cell-type-specific immunomodulatory molecular phenotypes in infectious disease. We used Covid-19 as an exemplar given the availability of deep genotype data from the Covid-HGI, and corresponding cellular and molecular data. However, the framework is broadly applicable across infectious disease contexts.

We combined genotype data for COVID-19 disease occurrence and severity with a structurally resolved reference human protein interactome network to identify coding mutations at and away from the interfaces of specific protein-protein interactions. We then compared the cell-type-specific functional impact of these variants to non-coding regulatory variants that attain/do not attain genome-wide significance. The functional impact of these classes of mutations on different specific cell types was inferred via network propagation of the effects of these mutations on cell-intrinsic and cell-extrinsic signaling networks inferred from scRNA-seq and scATAC-seq data. Mutation effects within cell types were also ensembled to evaluate the relative importance of different cell types in Covid-19 pathogenesis. We recapitulated the well-known roles of classical and non-classical monocytes in Covid-19 pathogenesis. However, surprisingly, we found that a large component of the signal was driven by only a handful of prioritized coding variants at specific protein interaction interfaces. These were functionally as important as genome-wide-significant regulatory variants, and both sets were far more important than all other classes of variants.

Of particular interest, was a common coding variant, with a minor allele frequency of 40%, at the interface of the OAS3-CHMP1A interaction. This locus is tightly linked to the OAS1 locus which has recently been reported to have critical immunomodulatory roles in Covid-19. However, while the OAS1 variants modulate function by altered gene regulation and/or splicing, the variant we uncovered is immunomodulatory via the disruption of a specific protein interaction between OAS3, which has key antiviral properties and is involved in dsRNA sensing, and CHMP1A, a part of the ESCRT-III protein complex essential for the envelopment of viruses. Thus, our framework converged on a novel molecular phenotype that explains a key component of the variance in the host immune response to Covid-19.

SARS-COV-2 INFECTION AND mRNA VACCINATION INDUCE MOLECULARLY DISTINCT MEMORY CD4+ T CELLS

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Adaptive immune responses are induced by vaccination and infection, yet little is known about how CD4+ T cell memory differs between these two contexts. Notable differences in humoral and cellular immune responses to primary mRNA vaccination were observed and associated with prior COVID-19 history, including in the establishment and recall of Spike-specific CD4+ T cells. It was unclear whether CD4+ T cell memory established by infection or mRNA vaccination as the first exposure to Spike was qualitatively similar. To assess whether the mechanism of initial memory T cell priming affected subsequent memory T cell responses to Spike protein, 14 people who were receiving a third mRNA vaccination were stratified based on whether the first exposure to Spike protein was by viral infection or immunization (infection-primed or vaccine-primed). Using multimodal scRNA-seq of activation-induced marker (AIM)-reactive Spike-specific CD4+ T cells, we identified 220 differentially expressed genes between infection- and vaccine-primed individuals post-booster. Infection-primed participants had greater expression of genes related to cytotoxicity and interferon signaling than vaccine-primed participants. Gene set enrichment analysis (GSEA) revealed enrichment for multiple interferon-response gene sets in Spike-specific CD4+ T cells from infection-primed individuals, whereas Spike-specific CD4+ T cells from vaccine-primed individuals had strong enrichment for proliferative pathways by GSEA. Finally, SARS-CoV-2 breakthrough infection in vaccine-primed participants resulted in only subtle changes in the transcriptional landscape of Spike-specific memory CD4+ T cells relative to pre-breakthrough samples but did not result in the inflammatory imprinting that was seen in infection-primed Spike-specific CD4+ T cells. Together, these data suggest that the inflammatory context during CD4+ T cell priming is durably imprinted in the memory state, which has implications for personalization of vaccination based on prior infection history.

INCREASED EXPRESSION OF HSP70.2 IN MULTIPLE SCLEROSIS AND ITS REGULATION OF T_H17 FUNCTION

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Multiple sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system. Autoreactive CD4 T cells, including T_H17 cells, are implicated in MS. Not all T_H17 cells are pathogenic. Studies in murine autoimmune models and humans suggest that IFN-γ-secreting T_H17 ($T_H1/17$) cells are pathogenic in autoimmune diseases. The basic immune mechanism underlying the development and function of pathogenic T_H17 cells in human autoimmunity needs to be better understood. To characterize pathogenic/proinflammatory T_H17 cells in MS, we established an experimental protocol to isolate IFN-γ-positive T_H17 ($T_H1/17$) and IFN-γ-negative T_H17 (T_H17) cells with high purity based on differential IL-17 and IFN-γ secretion for gene expression analysis. RNA-seq analysis of $T_H1/17$ and T_H17 cells isolated from untreated patients with MS and healthy controls showed that the mRNA levels of heat shock protein (HSP) Hsp70.2 and small GTPase Rab-35 were upregulated in $T_H1/17$ cells in MS but not in T_H17 cells. It has been reported that Hsp70 polymorphisms are associated with MS and Hsp70.1 deficient mice are more resistant to developing experimental autoimmune encephalomyelitis, an animal model of MS. Yet, it is unclear whether Hsp70.2 is involved in MS pathogenesis. Analyzing a public single-cell RNA-seq dataset, we found an association of Hsp70.2 expression with RORγt, the master transcription factor of T_H17 cells. We found that the gene expression of both Hsp70.2 and Rab-35 was induced during pathogenic T_H17 cell polarization. Knocking down Hsp70.2 by shRNA in pathogenic T_H17 cells led to reduced gene expression of Rab-35, suggesting Hsp70.2 is upstream of Rab-35. In these Hsp70.2-knockdown pathogenic T_H17 cells, we also found that the mRNA levels of proinflammatory cytokines IFN-γ, TNF-α, and GM-CSF were reduced. RNA-seq analysis of Hsp70.2-knockdown pathogenic T_H17 cells showed that Hsp70.2 regulated at least 37 other HSPs (about 40% of HSPs), which provides the basis for another finding that the HSP gene set was concordantly enriched in $T_H1/17$ cells in MS. However, Hsp70.2 did not appear to interfere with the initiation of T_H17 differentiation. Rather, it was critical for sustaining proinflammatory cytokine expression at a late stage of T_H17 development. Thus, our results suggest Hsp70.2 plays a role in MS pathogenesis by regulating T_H17 function.

T-DEPENDENT B-CELL SELECTION AND PROLIFERATION: A SYSTEMS ANALYSIS OF SIGNAL I AND SIGNAL II INTEGRATION

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In response to vaccination or infection, a successful antibody response must enrich high-affinity antigen-reactive B-cells through positive selection, but eliminate autoreactive B-cells by negative selection. Reactive B-cells undergo proliferative bursts that are governed by signaling from the B-cell receptor (BCR) which binds the antigen and the CD40 signal provided by neighboring T-cells that recognize the antigen via its own MHC T cell receptor complex, when it is presented with the antigen. Both signals are a function of the BCR's affinity to the antigen. Little is known about the mechanism by which BCR and CD40 signaling are integrated, and thus jointly determine B-cell selection and proliferation. We quantitatively evaluated the population dynamics after stimulating B-cells *in vitro* through their BCR and CD40 receptors. We interpreted the data with a newly developed mathematical model of the BCR and CD40 signaling pathways and their control of B-cell fate decision machineries. Our results show that while CD40 and BCR costimulation induces more NFκB activity, no such potentiation is seen at the level of population expansion. Model simulations reveal that functional antagonism may be mediated by BCR-induced caspase activity triggering apoptosis in founder cells. We investigated *in silico* and *in vitro* the temporal relationship between these antagonistic signals and found that within a limited time window CD40 signaling may effectively rescue cell death triggered by BCR signaling. The window size depends on the strength of the BCR and CD40 signals, but a longer time gap does not allow for B-cell population expansion. We thus propose a form of kinetic proofreading that governs the T-cell dependent humoral immune response.

DISTINGUISHING TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION IN HUMAN NAÏVE AND MEMORY CD8$^+$ T CELLS

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CD8 $^+$ T cells are one of the major types of cells in the adaptive immune system. CD8$^+$ T cells, also known as cytotoxic T cells, recognize and kill cells infected with pathogens. T cells that have not been activated by an antigen are referred to as naïve T cells, whereas memory T cells are antigen-experienced T lymphocytes that respond to reinfection faster and have higher sensitivity to antigenic stimulation. The memory T cell pool is heterogeneous, mainly consisting of central memory (T_{CM}) and effector memory (T_{EM}) cells, which are characterized by distinct homing potentials and effector functions.

Recent studies have reported that naïve and memory CD8$^+$ T cell subsets differ in their transcriptome, but the regulatory mechanisms driving these differences remain unclear. We are investigating gene regulation pathways in human CD8$^+$ T cells, and focusing on the key regulators that contribute to the different cell-intrinsic properties of naïve and memory T cell subsets.

To rigorously characterize the regulatory pathways of differentially expressed genes, I applied both RNA-seq and PRO-seq to human naïve and memory T cell subsets. While RNA-seq profiles steady-state mRNA, PRO-seq measures transcription directly by only capturing nascent RNA. The combined analysis of PRO-seq and RNA-seq allows transcriptional and post-transcriptional regulation to be reliably differentiated and quantified. Moreover, PRO-seq data also identifies enhancer RNAs (eRNA), facilitating the identification of active cis-regulatory elements. Compared to other techniques of enhancer prediction, eRNA profiling is considered a more reliable marker to identify active enhancers genome wide.

The integrative analysis of PRO-seq and RNA-seq has identified immune-related genes undergoing cell type-specific gene regulation. Additionally, PRO-seq data showed that the activities of cis-regulatory elements are dynamic across different T cell subtypes. By associating putative enhancers to target genes, I have detected novel enhancers potentially involved in T cell differentiation and functions. I also identified a large group of genes undergoing post-transcriptional regulation, which suggests an extensive role for post-transcriptional regulation in CD8+ T cells. Collectively, the results of our study provide insights into regulatory pathways at both the transcriptional and post-transcriptional levels that are involved in establishing naïve and memory CD8+ T cell identity in humans.

INFERRING CELLULAR AND MOLECULAR PROCESSES IN SINGLE-CELL DATA WITH NON-NEGATIVE MATRIX FACTORIZATION USING PYTHON, R, AND GENEPATTERN NOTEBOOK IMPLEMENTATIONS OF CoGAPS

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Non-negative matrix factorization (NMF) is an unsupervised learning method well suited to high-throughput biology. Still, inferring biological processes requires additional post hoc statistics and annotation for interpretation of features learned from software packages developed for NMF implementation. Here, we aim to introduce a suite of computational tools that implement NMF and provide methods for accurate, clear biological interpretation and analysis. A generalized discussion of NMF covering its benefits, limitations, and open questions in the field is followed by three vignettes for the Bayesian NMF algorithm CoGAPS (Coordinated Gene Activity across Pattern Subsets). Each vignette will demonstrate NMF analysis to quantify cell state transitions in public domain single-cell RNA-sequencing (scRNA-seq) data of malignant epithelial cells in 25 pancreatic ductal adenocarcinoma (PDAC) tumors and 11 control samples. The first uses PyCoGAPS, our new Python interface for CoGAPS that we developed to enhance runtime of Bayesian NMF for large datasets. The second vignette steps through the same analysis using our R CoGAPS interface, and the third introduces two new cloud-based, plug-and-play options for running CoGAPS using GenePattern Notebook and Docker. By providing Python support, cloud-based computing options, and relevant example workflows, we facilitate user-friendly interpretation and implementation of NMF for single-cell analyses.

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CAR-T CELL INFUSION RESULTS IN ACTIVATION OF CD160+/NKG2D+/CCL5+ NON-CAR CD8+ CYTOTOXIC 'BYSTANDER' T CELLS IN BOTH NON-HUMAN PRIMATES (NHP) AND PATIENTS RECEIVING B-CELL-DIRECTED CAR-Ts

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Bystander T cell activation has not been well-characterized in CAR-T therapy. We use data from a NHP CAR-T cell model and human clinical studies to identify mechanisms of bystander activation and their potential antileukemic role.

Anti-CD20 CAR-T cells were transferred into lymphodepleted NHP and persisted for ~4 weeks. At this time, loss of CAR-Ts was followed by B cell recovery. Flow cytometry, scRNA- and scTCR Seq were performed on 5 NHP CAR-T cell recipients. These methods were also performed on T cells from 6 pediatric B-ALL patients receiving Tisangenlecleucel.

NHP recipients of CD20-CAR T cells demonstrated CAR-T cell maximum expansion on Days 7-11 post-infusion and a 5-fold expansion of activated CD8+ CAR^{neg} T cells. We performed scRNASeq and scTCRSeq on sorted CAR^{neg} T cells and CAR^{pos} T cells from multiple timepoints, and these data demonstrated that the CD8 CAR^{neg} T cell clones formed a unique cluster that emerged in parallel with expansion of CAR^{pos} T cells. This CD8 CAR^{neg} population made up <1% of T cells in the blood prior to CAR-T infusion or in the infused CAR-T product, suggesting development and expansion of these cells in the post-CAR-T cell infusion/CRS milieu. These 'bystander' CD8 CAR^{neg} T cells demonstrated high expression of receptors for IL15 and IL2, the cytokines IL18, IL2 and IFNg, and expression of cytotoxicity molecules, including Prf1 and Gzmm.

scRNA-Seq on samples from 6 patients receiving Tisagenlecleucel CAR-T cells identified a CD8 CAR^{neg} T cell population in some patients which mirrored the transcriptional signature observed in the NHPs. To determine if cytokines released by CRS could induce the transformation of human CD8 CAR^{neg} T cells into these cells, we stimulated primary human T cells with an array of CRS-associated cytokines, and found IL-2 and IL-15 induced a CD8+ T cell population with phenotypic markers (CD160, NKG2D and CCL5) observed in the bystander cells found in CAR-T cell patients.

These results demonstrate that a CAR-T-induced bystander effect can be elicited in response to CRS-associated cytokines in CD8 CAR^{neg} T cells.

TARGETING CNS LYMPHOMA WITH INTRAVENOUS AXICABTAGENE CILOLEUCEL AND ELUCIDATING THE TRANSCRIPTIONAL PROGRAM OF CAR-T CELLS TRAFFICKING TO THE TUMOR SITE

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Chemorefractory primary and secondary central nervous system lymphomas (CNSL) represent a major unmet need in oncology. We conducted a pilot trial of axi-cel CAR-T therapy in patients with relapsed/refractory CNSL. All patients had Ommaya reservoirs, such that after CAR-T infusion, paired CSF and blood could be collected and analyzed daily, allowing the interrogation of single-cell CAR-T cell transcriptional profiles at an unprecedented level of detail.

Adult patients with either primary or secondary relapsed/refractory CNSL were enrolled on the 'Axi-cel In CNS Lymphoma' Trial (Clinicaltrials.gov# NCT04608487). Blood and CSF were sampled daily between D0-14 after infusion, with data from CAR-T maximum expansion (Days 5-9) reported here. 5' 10x Single-cell RNA-Seq and TCR-Seq were performed on PBMC's, T cells, and CSF-derived cells.

Data from the first 7 patients on-study demonstrated no treatment-limiting toxicities and an overall response rate of 86%, with 6 of 6 responding patients achieving a CR by 3 months. 2 of the responding patients have progressed, 1 at 6 months and 1 at 15 months. We analyzed 129,088 CAR+ and non-CAR T cells from the peripheral blood (64,337 cells), the CSF (37,070 cells), and the axi-cel product (27,681 cells) from 5 enrolled patients, with blood/CSF collected around the time of maximum CAR-T expansion (Days 5-9 post-infusion). While blood CAR-Ts exhibited proliferation gene expression signatures (assessed by GSEA), CSF CAR-Ts, obtained on the same timepoints as those from the blood, exhibited strong enrichment for interferon-response pathway associated genes.

We find that IFN-response may play a role in CAR-T efficacy for the eradication of CNS disease in patients with CNS, and that axi-cel is effective for the treatment of both primary and secondary CNSL, and has an acceptable safety profile with no increased risk of neurologic events.

TCRen: PREDICTING TCR RECOGNITION OF UNSEEN EPITOPES BASED ON RESIDUE-LEVEL PAIRWISE STATISTICAL POTENTIAL

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TCR recognition of foreign peptides (epitopes) presented by MHC proteins is a crucial step in triggering the adaptive immune response. Prediction of TCR-peptide interaction is important for many clinical applications in cancer immunotherapy, autoimmunity studies, and vaccine design. Importantly, most of these tasks require predictions for novel (unseen) epitopes for which no specific TCRs are known. Several machine learning-based methods were developed for prediction of TCR-peptide recognition, however, all of them either do not support or have very low performance for unseen epitopes. To overcome this issue, we developed a novel computational method for prediction of TCR-peptide recognition, which estimates energy of peptide-TCR interaction based on TCR-peptide-MHC structures, that makes it suitable for unseen epitopes.

The key element of our method is a statistical potential TCRen (from "TCR energy") that describes interaction energies for all possible contact pairs of TCR and peptide residues. TCRen potential was derived based on statistical analysis on contact residue preferences in TCR-peptide interfaces of known TCR-peptide-MHC crystal structures from Protein Data Bank.

Our method takes as input the TCR sequence and a list of candidate epitopes. The method starts from homology modeling of TCR-peptide-MHC structure, then extracts a TCR-peptide contact map, estimates the TCRen score for all candidate peptides by convolving the contact map with TCRen pairwise energies, and finally, generates a ranked list of candidate peptides based on this score.

On several benchmarks we showed that TCRen significantly outperforms state-of-art methods for prediction of TCR specificity and other structure-based approaches for protein-protein interactions. We demonstrate that TCRen may be useful in identification of cancer neoepitopes recognized by tumor-infiltrating lymphocytes, enabling reduction up to several folds of the list of candidate neoepitopes for experimental validation.

GENOME-WIDE TRANSCRIPTIONAL REGULATORY NETWORK OF HUMAN IMMUNOLOGICAL MEMORY USING SINGLE-CELL MULTIOME-SEQ

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Immunological memory is induced by an initial exposure to a pathogen or vaccination and allows the organism to respond more rapidly and efficiently to a repeat encounter with the same pathogen. In T cells, effector memory cells can start secreting cytokines within minutes of reexposure compared to days for naive cells. Previous work demonstrated that memory correlates with the priming of genomic regulatory elements proximal to rapid-recall genes; however, causality is not yet established.
Here, we present a transcriptional regulatory network (TRN) of immunological memory using human CD4+ T cell single-cell multiome-seq data (parallel gene expression and chromatin accessibility). TRNs explain cellular behavior by describing interactions between transcription factors and their gene targets. Multi-modal clustering of the multiome-seq data revealed 22 cell populations, including both memory and naïve populations. We explored how responses to TCR stimulation differed across these populations. While naïve and memory populations shared a set of upregulated genes after TCR stimulation at 2h (850 genes), memory cells uniquely upregulated many more genes than naïve cells (2000 versus 150 genes). Thus, our data (1) captures dramatic differences as well as a small shared core of gene regulatory processes operational in memory and naïve cells and (2) present a rich resource to infer the underlying TRNs.
The TRN was constructed using the Inferelator, which incorporates transcription-factor binding predictions from chromatin accessibility data to guide TRN inference (gene expression modeled as a sparse linear function of TFAs). Our draft TRN for TCR stimulation in naïve and memory cell populations includes 56,725 TF-gene regulatory interactions. Cell-type-specific core networks were generated, which involves finding TFs whose predicted gene targets are enriched in a cell-type's signature genes. These core networks demonstrated a group of TFs, including RUNX1, RUNX2, and FLI1 that was core for resting memory cells but not naïve. Our preliminary data showed that binding motifs for these TFs were frequently found in the open chromatin of CD4+ memory T cells. Thus, we generated Cleavage Under Targets and Tagmentation (CUT&Tag) for RUNX1 to demonstrate differential binding of RUNX1 between memory and naïve cells.
Further analysis of the TRN will be used to delineate the transcription factors that induce and maintain the memory-dependent "poised" regulatory elements near rapid-recall genes, identifying potential molecular mediators of long-term maintenance of immune memory. These models will help improve understanding of immune memory and will guide the development of new vaccinations and therapies for autoimmunity.

COMPARISON OF ACTIVATION INDUCED MARKERS TO DETECT ANTIGEN SPECIFIC CD8+ T CELLS

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Background: Quantification and characterization of antigen-specific T cells are crucial to our understanding of immune responses as well as to the development of new immunotherapies. The Activation Induced Marker (AIM) assay has been validated to detect and quantify antigen specific CD4+ T cells. However, little work has been undertaken to validate this assay to quantify antigen specific CD8+ T cells. In this study, we investigated the kinetics of different AIM markers expressed by Flu specific CD8+ T cells.

Methods: To identify the best AIM markers combination to define antigen specific CD8+ T cells, PBMCs from five HLA-A2+ Flu vaccinated volunteers were stimulated with HLA-A2–restricted influenza M1 (Flu-M1) peptide GILGFVFTL for 12hr, 18hr, 24hr, 36hr, 42hr and 48hr at 37^{o}C. Cells were then stained with phenotype identification markers and different AIM markers including CD137, IRF4, CD69, HLADR, CD38, CD25 and OX40. HLA-A2-Flu-M1–specific CD8+ T cells were prelabelled with a specific pMHC class I pentamer. We then compared the frequencies of different AIM marker combinations within CD8+Pentamer+ T cells at different timepoints. Events were acquired on BD Fortessa and analysed using FlowJo v10.6. Statistical analysis was performed in GraphPad Prism V8/9.

Results: Significant differences in the kinetics of different AIM markers combinations were observed; CD69CD137 after 12 hr of stimulation exhibited the highest sensitivity (median of 80%) but had significantly high background ($p<0.01$) and low signal to noise ratio. IRF4CD137 had slightly lower sensitivity than CD69CD137 but displayed a lower background. Further, blocking the interactions between TCR and pMHC complexes confirmed that CD69CD137 co-expression was a bystander effect that was independent of TCR triggering. In contrast, IRF4CD137 expression decreased gradually with the blocking of TCR and pMHC complexes.

Conclusions:

Different AIM markers expressed by CD8+ T cells exhibit different characteristics. IRF4CD137 co-expression is more specific than CD69CD137 for detection of antigen specific CD8+ T cells. Although, CD69CD137 expression is slightly more sensitive this is attributable to a higher TCR-MHC interactions independent background. We recommend the use of IRF4CD137 co-expression for detection of antigen specific CD8+ T cells.

SINGLE-CELL TRANSCRIPTOMICS IDENTIFIES KEY COMPONENTS IN METABOLIC PATHWAYS OF DRUG PERTURBED CD4+ T CELLS

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CD4+ T cells play a critical role in the development of autoimmune diseases. During CD4+ T cell activation, remodelling of metabolic pathways is required for the cells to exert their effector functions. The importance of these pathways is highlighted by the successful therapies for immune diseases that target metabolic pathways. Although key metabolic processes have been recognized to affect T cell activation and lineage development, how metabolic interventions can skew the outcome of T cells activation and differentiation remain largely unknown. Here we perturbed both CD4+ naive and memory T cells with selected 19 compounds that target various metabolic pathways with demonstrated effects on T cells. We measured day 3 proliferation and performed single cell transcriptional profiling at three time points (resting, 16 hours and 3 days after stimulation). Our results showed compound perturbations targeting metabolic pathways have a significant impact on T cell proliferation. Single-cell transcriptomics revealed key genes and pathways altered by perturbations with cell type specific effects. For each of the three time points, we built a pseudotime trajectory from naive to effector memory cells and identified immune-mediated disease genes regulated by interactions of metabolic pathways perturbation and effector function. Leveraging RNA splicing information, we further demonstrated dynamic changes of genes targeted by compounds altered T cells lineage development. Finally, we showed a different metabolic landscape in control and perturbed cells. Our study presents a unique resource of metabolic perturbations in CD4+ T cells and provides insights into understanding the role of T cell metabolism in immune-mediated diseases.

DECIPHERING ANTI-TUMOR IMMUNE RESPONSES WITH SYSTEMS IMMUNOLOGY AND GENOMICS

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One of the major challenges in developing new cancer immunotherapies and identifying effective treatment biomarkers is the incomplete understanding of the molecular and cellular mechanisms that drive human anti-tumor immune responses. Despite the usefulness of animal models in studying cancer immunology, they fail to perfectly replicate the complexity of human immune responses. By analyzing genetic data from large groups of human patients, we can gain insights that cannot be obtained from animal models or reductionist experiments, particularly in the case of non-small cell lung cancer (NSCLC). The response rates to immune checkpoint blockade (ICB) in NSCLC vary greatly, and the mechanisms behind these responses are not fully understood. Somatic loss of heterozygosity at the HLA-I locus (HLA-LOH) has been identified as a mechanism that enables tumors to evade the immune system, but many patients with HLA-I disruptions in their tumors still have durable responses to ICB. By utilizing genomic sequencing and integrating it with single-cell profiling and ICB treatment outcomes, we can better understand the complex and dynamic systems underlying anti-tumor immune responses in patients with HLA-I disruptions. Our research has found that clonally expanded populations of CD4+ T cells with a cytotoxic phenotype can infiltrate tumors in NSCLC patients and may have an underappreciated role in contributing to anti-tumor immune responses through MHC class II mechanisms. When we integrate these findings with tumor mutational burden, we found a significant association with progression-free survival, including in patients with HLA-LOH. These results demonstrate how a systems immunology approach can leverage genomic profiling to generate insights into human immune responses and potentially inform strategies for exploiting other genetic variations to decipher human immune functions.

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EXPLOITING CRISPR ACTIVATOR ASSOCIATED REPORTER SYSTEM TO STUDY GENETIC ESSENTIALITIES INVOLVED IN B CELL LYMPHOMAGENESIS

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Genomic data of B cell non-Hodgkin lymphomas (B-NHL) reveals two main types of genetic alterations- chromosomal translocations and aberrant somatic hyper mutation (ASHM). Chromosomal translocation in GC derived NHL involve BCL6 (t(3q;27)), BCL2 (t(14;18)), and MYC (t(8;14) loci. BCL-6 is a major regulator of GC B-cell development and has an established role in the etiology of the subset of non-Hodgkin's lymphoma (NHL) with a large-cell component. It is located at chromosome 3q27, which is a common site of translocation in NHL. Mice that were deficient in BCl-6 have normal B-cell development, but lack the ability to develop Germinal Centers and perform affinity maturation. This demonstrate the crucial role of BCl-6 in GC B cell proliferation and genomic remodeling, such as somatic hyper mutation and class-switch recombination. Interestingly, It was observed that resting B cells and activated B cells have comparable levels of BCL6 mRNA, but protein expression was much higher in germinal center B cells than in resting B cells. This suggests that BCL-6 protein levels are controlled by translational or post-translational mechanisms but yet to be explored.

CRISPR monitoring system which is comprised of CRISPR-activator-associated reporter—Suntag-P65-HSF1 (SPH) and optimized miniCMV-mCherry (SPH-OminiCMV). This system can be integrated into the 3'UTR of a gene and the expression of that gene can be readily monitored. We have modified the reporter system and Fast fluorescent timer (FT) was incorporated. Benefit of this system is the selection of FT positive cells in one step and transition of blue fluorophore to red occurs after 6 hours, that can give direct estimation of transcriptional gene expression. The system was stably established in A20 cell line (that resemble germinal center B cell physiology) and expression profile of housekeeping beta-actin gene was observed. Expression profile was monitored for 3 weeks with both miniCMV-mCherry and miniCMV-FT systems.

The next step is to study the expression profile of BCL6 at transcriptional and translational level in vitro in A20 cell line. Homology directed repair-based constructs were developed that will target 3'UTR of BCL6 and will activate the monitoring system in two different ways. The constructs are designed in way that will be able to detect very low signal. I will perform experiments in the following weeks and results will be shared at the conference. For in vivo studies, we are developing mouse models.

CYNOMOLGUS MACAQUE MODEL FOR COVID-19 OMICRON VARIANT INFECTION

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The Omicron variants, classified as variants of concern (VOC) by the World Health Organization (WHO), have high infectivity and immune evasion ability. These strains may invalidate existing covid-19 vaccines as well as reinfect individuals previously infected with other SARS-CoV-2 variants. The development of preclinical animal models is essential to validate the efficacy of vaccine and antiviral agents that can provide protection against these fast-evolving variants. The purpose of this study is to analyse pathology and cellular immune responses in a macaque model for the omicron BA.1 infection. Inoculation of cynomolgus macaques with the omicron variant showed infectious viruses and viral RNA in the nose, throat, and lungs during the acute phase of infection. After 3 days of infection, edema and hyaline membranes were observed in some lung lobes, but only alveolar wall thickening with mononuclear infiltration was observed after 21 days of infection. For the immunological phenotype, all macaques showed transient lymphopenia and neutrophilia during the earliest phase of infection. The level of anti-SARS-CoV-2 Omicron variant spike protein-specific IgM, IgG, and IgA antibody responses in plasma were significantly increased 14 days post infection, consistent with the GC formation in lymphoid tissues. This nonhuman primate Omicron variant infection model can be useful for evaluating the efficacy of vaccines and universal antiviral agents against rapidly evolving mutations.

SINGLE-CELL RNA SEQUENCING IDENTIFIES DISTINCT TRANSCRIPTOMIC SIGNATURES BETWEEN PMA/IONOMYCIN- AND ACD3/ACD28- ACTIVATED PRIMARY HUMAN T CELLS ACTIVATION

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Immunologists have activated T cells in-vitro using various stimulation methods including phorbol myristate acetate (PMA)/ionomycin and αCD3/αCD28 agonistic antibodies. PMA stimulates protein kinase C, activating NF-κB, and ionomycin increases intracellular calcium levels, resulting in activation of NFAT. On the other hand, αCD3/αCD28 agonistic antibodies activate T cells through ZAP-70, which phosphorylate LAT and SLP-76. However, despite the use of these two different in-vitro T cell activation for decades, the differential effects of chemical-based and antibody-based activation of primary human T cells have not yet been comprehensively described. Using single-cell RNA sequencing (scRNA-seq) technologies to unbiasedly dissect gene expression unbiasedly at the single cell level, we compared the transcriptomic profiles of the non-physiological and physiological activation methods on human PBMC-derived T cells from four independent donors. Remarkable transcriptomic differences in the expression of cytokines and their respective receptors were identified. We also identified activated CD4 T cell subsets (CD55+) enriched specifically by PMA/ionomycin activation. We believe this activated human T cell transcriptome atlas derived from two different activation methods will enhance our understanding, highlight the optimal use of these two in-vitro T cell activation assays, and apply as standard when we analyze activated specific disease originated T cell through ScRNA-seq.

PROTEIN EXPRESSION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TdT) IDENTIFIES A LYMPHOID-PRIMED PROGENITOR POPULATION IN HUMAN BONE MARROW

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Lymphoid specification in human hematopoietic progenitors is not fully understood. To better associate lymphoid identity with protein-level cell features, we conducted a highly multiplexed single-cell proteomic screen on human bone marrow progenitors. This screen identified terminal deoxynucleotidyl transferase (TdT), a specialized DNA polymerase intrinsic to VDJ recombination, broadly expressed within CD34+ progenitors prior to B/T cell emergence. While these TdT+ cells coincided with granulocyte-monocyte progenitor (GMP) immunophenotype, their accessible chromatin regions showed enrichment for lymphoid-associated transcription factor (TF) motifs. TdT expression on GMPs was inversely related to the SLAM family member CD84. Prospective isolation of CD84lo GMPs demonstrated robust lymphoid potentials ex vivo, while still retaining significant myeloid differentiation capacity, akin to LMPPs. This multi-omic study identifies previously unappreciated lymphoid-primed progenitors, redefining the lympho-myeloid axis in human hematopoiesis.

PAN-CANCER SINGLE CELL RNA-SEQUENCING ANALYSIS OF THE TUMOR MICROENVIRONMENT REVEALS A BROAD-SPECTRUM OF BALANCE BETWEEN IMMUNE SUPPRESSION AND ACTIVATION AMONG DIFFERENT CANCER TYPES

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The presence of CD8+ T cells in the tumor microenvironment (TME) has favorable clinical implications for many cancer types including breast (BrCa), head and neck (HNSCC), and lung cancers (NSCLC). In contrast, the opposite is true for renal cell carcinoma (RCC). Higher tumor mutational burden (TMB) is typically associated with increased responsiveness to immune checkpoint blockade (ICB). Although NSCLC and HNSCC harbor similar TMB, response rates to ICB are much higher in NSCLC. RCC harbors much lower TMB, nonetheless, it responds well to ICB. RCC also uniquely responds to high dose IL-2. Therefore, we hypothesized that a balance between immune activation and suppression determines, in part, the prognosis of primary cancer. We designed pan-cancer single cell RNA-sequencing (scRNA-seq + CITE-seq + scTCR-seq) experiments for tumor-infiltrating lymphocytes (TILs) from primary surgical specimens of BrCa (n=12, age 32-87, median=64.6, mostly HR+HER2-), HNSCC (n=11, age 51-77, median=62.1), NSCLC (n=13, age 45-76, median=59.8), and RCC (n=12, age 24-93, median=62.9). To avoid making the cancer type a confounding factor, we conducted each scRNA-seq experiment with a mixture of cancer types. After staining with CITE-seq antibodies, CD45+ cells were isolated by cell sorting and then cDNA libraries were generated using the 10X chromium system. We obtained a total of 150,598 cells for T cell subclusters. We found prominent presence of Treg in HNSCC. While RCC showed elevated effector (Teff), effector memory (Tem), and exhausted CD8 T cells (Tex), NSCLC had higher tissue-resident memory CD8 T cells (Trm). DGE analysis of Tex between NSCLC and RCC revealed that NSCLC Tex expressed higher level of ZNF683, a Trm marker, whereas RCC Tex expressed higher GZMK and HLA-DR, makers for activated Tem. Diffusion maps illustrated that CD8 T cells in RCC were more advanced toward a terminally exhausted state than those in NSCLC. Using markers for neoantigen-specific CD8 T cells (CD39+CXCL13+, Hanada et al. Cancer Cell. 2022), we found they were most enriched in NSCLC followed by HNSCC. Neoantigen-specific CD8 T cells in NSCLC and HNSCC were mixture of Trm and Tex whereas those in RCC were mostly Tex. For myeloid subclusters, we obtained 36,926 cells. While inflammatory monocytes were enriched in RCC, regulatory DCs were enriched in HNSCC and lower in RCC. Finally, we conducted whole exome sequencing and bulk RNA-seq analysis and observed a lower TGF-beta signature in RCC. Immune checkpoint ligands (PDCDLG2, HMGB1, and FGL1) were all downregulated in RCC. Together, we found deeper immune suppression in HNSCC than in NSCLC owing to prevalent presence of regulatory cells. We also found RCC is largely devoid of immune suppression that might result in preferential Tex differentiation with a concomitant lack of Trm, which may contribute to a poorer prognosis if not treated.

INVESTIGATING THE RELATIONSHIP BETWEEN HLA GENOTYPE AND MUTATIONAL SIGNATURES IN CARCINOGENESIS: A LARGE MUTAGENESIS STUDY.

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The human leukocyte antigen (HLA) plays a crucial role in the antitumor immune response. During carcinogenesis, non-synonymous mutations occur within a cell, resulting in the formation of novel peptides that can be presented by HLA molecules. Different mutagenic agents cause different changes in DNA, which leads to different amino acid (AA) substitutions. As a result, carcinogens can be classified based on their specific mutational signatures. At the same time, different HLA variants are specific for different AA substitutions. We propose that the HLA genotype and the mutational signatures jointly determine the immunogenicity of tumors. In this study, we utilized a novel approach to create a large number of mutations in the exome of cell lines by applying different carcinogens in a cyclic manner. This allowed us to examine the relationship between mutational signatures and specific AA changes. Additionally, we performed a pan-tumor examination and compared AA changes generated *in vitro* with those found in real tumor samples. We considered the depletion of AA substitutions in real tumor samples compared with treated cell lines as a consequence of immunological selection. We found that immunogenic and non-immunogenic tumors can be clearly distinguished based on the depletion of certain AA substitutions. Furthermore, we observed that certain changes are underrepresented only in specific tumors and are dependent on the HLA genotype. Interestingly, the I>F, L>P, M>K, D>E, G>W substitutions were underrepresented in all tumor types suggesting that they are generally immunogenic. At the same time, P>H and P>T substitutions showed a lack of depletion suggesting a weak immunological selection. In conclusion, our findings suggest that the combination of mutational signatures and HLA genotype determine the immune recognition of tumor cells. Our results could offer a framework for predicting the immunogenicity of tumors and developing novel biomarkers.

INFLUENCE OF THE MICROBIOTA ON LUNG-SPECIFIC MACROPHAGE PROGRAMMING

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Macrophage function is shaped by diverse tissue stimuli, including microbial products, lipids, cytokines, and growth factors. These signals program macrophages and maintain tissue homeostasis through a network of interactions, as various cell types will respond to given stimuli and alter their expression of other soluble proteins and membrane bound ligands to support intercellular communication. The signaling processes by which macrophages integrate complex tissue stimuli to control the balance of identity shaping transcription factors are largely unexplored. Our goal is to examine the effect of the microbiota on the functional programming of alveolar macrophages and to identify lung expressed factors that transmit this environmental information. We compared two complimentary RNA-seq datasets from germ-free and specific pathogen-free mice: the first from sorted CD11c+SiglecF+ alveolar macrophages and the second from whole lung tissue. Using these data we performed ligand-receptor analysis, with an initial focus on differentially expressed receptors in alveolar macrophages, for which we matched with known ligands differentially expressed in the whole lung. Using this approach, we identified several pathways of interest, including insulin-like growth factor signaling (*Itga6*/*Itgb3* and *Igf1*) and Wnt signaling (*Fzd1*/*Fzd5* and *Wnt5a*/*Wnt10b*). We are currently linking these pathways to alveolar macrophage programming via predicted transcription factor activity, based on the enrichment of transcription factor motifs in microbiota-dependent alveolar macrophage genes. Given that IGF1 and Wnt have been described to promote tolerogenic macrophage behavior in other contexts, we are also working to determine the functional consequences of altered ligand/receptor expression and changes in the activity of associated transcription factors. Our preliminary data suggest that alveolar macrophages from germ-free mice have a dysregulated inflammatory state, including higher expression of MHC-II and altered cytokine expression upon challenge. Defining signaling processes that underlie AM programming will provide mechanistic insights into lung homeostasis and support our ability to manipulate AMs therapeutically.

DIFFERENT B CELL ACTIVATION PATTERNS IN ASYMPTOMATIC AND SYMPTOMATIC COVID-19 PATIENTS

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Introduction: Recent COVID-19 studies showed a correlation between delayed and weak adaptive immune responses with poor clinical outcomes in severe patients. Low B cell counts of some subsets such as memory B cells and expression of B cells related genes are observed in severe COVID-19 patients. In this study, we aim to create a molecular pathway model of B cell activation in COVID-19 to comprehend the transformation of B cells into different subsets through different differentiation routes. The model will then be used for data analysis to identify key regulators of the pathway activation in COVID-19 in correlation to disease severity.
Approach: The pathway model was constructed following standard guidelines using an open-source pathway analysis and drawing software PathVisio version 3.3.0. A single-cell transcriptomic dataset obtained from B cells of 130 patients infected with SARS-CoV-2 with varying severity (asymptomatic, mild, moderate, severe, and critical) was used to study the activation of B cells in the curated pathway model.
Results: We have created a machine-readable molecular pathway model to analyze B cell development during SARS-CoV-2 infection. The pathway model depicts the initial B cell activation inside the lymph node and the following activation routes to differentiate to either plasmablast cells following the extrafollicular pathway or memory B cells through the follicular pathway (WP5218 in WikiPathways). The pathway showed a distinct gene expression profile between asymptomatic and symptomatic patients. In asymptomatic patients, B cells tend to go toward short-lived plasmablast cells with a high expression of antiviral interferon-stimulated genes such as ISG15, IFITM1, and NEAT1. In symptomatic patients, the results suggest an inhibition occurring at the germinal center hinting at a reduction in memory B cell production. The driver gene CXCR5 involved in germinal center development is one of the most downregulated genes. This could contribute to the shortage in the formation of memory B cells in COVID-19.
Conclusion: In SARS-CoV-2 infection, B cells follow different activation routes in asymptomatic and symptomatic patients. In this study, we constructed a pathway that allowed us to analyze and interpret activation patterns of B cells in COVID-19 patients and their link to disease severity. Importantly, the pathway and approach can be reused for further research in COVID-19 or other diseases.

NASAL αCD3 UPREGULATES TYPE-I IFN RESPONSES IN T-CELLS IN EAE

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Multiple Sclerosis (MS) is a debilitating autoimmune disease of the central nervous system (CNS) characterized by demyelination of the brain and spinal cord. Driven by chronic inflammation and immune self-stimulation, this demyelination leads to irreversible clinical and cognitive decline if untreated. The nature of this pathology can be traced back to the immune system, notably self-reactive T, B, and myeloid cells, which infiltrate the central nervous system. Modulating the immune system in MS has become a significant focus of therapeutics in recent years, with the rise of immunomodulatory monoclonal antibodies (mAb) leading the way. Currently, only intravenous mAbs for MS are clinically approved, with the majority of their targets being B lymphocytes. Here, we investigate the effects of a nasal αCD3 mAb to target T-cells in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. Specifically, we analyze the transcriptomic differences of αCD3 and isotype control mouse T-cells through single-cell RNA sequencing. The resulting differences elucidate how T-cell-targeted mAbs modulate an inflammatory environment to dampen CNS autoreactive T-cells and provide a view into potential new targets for cellular therapies in autoimmune disorders.

TIME, TISSUE AND TREATMENT ASSOCIATED HETEROGENEITY IN TUMOUR-RESIDING MIGRATORY DCs MODULATE ANTI-TUMOUR CYTOLYTIC ACTIVITY

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Background
Dendritic cells (DCs) in tumours internalise antigen, upregulate CCR7, and migrate to the tumour-draining lymph node (dLN), where they activate tumour antigen-specific T cells. New data suggests that CCR7 expression in DCs is coupled to a maturation programme enriched in immunoregulatory molecules (mRegDCs), such as PD-L1, which have been identified in tumours and draining lymph nodes (dLN). However, their spatio-temporal dynamics and role in anti-tumour immune responses at these two sites remain unclear.
Methods
To investigate the in vivo dynamics of tumour DC trafficking, we utilised a photoconvertible Kaede transgenic mouse model. Syngeneic MC38 tumours were introduced subcutaneously and anti-PD-L1 or isotype control administered on day 7, 10 and 13 following tumour injection. Tumours were 'photoconverted' with violet light enabling the distinction of newly-infiltrating Kaede-green cells from retained Kaede-red cells. Forty-eight to hours after photoconversion, myeloid cells were FACS-isolated and separated on Kaede green/red expression, and single-cell RNA sequencing performed on sorted cells. Transcriptomic findings were validated using flow cytometry, immunofluorescence microscopy and spatial transcriptomics in murine and human tumours.
Results
We found that mRegDCs comprised the dominant DC population arriving in the dLN, but a subset remained tumour-resident despite CCR7 expression. These tumour-retained mRegDCs were phenotypically and transcriptionally distinct from their dLN counterparts. There was remarkable heterogeneity among tumour mRegDCs, with increasing features of an "exhausted" phenotype with more prolonged tumour dwell time, characterised by reduced expression of antigen presentation and pro-inflammatory transcripts, an effect attenuated by anti-PD-L1 treatment. Tumour mRegDCs spatially co-localised with effector CD8+ T cells in human solid tumours, and anti-PD-L1 treatment enhanced their expression of several T cell-stimulatory molecules, with the potential to regulate T cell expansion and activation.
Conclusions
Our data provides fundamental insights into the biology of mRegDCs in cancer, including an mRegDC-cytotoxic T cell communication axis which may influence response to immune checkpoint therapy. We propose that mRegDC heterogeneity underpin variation in the capacity to support an intratumoral cytotoxic T cell niche, and that this presents new opportunities for intervention.

IMMUNE IMPRINTS BY ACUTE INFLUENZA INFECTION IMPACT THE OUTCOME OF SUBSEQUENT LOCAL IMMUNE RESPONSE

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The immune system's ability to respond to challenges is influenced by various factors. Infection history is one of them, as recent studies suggest that infection history could leave 'imprints' in the immune system. However, our understanding of this overwhelmingly came from the antigen-specific memory response. At the organism-level, the molecular and cellular regulators that can shape subsequent heterologous responses remain largely unknown. Here we use mouse acute influenza infection as a model to study these issues. Combining cellular and transcriptomic approaches, we found that complete immune resolution was not achieved after the clearance of the virus, even after more than three months post infection. Imprints are retained comprising adaptive and innate immune components in different organs, indicating a new immune baseline status after infection. Importantly, this altered immune state affected local IL-17 inflammatory response and secondary heterologous vaccination response in distal organs. We are currently exploring the mechanisms behind the altered immune states. Our study suggests that prior perturbations can shape an individual's immune response in an antigen-agnostic manner. This multi-organ analysis of immune response has implications for understanding immune system resolution and memory and is thus potentially beneficial for future vaccine design.

PIXIE: A PIPELINE FOR ROBUST PHENOTYPING OF HIGHLY MULTIPLEXED TISSUE IMAGING DATA USING PIXEL-LEVEL CLUSTERING

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While technologies for multiplexed imaging have provided an unprecedented understanding of tissue composition in health and disease, interpreting this data remains a significant computational challenge. To understand the spatial organization of tissue, imaging studies typically focus on cell-level phenotypes. However, images can capture biologically important objects that are outside of cells, such as the extracellular matrix. Here, we developed a pipeline, Pixie, that achieves robust and quantitative annotation of pixel-level features and show its application across a variety of biological contexts and multiplexed imaging platforms, including multiplexed ion beam imaging by time-of-flight (MIBI-TOF), co-detection by indexing (CODEX), cyclic immunofluorescence (CyCIF), and matrix-assisted laser desorption/ionization (MALDI-TOF). Furthermore, current cell phenotyping strategies that rely on unsupervised clustering can be labor intensive and require large amounts of manual adjustments. Cell phenotyping of intact tissue poses challenges that are not encountered when analyzing data from assays that use dissociated single cells. For example, bright signal along the border of a cell can be erroneously assigned to the neighboring cell. We demonstrate how pixel clusters that lie within cells can be used to improve cell annotations and decrease the amount of manual adjustments needed. To demonstrate the ability of Pixie to discover new biological insights, we used Pixie to characterize the myoepithelium in the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer (IBC). Normal breast myoepithelium is a thick, highly cellular layer between the stroma and ductal cells. In DCIS, the myoepithelium becomes stretched out in a thin layer with few, elongated cell bodies. Therefore, classical cell phenotyping strategies, which rely on detecting cells with a strong nuclear signal and are often optimized for conventional cell shapes, fail to capture the myoepithelial phenotype. Pixie allowed us to assign phenotypes to the acellular features of the myoepithelium that could stratify patient groups. We found that normal breast myoepithelium exists in a luminal, E-cadherin (ECAD)-positive phenotypic state, which transitions to a more mesenchymal, vimentin-positive state in DCIS, which aligns with an analogous shift in tumor cell differentiation. Importantly, a high abundance of ECAD+ myoepithelium pixels was the number one predictor of IBC recurrence in this study, highlighting the utility of Pixie.

INTERFERON REWIRING DURING IMMUNE AGING

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Alterations in the cellular composition of the immune system are an intrinsic part of immune aging. We have previously defined a directed trajectory quantitatively describing the progression of an individual through these changes over time. Here, we use this quantitative metric to investigate changes in regulatory networks during immune aging. We find a major rewiring of interferon signaling in healthy subjects at steady state. In healthy immune-young subjects, elevated interferon signaling is connected to inhibition of mRNA splicing and export, while in immune-old individuals, it predominantly inhibits translation. The interferon rewiring coincides with a decrease in expression of Toll-like receptors, connecting to interferon signaling through the MyD88 adaptor pathway. This rewiring occurs alongside with changes in cellular frequencies, but it cannot be accounted for solely by changes in major cellular subsets, suggesting a cell-intrinsic change in regulation. We hence propose that the rewiring of interferon signaling away from the suppression of mRNA maturation and towards the suppression of translation be investigated as a candidate driving force behind age-related changes in the immune cell composition in peripheral blood.

COMPREHENSIVE MAPPING OF THE SARS-COV-2 VIRUS-HOST MICRORNA INTERACTOME

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MicroRNAs (miRNAs), along with Argonaute (AGO) and other accessory proteins, constitute the RNA-induced silencing complex (RISC), which is essential for post-transcriptional gene regulation. Relying on base complementarity with the respective miRNA seed site, RISC binds specific messenger RNAs (mRNAs), which generally results in their degradation or inhibition; a delicate system that can be directly or indirectly perturbed by viral infection. For instance, some RNA viruses, such as the hepatitis C virus, hijack and repurpose RISC by binding specific endogenous miRNAs required for their replication. In the present work we investigated the miRNA interactome during infection with SARS-CoV-2, the single-stranded RNA virus responsible for the COVID-19 pandemic, aiming to experimentally 1) evaluate whether SARS-CoV-2 interacts with miRNAs, 2) delineate the resulting miRNA interactome, 3) study its effect on viral replication and 4) determine the repercussion on cellular miRNAs and their endogenous mRNA targets. Using the "Argonaute cross-linking immunoprecipitation combined with RNA proximity ligation" (AGO CLEAR-CLIP) methodology, we characterized the miRNA binding landscape in infected VeroE6 monkey kidney and A549-hACE2 human lung cells. Doing so, we showed that a wide variety of miRNAs can interact with SARS-CoV-2 RNA and that despite a complex binding landscape, this is highly conserved between cell lines. Targeted mutagenesis of miRNA binding seed sites and AGO1-3 knockdown revealed no major effects on viral replication or particle production. Intriguingly though, SARS-CoV-2 infection perturbed miRNA binding to their cellular targets. While the imbalance was largely compensated for via miRNA upregulation, perturbation of the miR-15-5p/miR-16-5p family remained non-compensated in both cell lines. In accordance, transcriptional expression analysis revealed global upregulation of miR-15-5p/miR-16-5p mRNA targets, exhibiting a seed specificity-dependent enrichment, indicating functional de-repression of cellular host targets. Altogether, this work supports a putative functional sponging mechanism and provides an important piece to the complete characterization of the SARS-CoV-2 molecular interactome.

HDAC7 INSTRUCTS THE GERMINAL CENTER PROGRAM AND ITS UNDEREXPRESSION IS INVOLVED IN DIFFUSE LARGE B CELL LYMPHOMA

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In germinal centers (GC) activated mature B cells proliferate and, after diverse rounds of cyclical transit, generate a hugely diverse repertoire of antibodies, enabling for a specific immune response against pathogens. Molecularly, this is triggered by two mechanisms named class switch recombination (CSR) and somatic hypermutation (SHM). During the GC reaction B cells cycle between two physical compartments in the GC: the dark zone (DZ) and the light zone (LZ). A coordinated network of transcription factors (TF) is fundamental for the correct GC reaction and terminal B development. The transcriptional regulator HDAC7 is essential in early B cell development and derived leukemias but its role remains still unknown in GC and B cell lymphomas such as Diffuse Large B Cell Lymphoma (DLBCL). Here, we demonstrate that HDAC7 is necessary for the correct GC reaction. Phenotypic analysis of a conditional knock-out mouse model for HDAC7 revealed lower number of GC B cells and Plasma cells (PC) in the spleen. Moreover, in the absence of HDAC7 the remaining PCs are blocked in a dividing plasmablast phase, uncapable of reaching a mature resting stage. Upon ex-vivo treatment of splenic B cells from wild-type and HDAC7-deficient mice with specific stimulus, IgG production is reduced, indicating a CSR impairment in the absence of HDAC7. In addition, we found that HDAC7 maintains a proper cell cycle since its deficiency results in the arrest of GC B cells in G1 phase and reduction in the number of cells in S phase. This is concomitant with downregulation in the proliferation ontology observed in transcriptomic analysis. Additionally, the developmental arrest results in decreased DZ/LZ ratio in HDAC7-deficient GC B cells. Importantly, a significant number of DLBCL patients present HDAC7 underexpression, that correlates with poor clinical outcome. Induced exogenous HDAC7 expression in DLBCL cell lines promotes programmed cell death and compromises proliferation both in-vitro and in-vivo. In summary, our findings identify HDAC7 as a critical regulator of the GC reaction and a valuable potential novel biomarker in DLBCL.

HUMAN SARS-COV-2 CHALLENGE RESOLVES INNATE AND ADAPTIVE IMMUNE RESPONSES AT SINGLE CELL RESOLUTION

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The COVID-19 pandemic remains an ongoing global health threat, yet our understanding of the dynamics of this infection at the cellular level remains limited. As the first of its kind, the Human Challenge Study allowed us to study the cellular dynamics of COVID-19 using single cell genomics of nasal swabs and blood, charting events from one day before the infection up to 28 days post-infection. This allowed us to distinguish abortive, transient and sustained infection in 16 seronegative individuals challenged with preAlpha-SARS-CoV-2.

Our analysis revealed rapid changes in cell type proportions and dozens of highly dynamic cellular response states in epithelial, nasopharyngeal resident and circulating immune cells associated with specific infection timepoints that differed with infection status. Sustained infection displayed a strong interferon response in blood before the nasopharynx. Global nasal immune infiltration occurred early in transient and later in sustained infection. Nasal ciliated cells showed an acute response phase, that included antigen presentation, and these cellls were most permissive for productive infection, whilst nasal T cells and macrophages were infected non-productively. Our detailed cell type annotations allowed us to follow innate as well as adaptive T and B cell responses at great resolution over time, distinguishing local and systemic responses. This detailed time series data can serve as a “Rosetta stone” allowing time inference of SARS-CoV-2 samples.

A LYMPH-NODE MICRODOMAIN THEORY FOR T CELL QUALITY CONTROL

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The adaptive immune system should mount an effective and robust response against invading pathogens while maintaining tolerance towards its host's constituents. Thymic selection plays a major role in this task, eliminating T cells with high affinity towards self-peptides and driving differentiation of intermediate self-reactive ones into regulatory T cells (Tregs). However, the distributions of self-affinity strength of conventional T cell (Tconv) and Treg populations overlap, suggesting a risk for autoimmunity when Tconvs are activated more strongly than Tregs. In addition, Tconvs and Tregs probably have non-self affinity distributions with even greater overlap region since they are not selected against non-self-peptides, limiting the potential for pathogen response. Here we propose a mathematical model for local interactions between Tconvs and Tregs in lymph node (LN) microdomains. Competition within each microdomain between activated Tconvs and Tregs leads to each cell type winning in separate spatial compartments. We show that effective immune responses to pathogens can originate from microdomains where high-affinity Tconv clones win while autoimmunity is still prevented due to Treg's advantage in consuming IL-2. Thus, this microdomain theory suggests a LN design that can both facilitate quality control of Tconv clones by Tregs during infections and avoid autoimmunity, despite constraints of the thymic selection process.

ROLE OF REGULATORY T CELLS IN COLORECTAL CANCER

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The colon immune system is the largest part of the immune system. It is a highly complex system that maintains a balance between immunity and tolerance by providing protection from pathogens while supporting the gut microbiota by limiting inflammatory responses. Carcinogenesis in the colon results in colorectal cancer (CRC) which is the third most common cancer in the US and worldwide, and the second most common cause of cancer death in the US. CRC can be broadly classified into mismatch repair-deficient (dMMR) CRC and mismatch repair-proficient (pMMR) CRC. Unlike dMMR CRC, pMMR CRC fails to respond to immune checkpoint therapy. We hypothesize the resistance is due to the role of immunosuppressive T cells, in particular regulatory T cells (Treg). To investigate this, we applied high-throughput multiomic single-cell sequencing of RNA+TCR and RNA+ATAC to investigate the dynamic changes in chromatin and transcriptomic landscape of T cells as well as TCR clonality in a mouse orthotopic pMMR CRC model. We first identified subsets of Treg cells in the tumor and the adjacent intestine samples based on scRNA-seq expression. Specifically, we noticed differential enrichment of thymic Treg (tTreg) and peripherally derived Treg (pTreg) cells in the samples. By leveraging the multiomic chromatin accessibility and transcription data, we derived unique dynamics of transcription factor binding that govern the functional activities of each Treg subset. Furthermore, using our time-series multiomic RNA and TCR sequence data, we noticed differential clonal expansions in the Treg cells of tumor and intestine samples that suggested altered cell-cell interactions. Following this, we applied a similar analysis to single-cell data obtained from metastases to the liver and lymph node of the pMMR CRC mouse model and discovered new Treg states that are not present in healthy tissue. Our comparative analysis of Treg subsets in the intestine, primary, and metastasis tumor samples using multiomic single-cell data will provide insight into the role of the Treg cells in pMMR CRC progression and metastasis.

ACTIVATION-NEUTRAL GENE EDITING OF CD4 T CELLS IN HUMAN EX VIVO TONSIL CULTURES

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The ability to rapidly change activation states to exogenous T cell receptor (TCR) stimulation is a key property of CD4 T cells that is essential for their function. The majority of CD4 T cells in the body are typically in a homeostatic, non-proliferative resting state with limited transcriptional activity and cell proliferation and gene expression is induced upon activation by specific TCR engagement. Molecular processes in activated CD4 T cells have been studied intensively. In contrast, resting CD4 T cells are refractory to gene-editing transduction and transfection methods without prior activation and knowledge on the molecular biology of truly resting CD4 T cells is therefore lacking.

Resting CD4 T cell biology is particularly relevant for the dissection of HIV pathogenesis since productive infection and establishment of latency only occurs in activated CD4 T cells while resting CD4 T cells are refractory to infection. We recently reported a highly efficient activation-neutral gene-editing approach of resting CD4 T cells for human peripheral blood cells (Albanese et al., 2022 Nature Methods 19: 81-89). However, HIV mostly replicates in lymphoid tissue, which can be recapitulated in tonsil explant cultures. While tonsillar CD4 T cells are phenotypically resting, they are permissive to HIV infection. Which molecular differences govern the permissivity of peripheral and tonsillar CD4 T cells to HIV infection is unclear and methodology for gene editing of CD4 T cells in tonsil explants is lacking.

To overcome this limitation, we developed a gene-editing workflow based on CRISPR/Cas9 RNP nucleofection in ex vivo tonsil cultures. Optimization of culture conditions and concentrations of added cytokines resulted in knock out efficacies of over 90%. Importantly, editing of tonsil CD4 T cells did not impair viability, activation state or immunocompetency of the cells such as their ability to provide help to a secondary CD8 T cell response, and efficient knock out did not require exogenous activation. In the context of HIV infection, complete prevention of HIV-1 infection and CD4 T cell depletion by knock out of the HIV-1 co-receptor CXCR4 validated the functionality of this method. This approach allows gene-editing of multiple cell types in bulk cultures or of CD4 T cells previously isolated from tonsil that can be added back to the non-CD4 T cell fraction post gene editing. In summary, we developed a highly efficient and versatile workflow for gene editing of tonsillar CD4 T cells that opens avenues towards the dissection of molecular mechanisms in ex vivo cultures of human lymphoid tissue.

MULTICELLULAR IMMUNE NETWORKS OF DISEASE ACTIVITY AND CLINICAL OUTCOME IN VASCULITIS

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Autoimmune vasculitides (AAV) associated with anti-neutrophil cytoplasmic antibodies (ANCA) are diseases characterized by severe systemic inflammation of small blood vessels. Current cytotoxic therapy to induce remission does not cure the underlying condition. Furthermore, certain patients have a comparably poor longitudinal outcome characterized by treatment resistance and debilitating disease relapses. We profiled the blood transcriptomes of sorted immune subsets from a prospective cohort of patients presenting to the clinic for the first time with active, untreated ANCA-associated AAV. We found distinct T cell states formed hubs in a multicellular immune network of vasculitis disease activity. Additional analysis of T cell subsets using diffusion map embeddings of patient scores for in vitro-derived T cell differentiation states revealed distinct ANCA-AAV T cell effector gradients compared to patients with other active/untreated autoimmune and inflammatory disorders. Expression of canonical signaling pathways also stratified AAV patients into sub-groups at presentation. Drug reversal signature screening nominated concordant targets of these processes, suggesting strategies to tailor induction treatment. In addition, unsupervised analysis of clinical data identified a group of patients with poor long-term outcomes, including a high frequency of disease flares. To predict clinical outcome from these initial presentation subset transcriptome profiles, we scored the activity of gene modules derived from co-expression analysis and curated from cell type-specific in vitro stimulation results. In an ensemble of machine learning algorithms, linear methods had the best performance and identified interpretable cell states associated with poor outcome. High dimensional conditional independence analysis screened the predictive molecular states for those directly linked to outcome. These approaches revealed strategies to target the global immune networks of vasculitis with personalized medicine approaches for induction therapy and maintenance.

COMPARATIVE ANALYSIS OF NORMALIZATION METHODS FOR SPATIAL TRANSCRIPTOMIC DATA

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Spatial transcriptomics (ST) is a cutting edge technology that holds great potential in identifying spatial patterns of gene expression, uncovering micro-environments and constructing cell-cell communication networks. Normalization, used to adjust for technical variation and artifacts, is a crucial step in data preprocessing. Importantly, the choice of normalization method can have a profound, significant impact on downstream analyses – and the same method can perform differently on data from similar technologies, such as RNA-seq and scRNA-seq. Yet, despite this, there is no consensus regarding which normalization method(s) should be applied to ST - which, to date, is regularly normalized using algorithms built for scRNA-seq.

Here, to address whether or not such algorithms are appropriate for ST data, we benchmark a multitude of frequently applied normalization methods (including SCTransform, dca, Sanity, DESeq2 and scran). Specifically, we constructed a two-step simulation platform which: (i) initially simulates gene expression at the single-cell level across two groups (ii) subsequently combines cells into 10X Visium-like spots, with a specified density and uniformity in 2D space, and downsamples each spot to generate realistic ST data. Critically, a number of parameters are variable and thus the platform enables us to assess the performance of normalization methods from multiple aspects.

A key downstream analysis in biology is the detection of differentially expressed genes (DEGs). Using our platform, and a myriad of DEG-algorithms, we show that all normalization methods tested have decreased performance for ST data across multiple metrics – including sensitivity, specificity, accuracy and the percentage of genes detected as differentially expressed. Moreover, this trend holds true when tissue characteristics (e.g. density) are modulated.

Overall, the results of this study will allow us to appraise and rank the suitability of current normalization methods as a function of tissue characteristics, and – more importantly - lay the groundwork for future method development by identifying the specific properties of ST data that cause a failure in normalization.

DYNAMIC FRACTIONAL CONTROL OF INTERFERON PRODUCTION IN VIRAL INFECTION

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Upon viral infection, infected cells produce type I interferon (IFN) as the first line of defense against viruses. The amount of IFN produced in an infected tissue requires tight regulation as an elevated level correlates with massive inflammation and tissue damage, as shown in lethal influenza and COVID-19 infection. However, how individual infected cell decides how much IFN to produce and whether a population of infected cells within a tissue make the decision individually or collectively remain poorly understood. To answer these questions, we infected a confluent layer of human lung epithelial cells with influenza A virus (IAV) and quantified viral/host gene expression using multiplexed single-molecule RNA fluorescence in situ hybridization. We find that IFN production is highly stochastic, with only ~10% of infected cells producing IFN during primary infection. To determine whether this fraction represents the IFN-producing probability that is completely intrinsic to each cell, or whether the cells within the population influence each other's decision, we blocked a potential cell-cell communication circuit mediated by IFN using neutralizing antibodies against IFN receptors and chemical inhibitors. Blocking IFN signal response reduces the fraction of IFN-producing cells to 1-2%. Furthermore, at the single-cell level, the probability of IFN production, but not the level of IFN, linearly correlates with the viral load. We further confirmed this phenomenon in influenza-infected mouse lungs at 36-hour post infection. Together, we demonstrate that during early infection, tissues can adjust the level of IFN to the viral load by tuning the fraction of cells that switch on IFN, rather than the IFN level per cell. The stochastic binary switch of IFN production among infected cells prompted us to ask what determines the intrinsic probability of this switch at the single-cell level. Upon quantifying the levels of upstream viral sensing and signal transduction components, we observed that the expression level of a key IAV sensor, RIG-I, correlates strikingly well with the cell's probability of producing IFN. Furthermore, RIG-I expression level is not fixed. The abundance of RIG-I increases rapidly in a small fraction of infected cells, forming a feedback circuit that sensitizes the cells' detection of viral RNA, and thus, increases the probability of switching on IFN in those cells. This viral-RIG-I feedback circuit acts as a novel and potentially crucial knob for controlling the fraction of infected cells to produce IFN. We speculate that this dynamic fractional control mechanism allows healthy tissues to maintain a low sensitivity to low levels of molecules released by pathogens or damaged cells at homeostasis, to avoid overreaction of the immune system, and ramps up pathogen detection and IFN production upon infection.

DISCOVERY AND INHIBITION OF TREG TUMOR-INFILTRATION MASTER REGULATOR PROTEINS BY VIRTUAL INFERENCE OF PROTEIN ACTIVITY

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Regulatory T-cells (Tregs) are immune-suppressive cells normally responsible for suppression of auto-immune disease, but frequently co-opted as mechanisms of tumor immune evasion. This makes them highly attractive immuno-oncology targets. To date, treatments targeted against Tregs have been unable to specifically inhibit tumor-infiltrating Tregs (TI-Tregs) while preserving the necessary function of peripheral Tregs (P-Tregs). To this end, we have leveraged an RNA-Sequencing database of matched TI-Tregs and P-Tregs from thirty-six patients across four malignancies. Applying bioinformatics tools for virtual inference of protein activity and Random Forest feature selection, we identify 17 candidate Master Regulator proteins (MRs) of TI-Treg cell state, observed consistently across patients and tumor types. Multiple independent ex vivo and in vivo screening tests confirm the essentiality of these proteins for TI-Treg recruitment and maintenance of TI-Treg transcriptional state. Pooled CRISPR/Cas9 knockout screening using a chimeric hematopoietic stem cell transplant model confirmed 8 MRs as vital to TI-Treg recruitment and/or retention, without depletion of P-Tregs. Over-expression of any one of these MRs in an ex vivo screen induced upregulation of the entire transcriptional program corresponding to TI-Treg phenotype. Furthermore, single-gene knockout of the most statistically significant MR (Trps1) in hematopoietic lineage significantly reduced ectopic tumor growth in immune-competent mouse model. In order to identify drug compounds inhibiting the TI-Treg MRs, we performed a systematic ex vivo drug screen with an unbiased panel of 1,554 FDA-approved and experimental compounds coupled to RNA sequencing (PLATE-Seq) on human TI-Tregs and P-Tregs. Drugs with preferential toxicity to TI-Tregs versus P-Tregs that also inhibited TI-Treg MR activity were thoroughly validated in vivo in MC38 tumor model. These studies reveal that Gemcitabine, at low dose, exhibits preferential activity against TI-Tregs versus P-Tregs, and against TI-Tregs versus cytotoxic effector T-cells— confirmed in vivo by flow cytometry and single-cell RNA-Sequencing. Low-dose Gemcitabine was found to significantly inhibit tumor growth in immune-competent mice only, improving response to anti-PD1 checkpoint inhibitor immunotherapy. Taken together, these studies provide key insight into regulators of TI-Treg cell state, identify Gemcitabine as a readily available therapeutic candidate against TI-Tregs meriting further clinical evaluation, and presents a generalizable strategy to elucidate and target MR proteins in other immunosuppressive cell populations.

DIGITAL SPATIAL PROFILING IN LUNGS OF CYNOMOLGOUS MACAQUES EXPERIMENTALLY INFECTED WITH SARS-CoV-2

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Molecular analysis of SARS-CoV-2 infection in the respiratory organs including lungs is necessary to understand viral pathogenesis and discover clinical biomarkers. Gene expression patterns have been reported in the lungs of mice infected with SARS-CoV-2 using a recently developed spatial transcriptome analysis. However, similar experiments on non-human primates have been challenging to perform because of limited subject-appropriate experimental resources. Here, we reveal gene expression patterns in the lungs of cynomolgous macaques experimentally infected with SARS-CoV-2 using GeoMX ® Digital spatial profiling. The virus infected lungs showed a significant upregulation of genes associated with the inflammatory response, interferon and interleukin signaling, TNF alpha signaling via NFκB, TGF-β signaling, coagulation, complement, hypoxia and apoptosis pathways. The types and amounts of genes expressed in each pathway were different for alveoli, bronchioles, and blood vessels. This study revealed the spatial transcriptome profile of SARS-CoV-2-infected macaques' lungs using the human whole transcriptome atlas probe following histopathological classification of major tissue structures. These findings may assist in facilitating efforts of designing spatial transcriptome analysis in macaque models to aid in understanding the pathogenesis of SARS-CoV-2 variants or in evaluating therapeutics and vaccines against the virus.

CROSSTALK AND PLASTICITY IN INFECTION-INDUCED PROGRAMMED CELL DEATH

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Programmed cell death (PCD) is an evolutionarily ancient and highly conserved type of antiviral defense. The importance of PCD as a defense mechanism is underscored by the fact that viruses have evolved myriad adaptations that interfere with it. How does the host maintain a functional defense in the face of these different adaptations? Our overarching hypothesis is that the different PCD types are functionally redundant and that crosstalk between the pathways confers robustness to viral interference. Specifically, we think that crosstalk among these pathways enables PCD to be triggered even when elements of a pathway are blocked. To test this hypothesis, we profiled death pathway activation in single cells at the molecular level, and used a computer vision-assisted approach to assign how cells ultimately die. Our work demonstrates that virus-infected cells promiscuously activate proteins associated with different PCD pathways. This molecular promiscuity in single cells leads to execution of heterogeneous death fates throughout the population. Thus, a single viral trigger in a homogenous population of cells can simultaneously activate each of the three major death pathways. We argue that the interconnected nature of the PCD pathways enables cellular immunity by providing flexibility in the death decision and robustness to viral pro-survival tactics.

INTEGRATIVE GENE REGULATORY NETWORK ANALYSIS OF IKAROS TUMOR SUPPRESSION IN IKZF1-MUTATED B CELL ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Altered gene regulatory mechanisms are characteristic of specific hematological malignancies, yet we still know relatively little about how transcription factors (TFs) in these diseases. Somatic deletions in the lymphoid transcription factor (TF) gene encoding IKZF1 have been strongly associated with a poor prognosis in B cell acute lymphoblastic leukemia (B-ALL), however the mechanisms that mediate Ikaros tumor suppressor function are unknown. Comprehensive characterization of disease-related gene regulatory networks (GRNs) can help clarify potential disease mechanisms and prioritize targets for novel therapeutic approaches. In this study, we used inducible expression of wild-type Ikaros (IK1) in patient-derived B-ALL cell lines that harbor a heterozygous IKZF1 deletion to study gene regulatory mechanisms associated with IKZF1-mutated B-ALL. Integrated ChIP-seq, RNA-seq and ATAC-seq analysis reveals mechanisms associated with IK1-mediated growth suppression in B-ALL, including the direct regulation of pathways associated with B cell development, genes that function downstream of the pre-B cell receptor (pre-BCR), as well as genes that limit the metabolic capacity of leukemia cells. We show that induction of IK1 results in decreased chromatin accessibility and altered histone modifications at Ikaros binding sites located at proximal and distal gene regulatory elements. To gain insight into GRNs associated with IK1, we performed paired RNA-seq and ATAC-seq analysis to study the gene regulatory networks of IKZF1-mutated B-ALL. Overall, we present a high-resolution map of the deregulated gene regulatory landscape in IKZF1-deleted B-ALL and insight into Ikaros tumor suppression mechanisms. This work further identifies characteristic regulatory features including putative oncogenic TFs that may represent therapeutic targets for B-ALL.

TISSUE-SPECIFIC OPTIMIZATION OF T CELL ACTIVATION IN DRAINING LYMPH NODES

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Depending on size, mammals can have dozens to hundreds of lymph nodes that drain lymph from specific tissues. Since different tissues have different functions and regenerative capacities, we hypothesize that immune activation in a given lymph node is regulated to effectively eliminate pathogens and concurrently avoid irreparable damage to a given tissue. If the proper balance is not maintained, there are drastic evolutionary fitness costs incurred upon the organism, such as death and infertility. To explore the trade-offs between host protection and tissue damage in different lymph nodes, we employ high-resolution multiplexed imaging and mathematical modeling to characterize the regulation of immune activation in different settings. We assess spatial parameters of T cell activation in the mandibular, lung, pancreatic, ovarian, popliteal, mesenteric, and colic draining lymph nodes in Nur77GFP mice. In parallel, we construct a dynamic model of T cell activation that outputs the clonal expansion and effector differentiation of CD4 T cells for a given antigen input and tissue-specific contextual signals. We explore the parameter space of this model by posing this trade-off as a multi-objective optimization problem. We show that the basic classes of lymph nodes that our model predicts shares commonalities with our imaging data.

A COMPARATIVE STUDY OF PRIMARY AND IMMORTALIZED IN VITRO AIR-LIQUID INTERFACE CULTURE MODELS OF THE HUMAN AIRWAY EPITHELIUM EVALUATING CELLULAR HETEROGENEITY AND GENE EXPRESSION AT SINGLE CELL RESOLUTION.

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The human respiratory epithelium maintains airway homeostasis and acts as a barrier to harmful agents. Human airway epithelial cultures at air-liquid interface (HAE) are a physiologically relevant *in vitro* model of this heterogeneous tissue, enabling numerous studies of airway disease. HAE cultures are typically derived from primary cells harvested from bronchial epithelium, which are differentiated into a pseudostratified architecture. However, primary cells are relatively short-lived, restricting the scope of potential experiments. BCi-NS1.1, a previously described basal cell line derived from bronchial epithelium and engineered for transgenic expression of hTERT, exhibits extended passage lifespan and retains capacity for differentiation to HAE. However, the effects of hTERT on HAE gene expression and innate immune function have not been fully characterized. Combining single cell RNA-Seq (scRNA-Seq), immunohistochemistry, and functional experimentation to compare BCi-NS1.1 (N=3) and primary HAE from distinct donors (N=3), we confirm that BCi-NS1.1 cells form HAE cultures with similar structure and cellular composition to those from primary cells. scRNA-Seq analysis resolved 12 airway cell populations, including rare airway cell types. Cell frequencies were largely similar between culture conditions, though a subset of MUC5AC^{hi} secretory cells were more frequently observed in BCi-NS1.1. In per cell-type gene expression analyses, data clustered primarily by cell type rather than source or donor. Differential gene expression contrasts revealed 1,052 differentially expressed genes across culture conditions, the majority of which were unique to a single cell type. While we observed cell-type specific elevated expression of several interferon stimulated genes in BCi-NS1.1 HAE, we found unremarkable differences in susceptibility to infection with influenza A virus. Taken together, our results indicate that despite modest differences in gene expression, BCi-NS1.1 and primary HAE are largely similar in morphology, cell type composition, and overall transcriptional patterns, and further support the BCi-NS1.1 cell line as a valuable tool for the study of airway infectious disease. *Equal contribution, #Co-corresponding

SELF-REACTIVITY DRIVES SELF-TOLERANCE IN THE PERIPHERY: A SUGGESTION BY FRACTAL IL-2-MEDIATED CD4+ T CELL REGULATORY CIRCUITS

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T cells provide effective host protection while limiting autoimmunity. However, experimental evidence suggests that a small fraction of conventional CD4+ T cells (Tconvs) are continuously activated in response to self-antigens presented by peripheral dendritic cells in secondary lymphoid organs (SLOs). These self-activated Tconvs are typically suppressed by regulatory CD4+ T cells (Tregs). Separate lines of evidence illustrate that maintaining a homeostatic Treg population in SLOs depends on a cytokine, IL-2, mainly secreted by CD4+ T cells, even in homeostatic conditions. These suggest that we still lack an integrated quantitative understanding of establishing immune homeostasis. To this end, we hypothesized that a major source of IL-2 supporting peripheral Treg maintenance is self-activated Tconvs and that such a homeostatic self-activated fraction of Tconvs is not dangerous to cause autoimmunity via IL-2-mediated paracrine activation by Treg-mediated suppression. In other words, the homeostatic frequency of self-activated and IL-2-secreting Tconvs can regulate the homeostatic population size of Tregs. Reciprocally, Tregs dynamically constrain the individual self-activated Tconvs and their homeostatic population size below quorum-regulated decision boundaries between tolerance and activation. To test these hypotheses, we developed a series of computational models of T cell activation dynamics, probabilistic IL-2-mediated quorum regulation, and reciprocal regulatory relationships between Tconvs and Tregs, describing intracellular, intercellular, and cell population dynamics of constituent molecules and cells across scales based upon published experimental data. By bridging these cross-scale models, we finally showed indeed those hypotheses are likely the case and derived a quantitative framework describing immune homeostasis as a dynamical equilibrium between self-activated Tconvs and Tregs, typically operating well below thresholds, beyond which could result in clonal expansion and subsequent autoimmunity. Our framework suggests the counterintuitive role of the peripheral self-reactive repertoire of Tconvs in maintaining immune homeostasis and may guide the therapeutic manipulation of immune homeostasis to treat cancer and autoimmunity.

MULTILEVEL PROTEOMICS REVEAL EPIGENETIC MARKERS INFLUENCING BCG-MEDIATED MACROPHAGE ACTIVATION

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Introduction: The bacille Calmette-Guerin (BCG) vaccine is currently administered at birth in endemic areas to prevent TB transmission [1]. BCG has been shown to be particularly immunogenic [2], and causes trained immunity. Monocytes display epigenetic memory of BCG vaccination at the histone level for up to three months, characterized by increased TNF-alpha and IL-6 production upon secondary stimulation with unrelated pathogens[3]. Mass spectrometry based proteomics allow for global profiling of histones mediating inflammatory cytokine expression, which can provide insight into mechanisms of sustained immunogenic signaling. **Methods:** THP-1 macrophages were infected with BCG for 24 hours, and protein extracted for total proteomic analysis. Histones were isolated to assess global changes in posttranslational modifications, and total protein and histone isolates were assayed using tandem mass spectrometry (Q Exactive). Bioinformatic analysis allowed for discovery of differentially regulated cytokines and histone post translational modifications in BCG infected and uninfected macrophages. These data were cross-referenced with phospho proteomics previously obtained phospho-proteomic data. **Results:** Bioinformatic analysis revealed decreased phosphorylation of four histone acetyltransferase (KAT) peptides, two demethylation (JmjC) peptides, a lysine demethylase peptide, and a lysine methyltransferase peptide in BCG infected macrophages. A total of 27 histone fragments showed significantly altered abundances between the control and infected macrophages, including 4 unmodified segments and 23 PTMs. Several identified PTMs are associated with increased cell proliferation and altered chromatin organization. Total proteome analysis showed 298 proteins differentially regulated between infected and non-infected macrophages. **Conclusion:** Proteomic analysis allows for robust systems analysis of and reveals activation of complex signaling cascades upon initial BCG infection. Our data implies that dynamic histone modification occurs upon BCG infection in macrophages and provides insight into epigenetic mechanisms that can mediate sustained immunomodulation after BCG exposure.

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ANALYSIS OF SIGNALING-COUPLED TRANSCRIPTION FACTOR NETWORKS REGULATING HUMAN B CELL FATE DYNAMICS

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Activated B cells bifurcate into antibody-secreting plasmablasts (PBs) or germinal center B cells (GCBC), the balance of which determines the quality and magnitude of humoral immune responses. While short-lived PBs provide a rapid burst of low-affinity antibodies, GCBCs delay differentiation in secondary lymphoid organs where their immunoglobulin genes undergo somatic hypermutation and affinity-based selection to generate precursors of long-lived plasma cells that secrete high-affinity antibodies. We are attempting to comprehensively elucidate the dynamic signaling-coupled gene regulatory network (GRN) that regulates the bifurcation of activated human B cells into PBs or GCBCs. We and others have previously demonstrated that reciprocal and sequentially acting negative feedback loops between pairs of signaling-induced TFs, IRF4 and IRF8 and Blimp1 and Bcl-6 regulate the primary bifurcation of murine B cells. While elevated IRF4 and Blimp1 expression promotes PB differentiation, elevated IRF8 and Bcl-6 expression restrain PB differentiation and enable GCBC formation instead. In striking similarity to these studies in mice, we show that in vitro activated human B cells bifurcate into distinct IRF4hi/IRF8lo/BLIMP1hi/BCL6lo (PB) or IRF4lo/IRF8hi/ BLIMP1lo/BCL6hi (pre-GCBC) regulatory states upon activation and several days of rapid cell division. The bifurcation occurs in the context of multiple signaling inputs including from the B cell receptor, Toll-like receptor 9, and T cell-derived ligands and cytokines. To analyze the signaling and TF networks that dynamically program human B cell fate specification, we are coupling structural and functional genomics with Cas9 mediated perturbations and predictive modeling approaches. Single-cell transcriptional profiling coupled with clonal tracking using BCR-seq reveals distinguishable trajectories with characteristic cell cycle dynamics. PB cell fate specification is promoted by slowing down of cell cycle rates. Use of bulk and single cell chromatin profiles and their integration with corresponding transcriptional states to generate a draft human B cell GRNs for naïve and activated human B cells and their bifurcated PB and GCBC progeny, will be presented. This work will provide new molecular insights into the dynamic regulatory apparatus that controls the quality and durability of humoral immune responses in humans and facilitate vaccine design.

INNATE IMMUNE RESPONSE AFTER CARDIAC ARREST: THE INNATUS STUDY

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Background: Treatment of cardiac arrest patients after successful resuscitation is complicated by post-cardiac arrest syndrome (PCAS). The syndrome is characterized by myocardial and neurological dysfunction, hemodynamic instability resembling septic shock, and multiple organ failure. Accumulating evidence is pointing at an inflammatory reaction resulting from whole-body ischaemia-reperfusion injury (IRI) as a triggering event for PCAS. However, previous studies on the topic have focused on finding isolated biomarkers for prognostication, ignoring the underlying complexity of the immune system responsible for the inflammatory response.
Objectives: The objective of this observational single-center study is to find associations between the evolving multiple organ failure after cardiac arrest and patterns of variables reflecting the activity of the innate immune system, the extent of tissue damage and disturbance in red/ox balance.
Patient population and sample size: Adult cardiac arrest patients admitted to the intensive care unit of Helsinki University hospital, Finland, starting from January 2022. Currently, we have recruited 28 of the planned 40 patients in the study. Control samples will be collected from patients scheduled for elective coronary artery bypass graft (CABG) operation.
Methods: We perform a deep immune profiling of repeated samples, combining transcriptomics and flow cytometry of peripheral blood leukocytes, measurement of nicotinamide adenine dinucleotide (NAD) metabolites in blood to assess metabolic red/ox balance, and analysis of a broad panel of inflammation-related plasma proteins with ELISA-based multiplex assays. A special focus will be given to innate-like lymphocytes (NK cells and innate-like T cells), which are rapid initiators and enhancers of inflammation. We will search for 2-4 groups of patients with latent class analysis and nonlinear stochastic embedding based on the measured inflammatory and metabolic variables and then test the difference in outcome between the groups.
Outcome measures: We will assess multiple organ dysfunction with emphasis on haemodynamic instability with modified Sequential Organ Failure Assessment (mSOFA) score including additional categories for severe haemodynamic instability compared to the standard SOFA. The temporal change of the mSOFA during the first 4 treatment days is the primary outcome measure.

CITR-SEQ: A ROBUST METHOD TO GENERATE PAIRED T CELL RECEPTOR REPERTOIRES IN MICE

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Recognition of foreign- and self-antigens by T cells and its receptors (TCRs) is key to adaptive immunity. To achieve effective immunity, millions of T cells must be able to match any given antigen through their unique, heterodimeric TCRs. This makes dissecting entire TCR repertoires challenging: conventional methods fail to pair both TCR chains, thus providing incomplete clonotype information. Further, single-cell transcriptome approaches are costly and only capture a small fraction (<1%) of the effective repertoire. Here we present our new method CITR-Seq (combinatorial indexing T cell receptor sequencing), which identifies TCR α and β pairings in hundreds of thousands of T cells from one individual. CITR-Seq combines two features: multiplexed TCR transcript amplification and single-cell combinatorial indexing. We bypass the need of specialized equipment, enabling processing of more cells and samples than before.
To test our approach we construct TCR repertoires of CD8+ T cells isolated from mouse spleen. Across samples we can consistently recover > 400,000 T cells with up to 70% successful TCR α and β pairing. By contrast, using the commercial 10X Genomic's Chromium Next GEM platform we were only able to confidently recover clonotypes of fewer than 10,000 cells per sample, thus clearly showing the boost in throughput. Next we applied CITR-Seq to mouse sister-species (Mus castaneus and Mus spretus). We can consistently recover diverged and evolved T cell repertoires confirming CITR-Seq's ability to better capture TCR diversity.
The method presented here greatly expands the scope of TCR repertoire studies, allowing for the generation of population-scale repertoire profiles. In the future, we expect CITR-Seq to be used to better understand the dynamics of TCRs, e.g. the role between public and private TCR molecules.

STAT1 GAIN OF FUNCTION MUTATION IMPAIRS IMMUNE RESPONSE TO VIRAL INFECTIONS

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The coordinated effort of the immune system in host defense is dependent upon the proper release and interpretation of different cytokine signals. Several examples of monogenic diseases targeting the JAK-STAT pathway reveal there is still a critical need to understand basic principles of cytokine signaling and cytokine output. For example, patients with STAT1 gain-of-function (GOF) mutations exhibit a type 1/IFN-gamma bias that antagonizes a type 3/IL-17 immune response important for controlling fungal infection, yet patients also, paradoxically, exhibit chronic and sometimes lethal viral infections. Using a novel conditional knock in STAT1-GOF mouse model, we demonstrate that STAT1-GOF mice respond poorly to viral infections that are easily controlled by WT mice, such as MCMV and LCMV-Armstrong. High-dimensional flow cytometry and scRNA-seq reveal an impaired NK and CD8 T cell effector response in STAT1-GOF mice that is not simply due to an exhaustion phenotype. Instead, STAT1-GOF mice respond poorly to viral infections due to an unexpected defect in IFN-gamma production early during the innate immune response, leading to a cytokine storm, immunopathology, and an impaired adaptive immune response. sc-Multiome-seq (GEX + ATAC) uncovers altered timing and usage of different STAT complexes in STAT1-GOF mice, leading to a misinterpretation of early innate-stimulating cytokines. These results highlight the importance of an early synchronized innate response during viral infection, as well as provides insight into why patients with interferonopathies or elevated interferons respond poorly to viral infections.

REGULATORY GENOMICS OF T CELL ACTIVATION AND DIFFERENTIATION

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T cells coordinate systemic immunity and are essential for defense against viral and bacterial infections and tumors. Systematic molecular characterization of T cell functional and differentiation states across contexts is important for better understanding of fundamental mechanisms of adaptive immunity and critical for developing better T cell modulation therapies.

I will present preliminary results of our multiple ongoing complementary efforts at developing and applying computational methods for studying regulatory mechanisms of T cell activation, differentiation and function.

First, we are building a comprehensive compendium of published ATAC-seq and RNA-seq data for T cells across mouse models and immunological challenges. This will provide systematic characterization of regulatory landscape in T cells at unprecedented resolution and will enable better informed and more accurate analysis of newly generated bulk and single-cell ATAC-seq and RNA-seq data.

Second, we are developing a transcription factor (TF) motif regression approach for analysis of single-cell ATAC-seq data. This will enable association of potential driver TFs with functional T cell states identified in published and our newly generated single-cell data.

Third, we are developing a computational method that will extend the RNA velocity approach to multi-omic scATAC+RNA-seq data. This will enable identification of activating and repressive TFs in dynamic processes of T cell activation and differentiation.

SLIDE: SIGNIFICANT LATENT FACTOR INTERACTION DISCOVERY AND EXPLORATION ACROSS IMMUNOLOGICAL DOMAINS

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Modern multi-omic technologies can generate deep multi-scale profiles. However, differences in data modalities, multicollinearity of the data, and large numbers of irrelevant features make the analyses and integration of high-dimensional omic datasets challenging. Here, we present Significant Latent factor Interaction Discovery and Exploration (SLIDE), a first-in-class interpretable machine learning technique for identifying significant interacting latent factors underlying outcomes of interest from high-dimensional omic datasets. SLIDE makes no assumptions regarding data-generating mechanisms, comes with theoretical guarantees regarding identifiability of the latent factors/corresponding inference, outperforms/performs at least as well as state-of-the-art approaches in terms of prediction, and provides inference beyond prediction.

Using SLIDE, we first sought to uncover altered cell-type-specific regulatory mechanisms underlying diffuse systemic sclerosis (SSc) pathogenesis. Using scRNA-seq profiles from skin biopsies of SSc subjects, SLIDE was able to accurately predict disease severity, and outperformed/performed as well as several benchmarks. Further, the interacting latent factors uncovered by SLIDE pointed to three distinct mechanisms. The first encompassed altered transcriptomic states in myeloid cells and fibroblasts, a well elucidated basis of SSc disease severity. The second included an unexplored keratinocyte-centric signature, which we validated using protein staining. Finally, SLIDE uncovered a novel mechanism involving an interaction between the altered transcriptomic states in myeloid cells and fibroblasts with HLA signaling in macrophages. This mechanism has strong support in recent genetic association analyses.

Next, we used SLIDE to elucidate latent factors underlying differences in clonal expansion of CD4 T cells in T1D. Using paired scRNA-seq and TCR-seq data on islet-derived cells in a non-obese diabetic (NOD) mouse model, we labeled cells based on their clonal expansion. SLIDE was able to accurately predict extent of clonal expansion, and outperformed/performed as well as the prior benchmarks. The latent factors uncovered by SLIDE included well-known activation markers, inhibitory receptors and intracellular regulators of receptor signaling, but also honed in on several novel naïve and memory states that standard analyses missed.

We also applied SLIDE in a range of other contexts including the study of immune cell partitioning by spatial localization from spatial transcriptomic data. SLIDE consistently outperformed benchmarks, and provided novel inference not afforded by other approaches. Thus, SLIDE is a novel versatile engine for biological discovery from modern multi-omic datasets.

HOW ARE THE GATEKEEPERS OF THE IMMUNE SYSTEM MAINTAINED THROUGHOUT LIFE?

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Strategically positioned as the gatekeepers between the circulation and the immune system, splenic marginal zone (MZ) B cells form a frontline of defense against blood-borne pathogens. They mediate early protective responses against diverse T-dependent and T-independent antigens, and their deficiency or functional impairment is linked to heightened susceptibility to sepsis and mortality related to encapsulated bacterial infections, and several autoimmune pathologies. Despite their importance, rules governing MZ B cell ontogeny, homeostasis, and their dynamics during immunogenic encounters, remain obscure. Here, we combine mathematical modeling with data derived from diverse experimental systems to quantify MZ B cell dynamics and clonal diversity, both at steady-state and during immune responses. Our analyses revealed that MZ B cells are a homogeneous population of slowly dividing cells that are continuously replenished by bone marrow-derived precursors. Further, modeling B cell differentiation during immune responses uncovered a novel Notch2-dependent pathway of MZ B cell generation from antigen-activated follicular B cells. Overall, these findings challenge the conventional view of an autonomously regulated MZ B cell compartment sustained by a high degree of self-renewal. Our results demonstrate a need for quantitative mapping of MZ B cell developmental trajectories during primary and memory responses, and for a comprehensive understanding of their establishment in early life.

PRE-VACCINATION CD56^{DIM} CD16+ NK CELL ABUNDANCE AND T_h1/T_h17 RATIO PREDICT RESPONSIVENESS TO CONJUGATED PNEUMOCOCCAL VACCINE IN OLDER ADULTS

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Older adults are at high risk of morbidity and mortality from *Streptococcus pneumoniae* (pneumococcus) infections. There are two available vaccines for pneumococcus: T-cell-independent capsular polysaccharide Pneumovax and T-cell-dependent conjugated Prevnar. However, it is not currently understood how older adults respond to these vaccines and if there are any baseline predictors for their responsiveness. To address this, we recruited older adults (>60+ years) who are vaccinated with the Prevnar (n=19) or Pneumovax(n=20) vaccine. Vaccine responsiveness was quantified using opsonophagocytic assays, which revealed that Prevnar induced marginally stronger response than pneumovax. Interestingly, sex was associated with Prevnar responses where women mounted stronger response than men. Pre-vaccination flow cytometry data showed that T_h1 cells positively and T_h17 cells negatively correlated with Prevnar responses. Furthermore, bulk RNA-seq data from PBMCs showed that baseline expression levels of cytotoxic genes (*NCAM1, GNLY, PRF1*) are negatively associated with Prevnar responses. scRNA-seq data from top responders and non-responders showed that this cytotoxicity signature stems from CD56^{dim} CD16+ NK cells, where having more of these cells are detrimental to responses. Interestingly, women had significantly higher T_h1, lower T_h17 and lower CD16+ NK cells compared to men, which explains their stronger Prevnar responses. This is the first study to uncover older adults' responses to two pneumococcal vaccines; we uncovered an activated immune phenotype of T_h and NK cell subsets that impedes responses to Prevnar. Interestingly, this phenotype only affected adjuvanted vaccine responses, providing an opportunity for precision vaccinology for pneumococcus disease.

IMMUNE SYSTEM PERTURBATIONS IN PATIENTS WITH SEVERE LONG COVID

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SARS-CoV-2 infections have different outcomes ranging from mild to severe acute infection as well as post-infectious multi-system inflammatory syndromes (MIS-C/A). Post-acute sequelae of COVID-19 (PASC) or long COVID is another outcome, which is poorly understood and range from mild to very severe persistent organ dysfunction and damages. To maximize our chances of identifying underlying mechanisms of long COVID we focus on the most severe cases with objective measures of disease such as lung damages, microvascular and autonomic nerve dysfunction. We are testing the following hypotheses that I) long COVID is a super-antigen mediated disease, II) an autoimmune condition driven by pathogenic autoantibodies, and/or III) a disease caused by viral persistence. A cohort of over 100 long COVID patients, 24 recovered long COVID patients as well as 9 COVID convalescent controls have been recruited thus far and blood samples analyzed for plasma proteome (Olink assays), immune cell composition and phenotypes (mass cytometry), T-cell repertoire (scTCR-seq), innate cell activation (circulating nucleosomes), and auto-antibody analyses (Luminex arrays) in order to understand immune perturbations in severe long COVID patients. Unlike children with hyperinflammatory MIS-C, we have not found any evidence of superantigen-mediated T-cell activation in long COVID, but instead substantial clonal expansion of memory T cell clones with effector memory states and shared clonality across patients. Additionally, cytokine perturbations such as elevated levels of IL8 and elevated levels of circulating nucleosomes indicate persistent activation of neutrophils in severe long COVID.

B CELL REPERTOIRE IN CHILDREN WITH SKIN BARRIER DYSFUNCTION SUPPORTS ALTERED IgE MATURATION ASSOCIATED WITH ALLERGIC FOOD SENSITIZATION

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The skin is a major immune organ and skin barrier dysfunction is a major risk factor for the development of the inappropriate immune response seen in allergic disease. Skin barrier disruption alters the landscape of antigens experienced by the immune system and the downstream impacts on the antibody repertoire remain poorly characterized, particularly for the IgE isotype responsible for allergic specificity and in early life when allergic disease is developing. In this study, we sequenced antibody gene repertoires from a large and well-characterized cohort of children with atopic dermatitis and found that food sensitization was associated with lower mutation frequencies in the IgE compartment. This trend was abrogated in children living with pets during the first year of life. These results elucidate potential molecular mechanisms underlying the protective effects of pet ownership and non-antiseptic environs reported for allergic disease and the hygiene hypothesis more broadly. We also observed increased IgE diversity and increased isotype-switching to the IgE compartment, suggesting that B cell development, particularly isotype-switching, is heavily altered in the those with food sensitizations relative to those without food sensitizations. Unlike sensitization to food antigens, aeroallergen sensitization exhibited no effect on IgE mutation or diversity. Consistent patterns of antibody rearrangement were associated with food sensitization in subjects with atopic dermatitis. Thus, we propose the Immune Repertoire in Atopic Disease (IRAD) score, to quantify this repertoire shift and to aid clinically in patient diagnosis and risk stratification.

SPATIAL PATTERNING ANALYSIS OF CELLULAR ENSEMBLES (SPACE): A GENERAL FRAMEWORK FOR CHARACTERIZING SPATIAL RELATIONSHIPS AMONG CELL TYPES IN TISSUES

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Immunological outcomes at the tissue scale emerge from local interactions among a variety of cell types. These local interactions, whether mediated by cellular contact or secreted signals, depend on the spatial arrangements and proximities of the involved cell types. Thus, spatial “ensembles” of cell types are crucial determinants of emergent tissue function, including immunity. However, quantitatively characterizing spatial patterns of cellular ensembles remains a challenge. As imaging techniques become increasingly high-plex (e.g. IBEX highly multiplex imaging), permitting detailed cell type and state identification (e.g. RAPID cellular phenotype learning), the set of observable spatial patterns grows combinatorically. Moreover, biologically relevant patterning occurs across a variety of spatial scales, from tissue-wide zonation to entanglements of just 2-3 cells. To address these challenges, we present a generalizable computational framework to discover and statistically validate cellular ensembles across scales in high-plex tissue images. This framework identifies spatial co-occurrence, mutual exclusion, and more complex patterns; it assesses patterns among pairs, triplets, quartets, and even larger groups of cell types; it distinguishes patterns that drive tissue architecture from patterns that are byproducts of it; and, it is compatible with spatial data captured and processed using a variety of modalities. We have validated this framework using lymph node images for which ground truth spatial patterning has already been defined, and we are now applying this framework to discover novel spatial patterns in new contexts, such as the tumor microenvironment. Rigorously identifying key spatial patterns of immune cell positioning in tissues will facilitate new insights into the emergence of immune outcomes.

This work was supported in part by the Intramural Research Program of NIAID, NIH.

STUDYING T-LYMPHOCYTE DYNAMICS IN DISEASE: INFERRING POPULATION REFERENCE FUNCTIONS NORMALIZING FOR NATURAL AGE-DEPENDENT CHANGES

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Lymphocyte numbers naturally change through age, but are typically measured as frequencies in blood samples. To allow one to quantify losses and gains of lymphocyte subsets in longitudinal data, such as the CD4+ T-cell decline in HIV-infected children, and/or the rate of T-cell recovery after the onset of treatment, requires reference functions accounting for these underlying age-dependent changes. These changes are most prominent in young children, but normalization functions are generally based on sparse measurements in healthy adults. In this study, we analyze cross-sectional measurements of T-cell populations (CD3+, CD3+CD4+ and CD3+CD8+, naïve and memory) in blood from 673 healthy Dutch individuals ranging from infancy to adulthood (0-62 years) with a special focus on children under one year of age. We described the trajectories of these measurements empirically and estimated parameters for each lymphocyte subset. Our modeling approach follows standard laboratory measurement procedures in which the size of a T-cell subset (expressed as cells per unit volume) is calculated by using its frequency within a reference population (typically total lymphocytes per unit volume) which is enumerated using a cell counter. Consequently, we obtain parameter estimates for each T-cell subset representing both the trajectories of their counts and percentages. We find that T-cell trajectories tend to increase during the first half a year of life before they decline exponentially approaching a homeostatic level in adulthood. The highly dynamic nature of these trajectories is often neglected in reference functions but is essential for quantifying T-cell dynamics across the human lifespan, in both health and disease.

INFERRING THE SINGLE-CELL TRANSCRIPTOME DYNAMICS OF MACROPHAGES REVEALS CONTEXT-DEPENDENT DYNAMICAL FEATURES THAT GENERATE HIGH STIMULUS-RESPONSE SPECIFICITY

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Macrophages respond in a context-dependent manner to appropriately neutralize immune threats, via the dynamic expression of hundreds of genes specific to the stimulus. However, technological limitations have precluded measurement of the heterogeneous dynamics of these response genes in single cells responding to stimuli. Here, to overcome this limitation we developed a method to reconstruct single-cell transcriptome trajectories in populations of responding macrophages, using time-point scRNAseq measurements. We then calculated dynamical features across hundreds of expression trajectories and found that specific combinations of gene expression dynamic features showed much greater stimulus-specificity than time-point measurements. Training statistical learning models on response dynamics, we also found that single-cell response dynamics were more accurate predictors of macrophage polarization state than either steady-state expression values or response measurements at any single time-point. Our findings point to the importance of transcriptome dynamics in both enabling highly stimulus-specific macrophage responses and revealing each cell's context-dependent functional state.

MODEL-AIDED DISSECTION OF THE INFLAMMATION-ASSOCIATED DYSREGULATION OF HEMATOPOIETIC PROGENITOR CELL FATE SPECIFICATION

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Aging, together with chronic low-grade inflammation ("inflammaging"), is associated with altered hematopoiesis resulting in increased myeloid cell and decreased lymphoid cell outputs, which increase infection susceptibility and reduce vaccine efficacy. It remains unclear, however, which hematopoietic stem and progenitor cells (HSPC) are affected by chronic inflammation and how their developmental dynamics are altered. To study hematopoietic dysregulation in inflammaging, we first formulate mathematical models of HSPC population dynamics to explore how perturbations in proliferation and differentiation parameters within HSPCs affect hematopoietic output. We then utilize a mouse model of NFκB-driven chronic inflammation to identify cell fate decision changes that are compatible with the observed myeloid expansion. We detect genes and pathways differentially expressed in HSPC subpopulations of this mouse model to identify the most likely dysregulated fate decisions and aim to further elucidate the molecular processes driving it. These processes could include cell-intrinsic elevated NFκB signaling within HSPCs, cell-extrinsic cytokine signaling originating from the bone marrow microenvironment, or both. Our studies aim to provide mechanistic insights about myeloid-biased hematopoiesis in inflammaging, and hence suggest strategies for therapeutic intervention.

NF-κB DYNAMICS ENCODE THE SPATIAL AND TEMPORAL INFORMATION OF LOCAL INFECTION AND IMMUNE CELLS

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Upon infection, local immune cells in tissue release multiple cytokines to alert nearby responding cells, which experience different cytokine level dynamics depending on their distance to the infection site and local immune cells, and the secretion profile by immune cells. To study how the inflammatory signaling network processes such spatially and temporally varying information, we simulated various infection scenarios by co-culturing macrophages and fibroblasts in a high-throughput microfluidic system that can form signal gradients, and monitored the dynamics of NF-κB, a central regulator of immune responses, in individual responding cells. Our early results indicate that the dynamics of NF-κB nuclear translocation encode spatiotemporally varying cytokine information. NF-κB dynamics not only carry information about the dose and duration of cytokine secretion, but also the distance to signal secreting cells. When we varied the concentration or distribution of macrophages in co-culture chambers, we observed that different NF-κB response patterns emerge, suggesting an additional capability of the NF-κB pathway to extract detailed information about tissue composition. Overall, our results demonstrate how the NF-κB network interprets various ligand dynamics to coordinate the inflammatory response during infection in complex environments.

LIVE BACTERIAL LIGANDS UNIVERSALLY REGULATE mTOR ACTIVITY BUT ARE NOT ESSENTIAL FOR HOMEOSTATIC INNATE IMMUNITY

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Multiple studies have reported significant impact of gut microbiota on hematopoiesis, including granulocyte development and functional defects. Germ-Free (GF) mice show developmental defects due to lack of live bacteria; underscored by the development of Altered Schaedler Flora (ASF), a community of 8 bacterial species sufficient to rescue developmental immune and gastrointestinal defects. Mimicking gnotobiosis by using antibiotics perturbs the microbiome systemically. Therefore, results from such studies may represent sequelae of the drug perturbation rather than simply modifying microbiota. We studied the impact of gut microbiota signals on murine hematopoiesis without antibiotics or fecal transfer. Namely, by using mice (from Taconic) generated by embryonic transfer of C57/Bl6 fertilized eggs into females with controlled microbiota, totaling 4 groups of mice: 1. GF; 2. ASF (no Gammaproteobacteria); 3. Excluded Flora (EF, includes Gammaproteobacteria); 4. WildR7 (wild mouse microbiome). We found no significant differences in either number or effector function of bone-marrow neutrophil granulocytes, myeloid progenitors, or stem cells. The single-cell genomic landscape of CD117+ bone-marrow progenitors confirmed flow analyses but revealed a profound defect in gnotobiotic hematopoietic signatures. Importantly, standard pathogen free C57/BL6 mice were inappropriate controls. While gnotobiotic mice likely receive inert TLR stimulation via bedding and food, the addition of any group of live bacteria was sufficient to rescue gene expression downstream of mTOR signaling. Such robust signatures could be considered so essential to life that they do not depend on any single bacterial clade.

HIGH-THROUGHPUT WORKFLOW LEVERAGING SPECTRAL FLOW CYTOMETRY AND CITE-SEQ TO CHARACTERIZE HUMAN BONE MARROW ACROSS RACE AND GENDER

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Characterizing human bone marrow is challenging due to heterogeneity of cell types, and the rarity of some populations. To address this, we developed a workflow that bridges CITE seq and flow cytometry. First, we rigorously titrated 277 TotalSeq-A antibodies (BioLegend) using Laminar Wash technology (Curiox Biosystems) to efficiently wash unbound antibodies in a hands-free manner and improve reproducibility. We resolved the utility of 133 TotalSeq-A antibodies (and 5 isotype controls) for CITE-seq of primary human bone marrow samples. We describe the application of these 133 markers in CITE seq of primary marrow samples (varying sex and race) to create a human bone marrow atlas with both transcriptional and antibody-based markers for over 70 cell states. Notably, we also discovered race-based differences in molecular markers and population frequencies. Next, we exploited a machine learning tool (pyInfinityFlow) to validate these molecular/informatics findings. We used a 22-color spectral flow panel tailored to human bone marrow (developed by Cytek Biosciences) as the backbone, then incorporated 111 PE-conjugated "Infinity markers" guided by the CITE-seq results. The analysis resolved a single final FCS file to co-visualize 133 markers, and provided concordance between CITE-seq values measured by sequencing and flow values measured by fluorescence intensity for marrow populations. In summary, we present a streamlined workflow widely adaptable to bone marrow disease studies and transferrable to other tissues. We deliver an atlas-level high-resolution reference with transcriptome-defined populations and immunophenotyping, enabled by both sequencing and spectral flow cytometry.

PD-L1 REGULATES INFLAMMATORY MACROPHAGE DEVELOPMENT FROM HUMAN PLURIPOTENT STEM CELLS BY MAINTAINING INTERFERON GAMMA SIGNALING

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PD-L1 (programmed death ligand 1) serves as a pivotal immune checkpoint in both the innate and adaptive immune systems. PD-L1 is expressed in macrophages in response to interferon gamma (IFNγ). We examined whether PD-L1 might regulate macrophage development. We established PD-L1-/- human pluripotent stem cells, differentiated them into macrophages, and observed a 60% reduction in CD11B+CD45+ macrophages in PD-L1-/-; this was orthogonally verified, with the PD-L1 inhibitor BMS-1166 reducing macrophages to the same fold. Single-cell RNA sequencing further confirmed the 60% reduction in macrophages as well as the downregulation of the macrophage-defining transcription factors SPI1, KLF6, and MAFB. Furthermore, PD-L1-/- macrophages reduced the level of inflammatory signals such as NFκB, TNF, and chemokines in the CXCL and CCL families. Anti-inflammatory TGF-β was upregulated. Finally, we identified that PD-L1-/- macrophages significantly downregulated interferon-stimulated genes (ISGs) despite the presence of IFNγ in the differentiation media. Mechanistically, PD-L1-/- macrophages reduced IFNGR1 expression, explaining why cells could not respond to IFNγ. These data suggest that PD-L1 regulates inflammatory macrophage development by maintaining IFNγ signaling.

PERSISTENT T CELL EXPANSIONS ARE FOUND IN THE PERIPHERAL BLOOD OF PATIENTS WITH PEDIATRIC SOLID TUMOR USING LONGITUDINAL SINGLE CELL PROFILING

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Background: Pediatric solid tumor is traditionally believed to be non-immunogenic, which leads to a lack of research on immunological changes on children with solid tumors. Consequently, children with solid tumors do not benefit from the development of immunotherapy in these years. Peripheral T cell expansion is an indicator of immune responses against cancer. By studying the longitudinal peripheral single cell profile, we aim to understand the immune response and find potential targets for immunotherapy of pediatric solid tumor.
Method: We collect 43 longitudinal PBMC samples from 8 patients with either osteosarcoma, Wilms tumor or neuroblastoma. Memory cells (CD45RO+) are sorted out with magnetic beads and then prepared for single cell T cell receptor (TCR) sequencing and targeted mRNA sequencing (259 genes T cell panel) using BD Rhapsody platform. Clonal expansion is identified as single cells with exact same CDR3 alpha and beta sequences. Virus specific clonal expansions are filtered out by clustering with TCR library of known specificity to common viruses. Single cell mRNA sequencing results are analyzed with Seurat pipeline.
Results: Massive clonal expansions are found mainly in CD8+ memory T cells beginning at different treatment steps with high individual heterogeneity. In this cohorts, 93.6% of the clonal expansions are not specific to common viruses. The TCR repertoires are also heterogeneous, with expanded public TCR only found in a maximum of 2 patients. Autologous stem cell transplantation leads to massive changes of the profile of expanded clones. Persistent clonal expansions alone do not correlate with good clinical outcomes. A transcriptomic signature about lymph node-blood-tissue migration is hypothesized to be a sign of successful immune response against solid tumor.
Conclusions: Persistent CD8 memory T cell expansions are found in the peripheral blood of patients with pediatric solid tumor. However, the relationship between peripheral T cell expansion and tumor eradication requires further research.

QUANTITATIVE DISSECTION OF T CELL AND CANCER CELL INTERACTIONS WITHIN THE TUMOR MICROENVIRONMENT

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In cancer, interactions between immune cells and cancer cells play a major role in nearly every step of carcinogenesis. Thus, the ability to genetically dissect immune cell-cancer cell interactions would facilitate mechanistic delineation of the role of the tumor microenvironment. Our initial work developed the GFP-based Touching Nexus (G-baToN) system which relies upon nanobody-directed fluorescent protein transfer to enable sensitive and specific labeling of T cell and cancer cell interactions. Here we described a new method, Anti-Tumor T cell Response Associated Colony Sequencing (ATTRAC-seq) to interrogate T cell and Cancer cell interactions within the tumor microenvironment in a multiplexed and quantitative manner. ATTRAC-seq enables high-throughput quantification of genotype- and tumor-size-dependent T cell and Cancer cell interactions in vivo by coupling G-baToN and tumor barcode sequencing. By combining ATTRAC-seq with multiplexed CRISPR/Cas9-mediated genome editing, we quantified the effects of 20 known and novel cancer immune regulators in T cell immune response in melanoma. ATTRAC-seq enables the broad quantification of the function of genetic drivers in cancer immune response and raises new possibilities for cancer treatment and prevention in adoptive immune therapy.

LIANA: A TOOL FOR 3D INTERACTION-BASED ANNOTATION OF DISTAL REGULATORY ELEMENTS TO GENES

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Transcriptional programs are orchestrated by a complex network of transcriptional regulators which exert gene regulatory function through binding to cis-regulatory elements (CREs). Considering the often distal location of these CREs relative to target genes, genome architecture emerges as another important factor in gene regulation, with chromatin loops enabling interaction between the elements and their target genes. Distal CREs are critical in lineage specific transcriptional regulation and with over 90% of disease- and trait associated variants located within non-coding regions of the genome, functional interpretation of CREs can also provide insights into disease mechanisms. However, identification of target genes to distal elements is not trivial and the ability to correctly annotate CREs using proximity-based methods, the current standard for assigning target genes to non-coding regions, is restricted by an upper distance limit for each gene, determined by the local gene density. This distance limit prevents identification of distal enhancers >100kb from TSS (low estimate of median enhancer-TSS distance) for the majority of genes using proximity annotation (or GREAT), highlighting the need for alternative approaches. With advancements in chromatin capture methods, the amount of available interaction data is steadily increasing and with that the potential to improve the functional interpretations of CREs and, in a cell type specific manner, assigning transcription factor occupancy and epigenetic features to genes. Despite this, the use of chromatin interaction in annotation is still limited, potentially related to the current need for manual processing and a lack of available tools.

To address this, LIANA was developed. LIANA uses a combination of interaction-based and conventional proximity-based annotation for improved accuracy of annotation of distal elements, while taking the resolution of interaction into consideration and allowing regions located near promoters to be annotated to the corresponding gene. To account for different types of data, LIANA provides several options, including the ability to identify and visualize co-occupancy of transcriptional regulators. Benchmarking LIANA against current annotation standards, revealed a superior performance of interaction-based annotation compared to proximity-based methods for identification of experimentally validated distal enhancers. In addition, interaction-based annotation enables the identification of enhancers with other genes located between the element and the target gene, representing a category of instances where proximity-based methods are inadequate. LIANA is available at: https://github.com/Tingvall/LIANA.

SYSTEMS-LEVEL ANALYSIS OF CO-STIMULATORY AND CO-INHIBITORY SIGNALING BETWEEN CD8+ T CELLS AND ANTIGEN-PRESENTING CELLS

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CD8+ T cells express a diverse repertoire of co-stimulatory and co-inhibitory receptors that, together with the ligands expressed on antigen-presenting cells (APCs), play important roles in modulating T cell receptor (TCR) signaling and T cell function. Analyzing multimodal single-cell profiles (CITE-seq) of peripheral blood mononuclear cells from multiple studies characterizing diverse human cohorts, we find that the expression of the well-known co-stimulatory and co-inhibitory receptors on CD8+ T cells is highly coordinated; the same is true for the expression of the corresponding ligands on different APC subsets, including monocytes and dendritic cells. Moreover, we observe correlation between the expression of receptors on CD8+ T cells and that of ligands on APCs across subjects. In response to influenza vaccination, the expression of the co-stimulatory / co-inhibitory receptors and ligands changes in a concordant fashion. We use mathematical modeling to show how such receptor-ligand expression levels can be established and maintained by certain signaling network motifs involved in the control of immune-related genes. Overall, we present a systems-level analysis of co-stimulatory / co-inhibitory CD8+ T cell signaling and describe a plausible immune set point sensitive to perturbations such as vaccination.

REGULATION OF NFκB cRel DYNAMICS IN B-CELLS MODULATES CLONAL EXPANSION AND SELECTION IN THE ANTIBODY REPERTOIRE

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B-cells produce an antibody repertoire by combining affinity-dependent selection signals with their intrinsic capacities to effect different fate choices. NFκB cRel transduces selection signals in B-cells, and induces the oncoproteins – cMyc, BclXL, and AID – that drive their proliferation, survival, and receptor mutation. Hence, the regulation of cRel is expected to exert complex emergent effects on the formation and properties of the B-cell repertoire. We developed a new fluorescent reporter mouse strain mTFP1-cRel, and found that steady-state cRel abundance is heterogeneously distributed in naïve B-cells, with the population enriched for high expressors. We modeled how cRel heterogeneity arises from balancing positive feedback by autoinduction with negative feedback by its inhibitor IκBe. Based on this, we used an IκBe$^{-/-}$ negative feedback mutant to perturb cRel dynamics and study the resulting functional effects on B-cell clonal expansion. We found that cRel activity *in vitro* drives rapid induction of cMyc but delayed expression of BclXL and AID, with this pattern of target gene expression resulting in two distinct phases of early proliferation and late survival. In the early phase, we showed that cRel abundance determines the intrinsic proliferative kinetics of B-cells *in vitro*. High-expressing cells are primed to divide faster, but at the cost of diminished proliferative capacity, which can modulate clonal expansion in the repertoire. In the late phase, undamped cRel activity increases BclXL for improved survival, and also raises AID activity. We extended these insights to *in vivo* responses by comparing repertoire sequences from immunized wild type and IκBe$^{-/-}$ mice. We correspondingly found smaller clonal families with excessive diversity in IκBe$^{-/-}$ mice, showing that appropriate negative regulation of cRel is required to sustain effective clonal selection. Thus, we demonstrate multi-scale dynamical effects in B-cell repertoire formation, where intracellular regulation of cRel determines the extent of clonal expansion and selection at the population level.

A VARIATIONAL DEEP-LEARNING APPROACH TO MODELING MEMORY T-CELL DYNAMICS

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Resident-memory T cells (TRM) provide direct immunological memory at the site of infection. As TRM specific for influenza A virus (IAV) in the lung target more conserved epitopes than neutralizing antibodies, they could potentially provide strain-transcending immunity. However, TRM in the lung are lost more rapidly than TRM in other tissues, making such protection short-lived. It also unclear whether there is ongoing differentiation within IAV-specific memory subsets after the resolution of infection. Using T-cell samples from mice infected with IAV, and sacrificed at varying times post infection, we seek to better characterize the phenotypic structure and dynamics of IAV-specific CD4 and CD8 memory T cells within the lung. We start with a more traditional, sequential work-flow in which single-cell flow-cytometry data is first aggregated into a time series using off-the-shelf unsupervised clustering and batch-correction methods, to which we then fit dynamical models in a Bayesian framework. However, we argue that such a sequential approach could introduce bias, and develop an integrated method in which dynamical model parameters and population structure is learned simultaneously. This method uses deep learning and stochastic variational inference and is trained on the single-cell flow-cytometry data directly. We find strong evidence for time-dependent loss rates of all T cell subsets in the lung between d9 and d70 post-infection. Effector cells are initially lost fastest, but on longer time scales, TRM that reside near the epithelial cells are lost at the highest rate. The integrated approach yields more precise parameter estimates than the sequential approach, and can distinguish T-cell populations that are phenotypically similar, but have diverging kinetics. Using the sequential method, we do not find strong statistical evidence for ongoing differentiation after the infection is cleared. However, the integrated approach suggests that central memory cells seed other compartments in the long run, consistent with findings from in vitro experiments with TRM from human skin.
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PROXIMITY SEQUENCING: A HIGHLY-MULTIPLEXED METHOD TO MEASURE EXTRACELLULAR PROTEINS, PROTEIN COMPLEXES, AND mRNA IN SINGLE CELLS

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Background: To maintain healthy function, cells must constantly respond to signals from their environment. In large part this information is processed through the formation and dissociation of protein complexes. Even for simple signals, the cell typically activates several signaling pathways involving hundreds of protein complexes. While there are many technologies that can measure mRNA and proteins from single cells, no single-cell technology can measure the hundreds of protein complexes relevant for cellular information processing. Current technology limits researchers to just a few protein complexes and often requires genetic manipulation. We sought to address this unmet technological need by developing a highly-multiplexed method that can simultaneously measure mRNA, protein, and protein complexes in single cells.
Methods: Custom DNA-functionalized antibodies were designed to couple a proximity ligation assay (PLA) to a DNA sequencing readout. In this design, prox-seq probes only generate measurable signal when their protein targets are close enough to allow for ligation of their DNA oligomers (50-70nm). Ligated oligomers can then be measured along with mRNA using commonly used single-cell sequencing methods such as Drop-seq, Chromium 10X, and plate-based methods. Finally, an end-to-end computational pipeline was developed to enable identification and relative quantification of protein complexes.
Results: Proximity sequencing dramatically increases the number of protein complexes that can be measured in single cells and can be readily applied to primary cells. We achieve proof-of-principle for protein complex detection by measuring the presence of several known protein complexes on the surface of individual Jurkat and Raji cells. We show that prox-seq can measure 741 potential protein complexes on the surface of single human peripheral blood mononuclear cells. Once again, we identify the presence of many known protein complexes. We also measure a novel complex between CD9 and CD8 on naïve, but not effector, CD8+ T cells. Finally, we show that proximity sequencing can be used to track the formation and dissociation of protein complexes during signaling in primary human macrophages stimulated with TLR ligands lipopolysaccharide (PLS) or Pam2CSK4 (PAM).
Acknowledgement: This research was supported in part by the Intramural Research Program of the NIH.

SINGLE-CELL NF-κB ACTIVATION DYNAMICS ENCODE INNATE IMMUNE MEMORY

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Sentinel cells respond to temporally evolving pathogenic and host signals. However, many of these stimuli converge on few intracellular networks. It is unclear how remodeling of these networks by prior history contextualizes subsequent signaling. Using microfluidics and live cell imaging, we examine the effects of innate immune memory on signaling in the canonical NF-κB network. We find that the identity, dose, and duration of the prior ligand reshape subsequent NF-κB response dynamics to produce both priming and tolerance. We show that, on a single-cell level, response to prior stimuli produces a deterministic effect on response to the subsequent stimulus. We use mathematical modeling to demonstrate how initial stochastic response variability can produce deterministic memory outcomes due to wiring of the NF-κB network. Furthermore, we find that these altered dynamics are encoded in alterations in the transcriptomic landscape. These results show that ligand-specific innate immune memory is encoded in NF-κB dynamics on a single cell level and gives rise to distinct phenotypic outcomes.

COMPREHENSIVE SINGLE CELL SEQUENCING ANALYSIS OF PAIRED TISSUE AND BLOOD SAMPLES FROM HCC PATIENTS TREATED WITH NEOADJUVANT ANTI-PD-1 THERAPY REVEALS TREATMENT-INDUCED CLONAL T CELL DYNAMICS

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Background: While immune checkpoint blockade (ICB) therapy has provided benefit to many cancer patients, the characteristics of anti-tumor T cell responses in non-tumor tissues and in circulation are not fully understood. Neoadjuvant ICB therapy provides a unique opportunity to deeply characterize pre- and post-therapy biomarker samples and identify features of drug activity across matched patient tissues. In a single-arm, open label phase 2 study, 21 patients with resectable (stage Ib, II, and IIIb) hepatocellular carcinoma (HCC) were treated with anti-PD-1 antibody, cemiplimab, in the neoadjuvant setting1. Patients were treated with two cycles of cemiplimab (350 mg Q3W) prior to resection (median time to resection = 29 days), and 6 of 20 patients who successfully underwent surgical resection has evidence of pathological response to therapy (Responders). **Methods**: Single cell RNA (scRNA) and T cell receptor (TCR) sequencing were performed on tumor, normal adjacent tissues (NAT), tumor draining lymph node (tdLN), and blood from 20 patients at the time of resection. Additionally, blood samples from many patients were analyzed by scRNA- and TCR- sequencing at baseline, during neoadjuvant therapy, at resection, and during adjuvant therapy. The characteristics of all tumor-expanded T cells were compared across tissues and longitudinally in the periphery. **Results**: We stratified patient tumors based on degree of T cell infiltration at surgery and identified several populations of PD-1high CD8+ T cells that were enriched in patient tumors compared to other tissues. Responder tumors were enriched with PD-1high Effector CD8+ T cells that expressed high levels of CXCL13 and markers of cytotoxic activity. In contrast, Non-responder tumors contained larger fractions of PD-1high terminally differentiated T cells that expressed multiple T cell dysfunction markers including KLRB1. Using TCR sequence as a fingerprint, we tracked tumor TCR clones across tissues, including longitudinal blood samples. Many of the most clonally expanded TCRs identified in Responders' resection tumors were present in baseline tumor biopsies. However, we also identified expansions of de novo T cell clones that were not detected in baseline biopsies, suggesting that both pre-existing and de novo T cell clones expanded in tumors following anti-PD-1 therapy. We also observed significant tumor-expanded TCR sharing across the tdLN, NAT, and blood at the time of resection in patients who responded to therapy. Finally, tumor expanded TCRs were identified in the circulation across treatment timepoints and were more expanded in the circulation of Responders. **Conclusions**: In conclusion, we identified several populations of CD8+ PD-1high T cells that were expanded in the tumors of patients who responded to cemiplimab. Both pre- existing and de novo T cell clones were expanded in the tumor at the time of resection, and many of these clones were identified in matched NAT and tdLN, particularly in Responders. Our study also suggests that tumor-expanded T cells are frequently found in the periphery, and peripheral expansion might correlate with response to neoadjuvant anti-PD-1 therapy.

AN ATLAS OF GENE REGULATORY NETWORKS FOR T MEMORY CELLS IN YOUTH AND OLD AGE

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Aging profoundly affects immune responses, rendering the elderly more susceptible to pathogens, cancers and chronic inflammation. Single-cell (sc)genomics studies have accelerated the discovery of age-dependent immune-cell populations, linking aging phenotypes to changes in diverse immune populations. We previously identified a population of IL-10-producing, T follicular helper-like cells ("Tfh10"), linked to suppressed vaccine responses in aged mice. Here, we use sc-genomics and genome-scale modeling to characterize Tfh10 – and the full $CD4^+$ memory T cell ($CD4^+$TM) compartment – in young and old mice. Unprecedented scRNA-seq coverage of the $CD4^+$TM compartment and parallel chromatin accessibility measurements (scATAC-seq) enabled identification of 13 $CD4^+$TM populations, which we validated through comprehensive cross-comparison to aging cell atlases and scRNA-seq studies reporting Tfh10 in other contexts. Beyond robust characterization of age- and cell-type-dependent transcriptional landscapes, we used integrative computational modeling to predict the underlying regulatory mechanisms: We inferred gene regulatory networks (GRNs) that describe transcription-factor (TF) control of gene expression in each T-cell population and how these circuits change with age. Among thousands of predictions: We identified TFs with increased activity across all $CD4^+$TM populations (e.g., ZBTB7A, ZFP773) and others unique to specific populations (e.g., STAT3, NFIA, ARNT2 in Tfh). Consistent with previous work in other IL-10-producing $CD4^+$TM populations, we predict that a PRDM1 (Blimp-1) and MAF circuit is responsible for IL-10 production in Tfh10 and that this circuit is age-dependent. Our GRN analyses also suggest that altered regulation of apoptosis genes contributes to Tfh10 accrual in old age. Furthermore, we integrated our data with prior, pan-cell scRNA-seq studies to identify intercellular-signaling networks driving age-dependent changes in $CD4^+$TM, discovering known (e.g., IL-6, CD30, osteopontin) and novel (e.g., NRP1, CAM) age-dependent cell-cell cross-talks. Our atlas of finely resolved $CD4^+$TM subsets, GRNs and cell-cell communication networks is a critical resource for analysis of biologic processes operative in memory T cells in youth and old age. The resource presents new opportunities to manipulate regulatory circuits in $CD4^+$TM, which, long-term, could lead to improved immune responses in the elderly.

A STIMULUS-CONTINGENT POSITIVE FEEDBACK LOOP ENABLES IFN-β DOSE-DEPENDENT ACTIVATION OF PRO-INFLAMMATORY GENES

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Interferon (IFN)-mediated immunity is a vital component of the innate immune response initiated during pathogenic exposure. Type I IFNs induce powerful anti-viral responses via the activation of IFN stimulated genes (ISGs) by the transcription factor, IFN-stimulated gene factor (ISGF3). In some pathological contexts type I IFNs are responsible for exacerbating inflammation. While the IFN-mediated cell-intrinsic anti-viral response is well known, it is less clear how IFNs may contribute to the cell-extrinsic inflammatory response. We hypothesized that IFN type-specific functions may be the result of IFN type-specific control of ISGF3 dynamics; hence we sought to develop a quantitative understanding of the mechanisms that control ISGF3 activity.

Using quantitative biochemical assays and next generation sequencing, we found that a high dose of IFN-β activates an anti-viral and inflammatory gene expression program in contrast to IFN-λ3, a type III IFN, which elicits only the common anti-viral gene program. We show that the inflammatory gene program depends on a second, potentiated phase in ISGF3 activation. Iterating between mathematical modeling and experimental analysis we show that the ISGF3 activation network may engage a positive feedback loop with its subunits IRF9 and STAT2. This network motif mediates stimulus-specific ISGF3 dynamics that are dependent on ligand, dose, and duration of exposure, and when engaged activates the inflammatory gene expression program.

Our results reveal a previously underappreciated dynamical control of the JAK-STAT/IRF signaling network that may produce distinct biological responses, and suggest that studies of type I IFN dysregulation, and in turn therapeutic remedies, may focus on feedback regulators within it.

THE EFFECT OF FINGOLIMOD ON CIRCULATING B CELLS IN CYNOMOLGUS MACAQUE

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Fingolimod (FTY720), an antagonist of the sphingosine 1-phosphate receptor, is an immunosuppressive drug that modulates the egress of lymphocytes from lymph nodes, resulting in decreased circulating T cells in the blood. However, their effect on B cells, including their subsets, has not been elucidated. Here, we investigated the B cell-related humoral immune response kinetics in macaques treated with daily doses of FTY720 for 7 days. All the B cell subsets, including naïve B cells, activated naïve B cells, resting naïve B cells, activated memory B cells, and plasmablasts, significantly decreased after a day of FTY720 treatment and were consistently low during the treatment. T cell subsets, including naïve and memory T cells, except for effector memory T cells, were significantly decreased. Interestingly, no substantial changes were observed in the circulating, total immunoglobulin (Ig) M and IgG levels. These findings indicate the role of FTY720 in promoting the migration of specific circulating lymphocytes and could help develop T and B cell depletion therapies with pathogen control in acute infectious diseases, such as influenza and severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 mRNA VACCINATION INDUCES B CELL RESPONSES IN THE TONSILS AND ADENOIDS OF CHILDREN

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We recently reported that SARS-CoV-2 infection triggers robust, persistent adaptive immune responses in the tonsils and adenoids of children. However, whether mRNA vaccination induces immune responses in these upper respiratory tract tissues is unknown. To evaluate these questions, we collected peripheral blood (PBMCs), tonsils, and adenoids from 21 children undergoing tonsillectomy/adenoidectomy in 2022 who had previously received a COVID-19 vaccination for in-depth multiparameter evaluation. From questionnaires and serology, we identified 10 vaccinated subjects (Vac) who had not been infected and compared them to 24 COVID-19 convalescent children (Inf) from our previous study collected in 2020-2021. We found that Vac children had higher neutralizing titers to WA-1 and Omicron (BA.1) compared to Inf children. We identified SARS-CoV-2-specific B cells in the tonsils and adenoids of nearly all Vac and Inf subjects, including cells recognizing the spike protein receptor binding domain (RBD) of the original WA-1 strain (S1+RBD+) and from Omicron. Furthermore, we found that a few Vac subjects had SARS-CoV-2-specific germinal center B cells in either the tonsil or adenoid. PBMCs trended towards having higher percentages of S1+RBD+ B cells post-vaccination than post-infection. Unsupervised analyses of the high dimensional flow cytometry data revealed differences in the characteristics of SARS-CoV-2-specific B cells post-vaccination and post-infection; S1+RBD+ B cells from Inf subjects had greater proportions of CXCR3+IgA+ memory B cells in the tissues and blood, while those from Vac subjects had more double negative (IgD-CD27-) atypical memory B cells in the blood, implying a greater extrafollicular response in the blood post-vaccination but stronger mucosal IgA and IFN-γ induced humoral responses post-infection. Our results provide evidence for tissue-specific immunity to SARS-CoV-2 in the upper respiratory tract tissue of children after mRNA vaccination, but also demonstrate that vaccination induces B cell phenotypes distinct from that of natural infection which may affect the quality and duration of immunity.

SINGLE-CELL GENOMICS STUDY OF AUTOIMMUNE LIVER DISEASE PATHOGENESIS

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Background: Primary sclerosing cholangitis (PSC) is a slowly progressive destruction of the biliary tree which commonly requires liver transplantation for complications of biliary fibrosis and cholangiocarcinoma. Development of targeted therapies is hampered by our incomplete understanding of the crosstalk between immune cells, bile duct epithelial cells, and stellate cells.
Methods: To understand the early pathological changes and identify contributing cell types and molecular mechanisms, we leveraged the established chronic cholestatic mouse model of 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (DDC) feeding that recapitulates hallmarks of PSC. We designed a perturbation time course that included two phases: DDC-induced liver injury 0,7,14 days and post-injury recovery for additional 7, 14, 28 days. We applied sc-genomics (sc-RNA-seq and scATAC-seq) to resolve the cell type-specific responses at genome scale. For validation, we performed single-cell multiome-seq, an assay that simultaneously measures gene expression and chromatin accessibility in single nuclei, on liver tissue samples from patients with early PSC (n=2), end stage disease (n=3), and from healthy controls (n=2).
Results: 22 major epithelial and immune cell types were identified from the scRNA-seq in the DDC mouse model. Cell-cell communication network analysis identified increased intercellular signals Platelet-derived growth factor (PDGF) from macrophage to hepatic stellate cells, a well-established pathway of liver fibrosis. Integrating scATAC-seq with scRNA-seq to refine cell type annotations and predict transcription factor binding we modelled transcriptional regulatory networks (TRN) of specific cell populations. TRNs help pinpoint the context- and cell-type-specific transcription factor regulators of gene expression patterns. From our CD4+ T cell network, we identified a regulatory T cell core that recovered known regulator Foxp3 and predicted Th17 polarization at peak injury (Day 14). Multiome seq data from human samples recovered seven major cell types, including epithelial, myeloid, and immune cells. Current studies investigate which of the TRN and cell-cell communications detected in murine sclerosing cholangitis are operational in early and late PSC.
Conclusions: Integrating longitudinal scRNAseq and scATACseq data from experimental sclerosing cholangitis with sc genomics of PSC patients may help to identify TRN and cellular crosstalks of immune mediated hepatobiliary injury which are conserved across species and can be targeted with novel therapeutics.

INVESTIGATING THE TNFα – IL-10 IMMUNE REGULATORY FEEDBACK DYNAMICS IN THP1 MACROPHAGES.

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Macrophages have been extensively shown to exhibit heterogeneity in the immune response, both at the transcriptional level, protein level, and secretion level. Following the Toll-like receptor 4 (TLR4) stimulation of macrophages, secretion of Tumor necrosis factor alpha (TNFa) drives the proinflammatory response in a positive feedback mechanism, while negative feedback is mediated by the anti-inflammatory cytokine interleukin-10 (IL-10), which limits the overall inflammatory response. Extensive cell-to-cell variability in the TLR4-stimulated cytokine secretion raises questions about how positive and negative feedback is robustly implemented during inflammation. We hypothesize that non-genetic cell-to-cell heterogeneity is perhaps a strategy that macrophages incorporate, in order to elicit their complex functional responses for optimal regulation of the inflammatory response. Previously, our lab characterized the TLR4-stimulated secretion program in primary murine macrophages using a single-cell microwell assay that enables evaluation of functional autocrine IL-10 signaling. High-dimensional analysis of single-cell data reveals three tiers of TLR4-induced proinflammatory activation based on levels of cytokine secretion. Surprisingly, while IL-10 inhibits TLR4-induced activation in the highest tier, it also contributes to the TLR4-induced activation threshold by regulating which cells transition from non-secreting to secreting states. Currently, we are characterizing the feedback dynamics of THP1 human mononuclear cells differentiated into macrophages, stimulated with lipopolysaccharide to elicit the TLR4 response to see if thresholding of the TLR4-activation is also conserved in human cell line. Emphasis was put on cell plating density to investigate the effects that quorum sensing-like activation has on cytokine signaling in macrophages. We have found that at high concentrations of stimulation, TLR4 activation is not cell density dependent, nor is it dependent on the variability of cytokine secretion; while at lower concentrations of stimulation, variability of cytokine secretion and quorum sensing-like activation may be at play. We anticipate on implementing these findings into a mathematical model that simulates inflammation spatiotemporally to further direct our research surrounding this TNFa-IL-10 regulatory feedback mechanism.

YELLOW FEVER VACCINATION AS A MODEL TO STUDY VARIABILITY IN IMMUNE RESPONSES TO RNA VIRAL INFECTIONS

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Vaccination with the live-attenuated yellow fever vaccine (YF17D) activates innate and adaptive immune responses resulting in life-long immune protection. Infection with YF17D thus provides an attractive model, which allows uncovering mechanisms of anti-viral immunity and identifying biomarkers that stratify patients in terms of their immune reactions. Sensing of viral particles by pattern recognition receptors (PRR) leads to the activation of antigen-presenting cells (APCs), elicitation of pro-inflammatory cytokines and chemokines, and the stimulation of a long-lasting adaptive immune response. However, the exact mechanism of this immune activation as well as the contribution of different factors including genetics, epigenetics, microbiome, or lifestyle, which modulate and alter the response across individuals is still poorly understood.

In this study, we have collected bio-samples from a cohort of 250 volunteers taken before the vaccination and then successively 3, 7, 14, and 28 days after the vaccination, which were analyzed using high-throughput, multi-omics approaches.

Infection with YF17D lead to differences in immune cell composition and pro-inflammatory cytokine production after the vaccination. Out of 21 cytokines measured using the Bio-Plex Luminex-100 system, 18 fell into the detectable range and were significantly induced on day 7. The profile of cytokine response on day 7 showed the greatest variability in CCL22, CXCL11, CXCL13, RANTES, and PDGF-bb levels across individuals modulated by host factors such as sex and genetic polymorphisms. To identify the genetic loci that influence cytokine levels upon YF17D vaccination, genotype data generated with the Illumina global screening array have been evaluated for associations with cytokine levels. We identified interesting trans-QTL candidates in the intergenic region (rs4385748) and in the BRE/FOSL2 locus (rs56048418), which influence CCL22, and CXCL11 levels.

MULTIMODAL CHARACTERIZATION OF ANTIGEN-SPECIFIC CD8+ T CELLS ACROSS SARS-COV-2 VACCINATION AND INFECTION

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The human immune response to SARS-CoV-2 antigen after infection or vaccination is defined by the durable production of antibodies and T cells. Population-based monitoring typically focuses on antibody titer, but there is a need for improved characterization and quantification of T cell responses. Here, we utilize multimodal sequencing technologies to perform a longitudinal analysis of circulating human leukocytes collected before and after BNT162b2 immunization. Our data reveal distinct subpopulations of $CD8^+$ T cells which reliably appear 28 days after prime vaccination (7 days post boost). Using a suite of cross-modality integration tools, we define their transcriptome, accessible chromatin landscape, and immunophenotype, and identify unique biomarkers within each modality. By leveraging DNA-oligo-tagged peptide-MHC multimers and T cell receptor sequencing, we demonstrate that this vaccine-induced population is SARS-CoV-2 antigen-specific and capable of rapid clonal expansion. Moreover, we also identify these $CD8^+$ populations in scRNA-seq datasets from COVID-19 patients and find that their relative frequency and differentiation outcomes are predictive of subsequent clinical outcomes. Our work contributes to our understanding of T cell immunity, and highlights the potential for integrative and multimodal analysis to characterize rare cell populations.

METATIME: META-COMPONENTS OF THE TUMOR IMMUNE MICROENVIRONMENT

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Recent advances in single-cell RNA sequencing have revealed heterogeneous cell types and gene expression states in the non-cancerous cells in tumors. The integration of multiple scRNA-seq datasets across tumors can reveal common cell types and states in the tumor microenvironment (TME). We developed a data driven framework, MetaTiME, to overcome the limitations in resolution and consistency that result from manual labelling using known gene markers. Using millions of TME single cells, MetaTiME uses machine learning to learn meta-components that encode independent components of gene expression observed in TME across cancer types. The meta-components are biologically interpretable as cell types, cell states, and signaling activities. New insights on TME are gained: first, investigation of each meta-component reveal co-expressed gene modules and help identify known and potential cancer immunology regulators. Second, the meta-components provide functionally distinct gene programs and reveal TME cell states with higher granularity. Third, leveraging epigenetics data, MetaTiME reveals critical transcriptional regulators for the cell states. Overall, MetaTiME learns data-driven meta-components that depict cellular states and gene regulators for tumor immunity and cancer immunotherapy. Finally, by projecting onto the MetaTiME space, we provide a tool to annotate cell states and signature continuums for TME scRNA-seq data.

SYSTEMATIC QUANTIFICATION OF IL-1-INDUCED HUMAN BLOOD RESPONSE OF IMMUNE CELLS WITH SINGLE-CELL MULTI-OMICS TO PREDICT IMMUNOTHERAPY PROGNOSIS

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The cytokine interleukin-1 (IL-1) family and its associated inflammasome pathway have been found to play an essential role in multiple immune diseases. In cancer, there is an association between a macrophage state that enriches the IL1B gene module and response to immune checkpoint blockade inhibitors (ICB) in cancer immunotherapy. However, a systematic understanding of how gene expression and regulatory networks change in a cell type-specific manner upon IL1B stimulation needs to be improved. This study systematically quantified the IL1B-induced response in Peripheral Blood Mononuclear Cells (PBMC) using scRNASeq and scATACSeq to measure gene expression and chromatin accessibility with single-cell resolution directly. We used an autoencoder-based machine learning method to integrate multiple single-cell multi-omic datasets and computationally quantify the expression changes and chromatin modifications. Our results showed different association to immunotherapy outcome of myeloid and lymphoid changes in gene expression and chromatin accessibility upon stimulus, as well as cell type-specific inter-cellular interactions through cytokine-receptor communications induced by IL1B treatment. This study develops a computational framework to quantify multi-omic changes in a dynamic immune system and provides insights on gene regulatory networks and cell interaction network rewiring upon cytokine stimulus.

USING PREDICTED IMMUNOPEPTIDOMICS TO TARGET ANTIGEN-SPECIFIC T CELL RECEPTOR REPERTOIRES

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Transplantation provides life-saving therapy for patients with end-stage organ failure. Targeting antigen-specific T cells is important for managing transplant rejection, both to assess disease progression and to develop therapeutic interventions. In contrast with viral immunology and increasingly, autoimmunity and cancer, the field of transplantation has been held back by the inability to detect T-cell epitopes and T-cell receptors (TCRs) involved in alloimmunity. Here we seek to close this gap by developing a robust system of computationally predicted T-cell immunopeptidomics, functional CD4+T cell epitope mapping, and grouping of antigen-specific TCR sequences into similar clusters in murine and human models.

To address this, we developed a computational platform to predict potential MHC-restricted epitopes recognized by CD4+T cells. To identify whether any peptide represented an immunodominant epitope, we used IFNγ ELISpot to experimentally map CD4+T cell reactivity to each predicted peptide. In the murine model of fully MHC mismatched rejecting mice, we discovered four immunodominant epitopes that resulted in robust IFNγ production. Peptide-conjugated tetramers were used to confirm the presence of antigen-specific CD4+T cells in the blood and draining lymph nodes of rejecting mice. TCR αβ sequencing of sorted tetramer-positive CD4+T cells demonstrated 112 public clones overlapped considerably in the TCR repertoire of rejecting mice (4 of 5) and were absent in naïve mice (5 of 5). Further TCR similarity analysis led to the detection of 13 different families with similar CDR3β sequences that we define as quasi-public TCR clones enriched within and across mice. To expand these analyses, we translated the same epitope prediction method into the human model and found one CD4+T cell-activating immunodominant epitope from antigen-stimulated PBMCs of three donors. TCRβ sequencing revealed no public clones shared among donors, but 3 families of quasi-public clones with similar CDR3β sequences. Overall, we demonstrate our system could be used to detect and characterize antigen-specific CD4+T cells both in murine and human models. Together, these results have implications for establishing a computational epitope design that expands our ability to target T cells from individual public clones to larger groups of quasi-public TCRs at the population-level in cross-disciplinary areas.

SINGLE CELL CHARACTERIZATION OF IMMUNE MEDIATED AUTOIMMUNITY IN PARKINSON'S DISEASE.

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Rapid eye movement (REM) Sleep Behavior Disorder (RBD) is a parasomnia characterized by dream enactment and loss of normal skeletal muscle REM atonia1, and it is a clinical marker for faster cognitive decline and motor progression in Parkinson's disease (PD) with up to 50% of people with RBD will develop PD2. While neuroinflammation has been implicated in PD, the immune system's role in the initiation and progression of the disease process remains unknown. Here, we sought to characterize immune perturbations in preclinical and early stages of PD by integration of paired single cell RNA sequencing (scRNA-seq) and single cell T cell receptor (scTCR-seq) data in peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid (CSF) cells from RBD patients, PD patients, PD patients with RBD (PDRBD) and age- and sex-matched healthy controls. Our data showed higher LRRK2 expression in CD14+ and CD16+ monocytes in the blood of RBD patients compared to controls, as well as in CSF myeloid cell populations, suggesting an increased probability of developing PD. From CSF samples, we identified five major myeloid cell populations with distinct transcriptional profiles. Specifically, myeloid cell population 1, which had a significantly higher proportion in RBD, PD, and PDRBD, compared to controls, showed higher level of complement genes such as C1QA, C1QB, and C1QC, as well as risk genes for Alzheimer's disease including APOE and TREM2. Further, to investigate T cell clonal expansion, we utilized scTCR-seq data and discovered over 65% of clonally expanded T cells in CSF were CD4 T cells, whereas around 77% of clonally expanded T cells in blood were CD8 T cells. After performing differential expression analysis between expanded T cells versus unexpanded T cells, we found clonally expanded CD4 T cells expressed higher levels of cytotoxicity genes such as CCL5, CCL4, and CST7 in RBD and PD. Together, our results provide a high-resolution view of immune landscape across the prodromal and early stages of PD, and future studies will reveal the gut-to-brain immune processes leading to a-synuclein autoimmunity in patients with PD.

IDENTIFYING DYSREGULATED MONOCYTES IN ME/CFS PATIENTS USING SINGLE CELL RNA-SEQ PROFILES

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Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS) is a serious disease characterized by diverse symptoms, including post-exertional malaise, impacting an estimated 65 million individuals worldwide. The disease is poorly understood, without known cause(s), diagnostic test, or FDA-approved treatment. Multiple lines of evidence suggest the role of immune system in the disease. Moreover, studies have found evidence showing dysregulation of homeostasis of the immune system in patients as well as changes in inflammation state. Furthermore, Long Covid, a chronic post-infectious condition, shares many symptoms with ME/CFS, which suggests an overlapping molecular basis. However, despite a multitude of studies examining different aspects of the immune system, there is currently no consensus on which components are most relevant to the disease.

To systematically investigate immune dysregulation in ME/CFS, we performed single-cell RNA-seq on immune cells in peripheral blood mononuclear cells of ME/CFS patients and controls. To identify dysregulated immune cells in patients, we performed clustering, identified immune cell types, and did differential expression comparing patients and controls for each type of immune cells respectively. Our analysis revealed that monocytes, a type of leukocyte, exhibited the strongest signals of dysregulation in patients. To examine monocytes dysregulation more closely, we performed bulk RNA-seq on purified monocytes. We observed extensive variation of monocytes among patients then within controls, which suggest a varied and heterogenous population of monocytes in patients, in contrast to a more consistent and homogenous population in controls.

To investigate the heterogeneity of monocytes in patients, we utilized a machine learning approach, positive unlabeled learning, on our single cell RNA-seq dataset, which accommodates mixed populations. Specifically, we constructed a classifier to group monocytes in patients as diseased or normal, given whether the cells possess similar features as monocytes in controls. The subsets of monocytes predicted as diseased in patients were shown to be more related to one another than those partitioned by disease status, sex, or the individual identity. Notably, we observed a correlation between the fraction of monocytes predicted as diseased for each patient and disease severity metrics. Finally, to examine the dysregulation in predicted diseased cells, we performed differential expression between predicted diseased and normal cells. We found that the predicted diseased cells upregulate expression of specific cytokines, suggesting aberrant monocyte recruitment to tissue that may result in local inflammation. Taken together, our data suggests that one aspect of ME/CFS pathology involves improper activation of a subset of monocytes.

IDENTIFICATION OF KEY TRANSCRIPTION FACTORS THAT SPECIFY DIFFERENT NAÏVE CD8+ T CELL SUBSETS

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The immune system protects the human body against infection and disease. In the adaptive immune system, T cells are a core component that both eliminate foreign pathogens and shape immune responses. CD8+ T cells, a major class of T cells, recognize and kill cells infected with intracellular bacteria or viruses, as well as cancer cells. Upon stimulation, naïve CD8+ T cells proliferate and differentiate into effector cells. We and others have shown that the population of naïve CD8+ T cells is heterogenous, with subsets of different origins, dynamics, and functional properties. For example, in the naïve CD8+ T cell population, a subpopulation exists that bears markers and certain traits of memory cells, which are referred to as 'Virtual Memory' cells (VM), as opposed to those traditionally defined as True Naïve (TN) cells. In addition, naïve T cells with fetal or adult stem-cell origins show distinct gene regulatory profiles and immune phenotypes. Interestingly, virtual memory cells and naïve cells of fetal stem-cell origin mount a more rapid response upon infection, compared to true naïve cells and cells of adult stem-cell origin. Importantly, our data has indicated that regulatory changes in the naïve cells establish the fast- or slow-acting response that manifests later in infection. Hence, elucidating transcriptional regulation within the naïve pool can lead to a better understanding of effector responses with distinct attributes post-infection.

Regulatory networks inferred with genome-scale measurements can provide a broad view of complex regulation. To investigate the transcriptional regulatory network in the naïve CD8+ T cells repertoire, we applied a cutting-edge network inference algorithm, called Inferelator, on genome-wide expression profiles of diverse naïve CD8+ T cell subsets. Using this approach, we identified key Transcription Factors (TFs) regulating each subpopulation of the naïve cell pool, as well as the TFs' putative targets. Identified TFs promoting the fast-acting phenotypes include well-studied regulators that control effector differentiation, including for example, Irf8 and Stat3. TFs typifying slow-acting phenotypes also include factors known to regulate memory functions, (e.g., Foxo1 and Bcl6). Notably, our network analysis also uncovered TFs that have not yet been associated with CD8+ T cells, such as Atf3, which is known to promote differentiation of human T-helper 1 cells. To validate the functions of certain TFs, we are currently generating knockout mouse models to assess their immune phenotypes. In conclusion, our work can not only uncover novel factors contributing to T cells responses, but also shed light on the mechanisms of how naïve T cells with different origins and functions shape immune responses.

CD8+ T CELL DIFFERENTIATION AND ACTIVATION REPRESENT DISTINCT AXES OF DIVERSIFICATION

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Priming of naïve T cells leads to their activation and differentiation into functionally distinct states. During the active response, the effector phase, the antigen-specific T cell population consists primarily of highly activated, highly differentiated 'effector' T cells with effector function. After antigen clearance, most antigen-specific T cells die, and the response enters the memory phase, which is dominated by resting, long-lived, minimally differentiated, stem-like 'memory' T cells. Although at the T cell population level, T cell activation and differentiation shift concurrently from low in naïve cells to high in the effector phase and back to low in the memory phase, it is unclear whether both processes are molecularly linked. We hypothesized that this confuses interpretation of the lineage relationship of effector and memory T cells.

By single-cell mRNA sequencing of virus-specific CD8+ T cells of a range of differentiation states from the effector and memory phases of an acute viral infection, we showed that T cell differentiation and activation status are molecularly distinct axes of T cell diversification. We identified an expression signature that classifies CD8+ T cells, irrespective of the T cell response phase, by their graded differentiation state. Additionally, we identified an expression signature that distinguishes cells in the effector and memory phases, irrespective of the cells' differentiation state. This phase signature was driven by genes related to recent TCR activation and apoptosis, and is a measure of T cell activation state. Translation of the transcriptional signatures to protein signatures allowed simple flowcytometry-based distinction of CD8+ T cells according to their differentiation and activation states. Temporal tracking of virus-specific T cells showed that while the differentiation state of the virus-specific population shifted gradually, the activation state sharply decreased after antigen clearance. Disentanglement of T cell activation status and differentiation furthermore revealed that all primed T cells, including the minimally-differentiated stem-like cells passed through a state of high activation during the effector phase.

In summary, we show that T cell activation and differentiation are molecularly distinct axes of T cell diversification, and provide transcriptional and flow cytometry applicable tools to determine the differentiation and activation state of individual T cells.

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VISITOR INFORMATION

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

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

GENERAL INFORMATION

Meetings & Courses Main Office

Hours during meetings: 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
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Use PIN# (On your registration envelope) to enter Library after hours.
See Library staff for photocopier code.

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

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.

CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

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

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

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

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

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

Similarly, meeting participants agree to refrain from:

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

2. Sexual harassment or misconduct.

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

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

DEFINITIONS AND EXAMPLES

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

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

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

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

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

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

REPORTING BREACHES OR VIOLATIONS

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

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

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

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

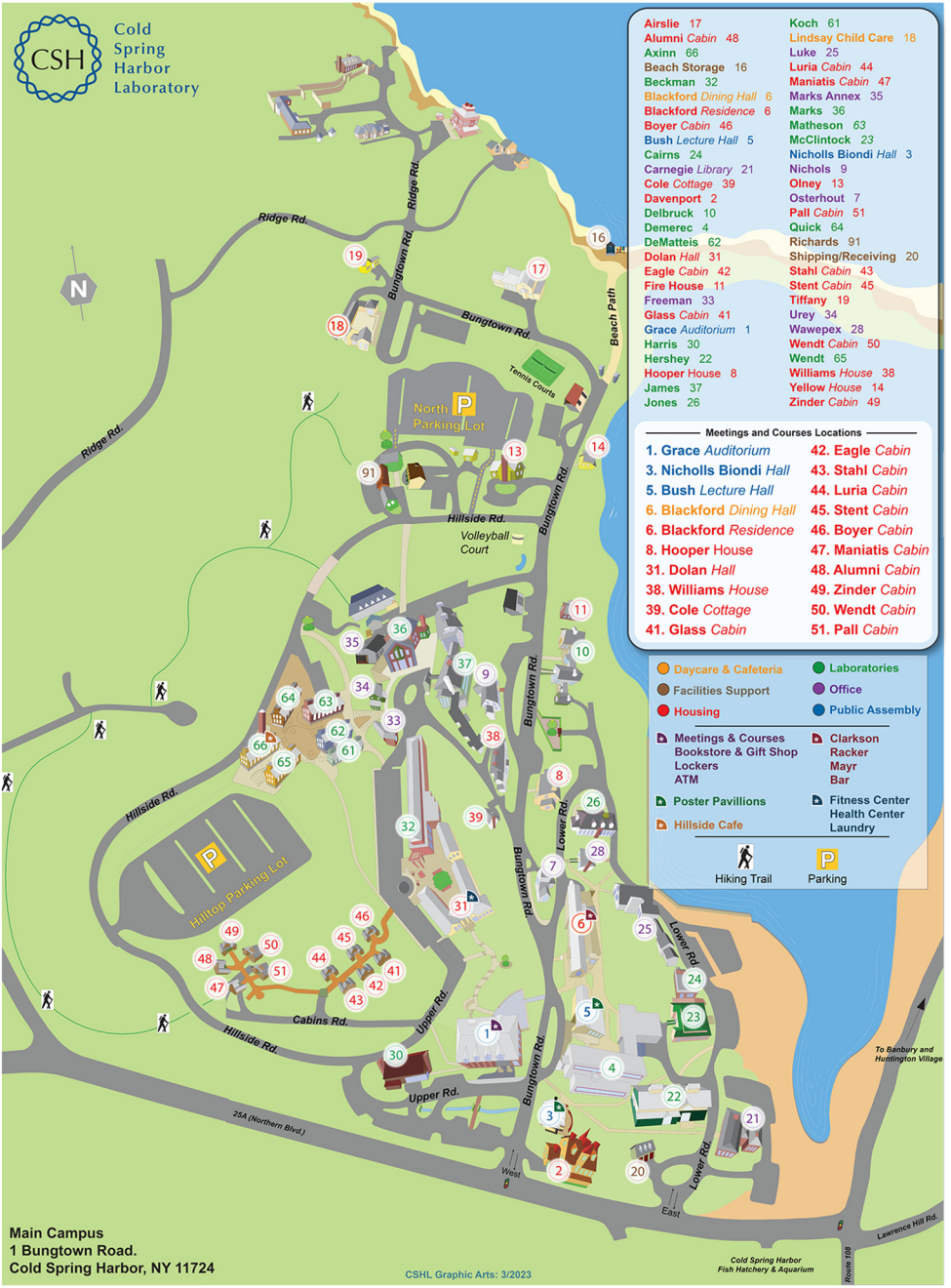
The Laboratory will act as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

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

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

CSHL Campus Map

CSHL Map



Main Campus
1 Bungtown Road.
Cold Spring Harbor, NY 11724

CSHL Graphic Arts: 3/2023