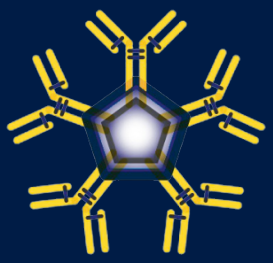

2026 Southeastern Immunology Symposium

Abstract Book

Table of Contents

53. IRF8 regulates T cell differentiation and anti-tumor effector function
54. RETRACTED Peroxisomes dictate anti-tumor immunity within the tumor microenviron…
55. Spatiotemporal Coordination of Kindlin3 and Talin1 during β2 integrin activation
56. Combination CD40 and TLR3 agonism drives durable anti-tumor immunity through cD…
57. Dendritic cells control inflammation through glutaminase-dependent metabolism
58. Chromobacterium violaceum bacterial effectors disarm neutrophil defenses ignit…
59. T cell–B cell interactions contribute to chronic lung immune responses after vi…
60. Identifying the correlates of protective adaptive immunity to Cryptosporidiosis
61. A non-redundant NFkB regulatory architecture governs CD8 T cell fate during exh…
62. Tfam-mediated metabolic perturbation in RORgt+ lymphocytes impacts intestinal t…
63. H-FIRE Triggers Antigen-Dependent CD8 T-Cell Activation and Abscopal like Immun…
64. Enhancing response in MHC-I low triple negative breast cancer with diacylglycer…
65. Naturally acquired immunity to Streptococcus pneumoniae is shaped by biofilm fo…
66. Themis integrates TCR, metabolic, and cytokine cues to control T cell developme…
67. Rational Design of a Broad Betacoronavirus Vaccine from a Cryptic Neutralizing…
68. Antigen-Specific Memory B Cell Responses Reveal Distinct Functional Architectur…
69. Role of IL-1b signaling in preventing the progression of primary amoebic mening…
70. Functional features of HLA-E restricted CD8 T cell responses associated with sp…
71. Targeting NADPH Oxidase 1 to Overcome T cell Exhaustion in T cell Immunotherapy
72. Pancreatic cell-intrinsic and innate immune response to acute viral infection o…
73. TTP is a Novel Sex-Specific Regulator of NGF
74. Characterizing the role of astrocytic IL-18R signaling in neuroinflammation in…
75. Lung-Resident B Cell Immunity in Pulmonary Tuberculosis
76. Title: Peroxisomal Control of Macrophage Senescence and Tissue aging
77. Non-canonical functions of PAD4 in regulating abdominal aortic aneurysm formati…
78. Acute Fasting Suppresses Neutrophil Metabolism and Effector Function
79. IL-26 increases Th1 effector polarization during mycobacterial infection
80. Folate Receptor Beta Orchestrates Tumor-Associated Macrophage Immunosuppressive…
81. Overcoming triple-negative breast tumor suppression of MHC-II to restore sensit…
82. Influenza-Specific Lung-Resident Memory B cells assist CD4 Recall Responses dur…
83. Single Cell Protein Synthesis Profiling Reveals Inhibitory Receptor-Independent…
84. Contributions of Neonatal-origin CD4+ T cells to the Adult Immune System
85. Radiation Therapy Remodels the Brain Immune Environment and Affects Brain Metas…
86. Interrogating the immunomodulatory role of Arginase-1-expressing neutrophils in…
87. Exhausted-like lung tissue-resident memory T cells are critical for protection…
88. Longitudinal Immune and Cytokine Changes Associated with Treatment Response in…
89. Assessment of the Impact of HLA-DO Expression on Endosomal pH Shifts
90. A Persistent Inflammatory Effector Neutrophil State in the Inflamed Colon
91. Innate immune memory as a determinant of glioma immunotherapy
92. Using CITE-seq to better understand the regulation of surface proteins by mRNA…
93. ER stress mediated mitochondrial dysfunction promotes Treg instability in coron…
94. Metabolic and proteomic GWAS-based dissection of genetic bases underlying preec…
95. A guard function of RIPK3 triggers catastrophic susceptibility to an intracellu…
96. Isotype-specific antibody secreting plasma cells harbor epigenetic and transcri…
97. Exploring vacuolar ATPase in APCs and its interaction with the non-classical MH…
98. Viral Microglia Reprogramming Clears Oligomeric Neurotoxic Debris
99. Guiding antibody evolution to elicit neutralizing antibodies against HIV-1 with…
100. Investigating the role of germinal center B cells in the anti-tumor immune resp…
101. Antibody Discovery Against Unique Influenza Neuraminidase Epitopes Using BCR Re…
102. Protection Against Type 1 Diabetes Development in Mice with 4E-BP2 Deletion
103. Olfactory Receptor 2 signaling Enhances Monocyte Chemotactic Migration in the A…
104. PVSRIPO ELICITS IMMUNE PRIMING IN MYELOID CELLS
105. RGS16 as a Therapeutic Target to Overcome TGFβ-Induced Resistance in Adoptive T…
106. IL32 drives an intracellular antimicrobial defense pathway
107. Rapid IFNg production by CD8+ TRM is necessary to slow viral propagation but is…
108. Treg infiltration in non-muscle-invasive bladder cancer is associated with prog…

109. Antigen properties and Metabolic Stress Determine B-Cell Functional Identity in…
110. Fc engineered malaria antibody therapeutic candidates for enhanced Fc receptors…
111. Targeting Tumor-TAM Interactions to Enhance STING-Mediated Anti-Tumor Immunity…
112. CD4 T Cells Drive the De Novo Activation of CD8 T Cells to Promote Vascular Inj…
113. Hypoxic Niches Enforce Glycolytic Dependence to Sustain FOXP3 and Suppressive F…
114. Early regulatory mechanisms governing stem-like CD4■ T cell differentiation in…
115. The XIST-RNP is a Novel Source of Autoantigens in Autoimmune Patients
116. Regulatory T-cells produce neuritin to drive tumor innervation and growth
117. Role of Olfr2 in Antigen Presentation and T Cell Activation During Atherosclero…
118. Macrophage diversity in response to Chlamydia muridarum infection in the upper…
119. Baseline Nasal sIgA Corresponds with Protection Against Influenza Infection
120. Exploring the Impact of Hyperglycemia on Macrophage Antimicrobial Effector Func…
121. B cells protect against Klebsiella pneumoniae following oral inoculation by con…
122. Sex-Specific Breaks in Tolerance in TLR7-Based Autoimmune Mouse Models
123. Alternative splicing regulates Z-nucleic acid sensing in Mycobacterium tubercul…
124. Tissues Guide Dependence of Regulatory T cells on the Transferrin Receptor
125. Investigating the Role of Type I Interferon Signaling in Calcium Mobilization a…
126. Antigen-specific effector B cells differentiate in the brain during CNS viral i…
127. Intrinsic Resistance to PD-1/PD-L1 Blockade in the LVRCC67 Clear Cell Renal Cel…
128. Impact of PARP inhibition on T cell dynamics and HIV persistence
129. PVSRIPO Immunotherapy Engages CNS Resident Border Macrophages
130. Antigen-Specific Heterogeneity of Human Lung CD8■ Tissue-Resident Memory T Cells
131. CD8+ T cells are required for maintenance of viral suppression under antiretrov…
132. Development of a Protective Cellular Therapy to Prevent Graft Versus Host Disea…
133. Dynamic Regulation of MLKL in Macrophages Drives Stage-Specific Processes in At…
134. HDAC9 Constrains Antibody-Secreting Cell Commitment Through Repression of MEF2…
135. Innate sensor responses set a supra-unitary threshold for viruses to successful…
136. Galactose Accentuates RLR-mediated Interferon Response in Human Monocyte-Derive…
137. TLR7/9–IFNα–LDHB Checkpoint Rewires NET Subtypes in Lupus
138. IL-5 signaling protects from acute lung injury
139. Targeting ADAR1 to harness the anti-tumor potential of PARP inhibitor-elicited…
140. Nr4a1E2 Deficiency Disrupts Vascular Wall Homeostasis Under Tumor-Associated St…
141. SARS-CoV-2 Alphavirus Replicon Particle Vaccine Induced Mucosal Immunity
142. Merkel cell carcinoma-derived macrophage migration inhibitory factor (MIF) may…
143. Sniffing Out Inflammation: Olfr2 Fuels Lipid Uptake and Foam Cell Formation in…
144. Regulatory T cell inspired engineering of CAR-T cells enhances anti-tumor effic…
145. Atherosclerotic Risk in Humans is Associated with Coordinated Monocyte-Neutroph…
146. Treg-Specific Iron Acquisition is Required for Colitis Inflammation
147. Impact of diet on host defense during Staphylococcus aureus skin infection
148. Characterizing cellular drivers of cytokine release during initial anti-CD20 th…
149. Overcoming macrophage hypophagia in monoclonal antibody therapies.
150. Antigen Presenting Cells Shape Regulatory T Cell Heterogeneity in Visceral Adip…
151. Functional T cell Imaging to Enhance Tumor Infiltrating Lymphocyte Therapy
152. Leveraging CD4 T cells to enhance antitumor immune responses in ovarian cancer
153. Differentiation of tolerogenic Fgl2+ CD8+ T cells is driven by CEBP/α and BASP1
154. Mechanisms of local regulation of alloimmunity by GM2A
155. Hemochromatosis Protein and Transferrin Receptor Endocytosis in T cells Regulat…
156. Subviral particles produced following immunization with a ZIKV M-E DNA vaccine…
157. Machine Learning Analysis of Spectral Flow Cytometry in Uveitis Vitreous Reveal…
158. Colonic Macrophage Dynamics During Clostridioides difficile Infection Are Resha…
159. Fine-tuning CD8+ T cell sensitivity: GM2A role in TCR expression and CD8+ reper…
160. Microbiome Drives Allergic Inflammation Independent of IL-33/ST2 Signaling
161. Modulation of regulatory T cell biology by GM2A in transplantation
162. Understanding the T-cell Subsets and Mechanisms of Interaction with Epithelial…
163. ArborMap: A Randomized Ensemble Tree-based Single-Cell Analysis framework
164. IgA Sialic Acid–Dependent Suppression of antiviral Fc Effector Function

165. Identification of TLR-based adjuvants suitable for polysaccharide antigen vacci…
166. The effect of aging on glioblastoma immunobiology and immunotherapy
167. A novel CAR-T therapy targeting tumor testis antigen LY6K against Solid tumors
168. Pancreas-derived lymphocytes exhibit increased glycolytic dependance in non-obe…
169. CRISPR-based functional genomics uncovers novel transcriptional regulators cont…
170. Persistent Lung-Resident CCR8■ST2■ Regulatory T Cells Are Essential for Restrai…
171. POU2F2 regulates abundance of nonclassical monocytes and their transcriptional…
172. T Cell Acly deficiency reduces atherosclerosis in female mice
173. G6PD deficiency decreases B cell production of IgM to oxidation specific neoepi…
174. Challenging Serological Assumptions: Antibody Responses to Influenza A Virus Ar…
175. The Role of Iron Metabolism in T-Cells in Celiac Disease
176. Omental microenvironment shapes memory properties of antigen specific CD8 T cel…
177. Understanding the role of tumor-intrinsic MHC-II in the anti-tumor immune respo…
178. An innovative robust assay platform to assess ADCC responses against distinct c…
179. Functional impact of the atherosclerosis-associated ID3 genetic variation on pr…
180. F5446 Epigenetically Reprograms T cells to Boost CEA CAR T Cell Immunotherapy E…
181. A Unified Human Immune Cell Atlas Spanning Cancer and Cardiovascular Disease Re…
182. CTLA-4Ig Immunosuppression Inhibits Alloimmunity but Maintains Anti-Tumor Immun…
183. Chemotherapy induced AML persister cells undergo immune remodeling and are sens…
184. CD11b: A novel marker for CD8+ T cell activation and potentially costimulation…
185. Stem cell memory T cells and how they interact with glioblastoma
186. Mitochondrial respiration is a critical vulnerability node in the immune respon…
187. Alternative splicing of RUNX transcription factors is an additional layer of tr…
188. Redistribution of Kindlin3 during neutrophil recruitment in vivo
189. NPY-Driven Fibrotic Signatures Across Skin Diseases: A Multi-Model Transcriptom…
190. The crucial role of VEGFR3 mediated lymphangiogenesis in colitis resistance, an…
191. NK Cells Drive Granuloma Formation against Chromobacterium violaceum in the Abs…
192. Engineered IgM and IgG cleaving enzymes for immunomodulation
193. Molecular titration of heterogeneous CD4 epitopes tunes target B cell fate dyna…
194. Human Apolipoprotein B Specific CD8 T Cells in Atherosclerosis
195. Memory Regulatory T Cells Reprogram into Protective Tfh-like Effectors in Recur…
196. Early-life exposure to antibiotics disrupts the development of the small intest…
197. A human multi-omic atherosclerosis atlas reveals distinct p300-associated regul…
198. Epithelial Cell Presentation of Injected Bacterial Antigens Drives Tissue-Resid…
199. TLR2 Activation Mediates Age- and Genotype-Dependent Microglial Activation and…
200. Attenuated Sindbis Virus Replicons Display Increased Immunogenic Potential In G…
201. Type 1 Interferons Regulate Cellular Redox Balance by Suppressing the Nrf2 Anti…
202. Humoral immunity leads to control of chronic Plasmodium infections
203. PFKP in immune evasion and tumor progression
204. Trem2■ macrophages and the epigenetic regulation of macrophage IL-10 in intesti…
205. Kynurenine-carboxyketoalkene (Kyn-CKA) regulates conventional dendritic cell ma…
206. PTEN increases lysosomal acidification and bacterial clearance in macrophages
207. Long-term IL12 induces immune dysfunction and T cell death
208. CD38-NAD+ axis rewrites the fate of CD8+ TRMs with age
209. Benchmarking Generative Large Language Models for de novo Antibody Design and A…
210. A Dual-Stream AI Framework for Multi-Perspective Antibody Functional Landscapes
211. Aberrant T Cell Glycosylation Drives Thymic Treg Erosion via B Cell-Derived Gal…

Abstract 1

Immunoglobulin A controls small intestinal bacterial overgrowth during undernutrition

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Childhood undernutrition and growth stunting present a major global health challenge and contribute heavily to childhood mortality. Stunted children have high rates of infection, intestinal inflammation, disrupted gut microbiota, and overall immune dysfunction. Immunoglobulin A (IgA) is a key immune regulator of the intestinal microbiota and is increased during stunting, though the functional basis for this increase is unknown. Growth stunting has also been linked to small intestinal bacterial overgrowth (SIBO), characterized by overgrowth of bacteria in the small bowel. Despite the prevalence of SIBO in stunted populations, the microbial drivers of SIBO are unknown, and the role of IgA during undernutrition-induced SIBO has not been studied. We hypothesized that IgA would be protective during undernutrition by suppressing the development of SIBO. To test this hypothesis, we modeled early-life undernutrition in SPF mice using a nutrient-deficient, humanized diet named the “Malawi-8” (M8) diet. M8-fed mice exhibit linear growth stunting and have robust increases in small intestinal microbiota-reactive IgA and Th17 cells. M8 mice have altered microbiota composition and exhibit bacterial overgrowth contained to the ileum. M8-fed RAG1-/- mice (lacking B and T cells) develop severe bacterial overgrowth throughout the entire small intestine, driving our hypothesis that IgA is protective against severe SIBO. Studies in IgA-deficient mice are ongoing, and we plan to use IgA-seq to identify specific IgA-targeted microbes driving intestinal inflammation during undernutrition. These results will uncover a novel role for IgA in undernutrition, guiding novel therapies to mitigate growth stunting in children.

Abstract 2

A nutrient deficient diet leads to intraepithelial lymphocyte loss and epithelial eosinophil infiltration in a murine model of undernutrition.

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Acute undernutrition remains a major area of public health concern and is associated with a dramatically elevated risk of mortality. Undernutrition is driven by microbial dysbiosis, immune dysregulation, and dysfunction of the small intestinal (SI) epithelial barrier, resulting in decreased nutrient absorption and inflammatory bacterial translocation. Enterocyte activity and function is regulated by tissue-resident intraepithelial lymphocytes (IELs) that can affect intestinal inflammation, nutrient sensing, and barrier maintenance. These lymphocytes also play a critical role in intracellular pathogen defense in the intestine. While IELs are sensitive to nutrient availability, the effect of undernutrition on their regulatory activity is poorly understood. To characterize the response of the SI epithelial immune compartment to undernutrition, we fed young mice a protein and micronutrient deficient (M8) diet for four weeks. M8 diet feeding led to fewer ileal CD8+, but not CD4+, IELs, with a particularly strong effect on regulatory “natural” IELs. M8-fed mice also showed lower levels of ileum IL15, a cytokine important for IEL maintenance in the epithelium. Unexpectedly, the M8 diet also resulted in an increase in CD11c-hi eosinophils infiltrating the epithelial layer of these mice. Similar results were observed in germ-free mice, suggesting that these phenotypes are driven by diet independent of the microbiome. Current work is focused on understanding the mechanism by which the M8 diet drives these phenotypes and the functional consequence of these changes on resistance to intestinal pathogens.

Abstract 3

Mice immunized with small extracellular vesicles from Salmonella-infected macrophages produce Salmonella antigen-specific circulating memory CD4 T cells

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Salmonella Typhimurium is a type of non-typhoidal Salmonella (NTS) that causes 93 million infections annually worldwide. Immuno-compromised adults and children are highly susceptible to systemic infections unresolved by antibiotics. To address the absence of an FDA-approved vaccine for NTS in humans, our lab utilizes small extracellular vesicles (sEVs) as potential cell-free vaccines. sEVs derived from Salmonella-infected macrophages (M0) have been previously shown to encapsulate Salmonella antigens and provide Th1-biased protection in vivo. To understand their role in eliciting antigen-specific adaptive immunity, mice were intranasally administered with sEVs from Salmonella-infected M0, or saline, in a three-dose regimen, followed by a Salmonella challenge at week 7. Serum ELISAs over the course of immunization demonstrated increasing IgG titers specific to Salmonella OmpA, CirA, and SopB antigens in sEV-immunized mice as compared to the control group. Post-challenge, mesenteric lymph node cells were stimulated ex-vivo for 72 hours with an antigen cocktail constituting recombinant Salmonella antigens (SopB, OmpA, OmpD, FliC, CirA). Flow cytometric analyses revealed that sEV-immunized mice had higher memory CD4 T-cell activation specific to the antigen cocktail. Both effector (TEM) and central (TCM) memory T-cell populations produced significantly higher IFNg and IL2 in response to the Salmonella antigen cocktail as compared to mice in the control group. The current study highlights the role of sEVs in long-term immunity; mice immunized with sEVs were able to elicit strong serum IgG responses and activate Salmonella antigen-specific memory T-cells, compared to control group. Collectively, results demonstrate the efficacy of sEVs as a vaccine against S. Typhimurium.

Abstract 4

Microbial functions driving Small Intestine Bacterial Overgrowth during childhood undernutrition

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Small intestine bacterial overgrowth (SIBO) is an increasingly common gastrointestinal disorder consisting of increased bacterial colonization of the small intestine. SIBO presentations range from diarrhea to gastrointestinal structural abnormalities. This disorder is prevalent among undernourished children and has a strong association with linear growth stunting. The etiology behind SIBO is poorly defined due to the difficulty of diagnosing this disorder combined with treatments limited to antibiotics. We aim to identify novel microbial metabolic pathways influencing the development of SIBO and impacting the immune system. We employed a model of undernutrition where germ-free mice were inoculated with human feces from a stunted or healthy infant and fed a nutrient and protein deficient diet. We profiled microbial community composition using 16S sequencing and shotgun metagenomics. Mice from the stunted donor displayed higher bacterial burden in the small intestine than the healthy group, a hallmark of SIBO, paired with an increase in secretory IgA and intra-epithelial lymphocytes. Suggesting an immune response directed towards microbes in the intestines and increased patrolling of immune cells. Additionally, there was a negative correlation between bacterial burden and linear growth. Escherichia coli was resolved in the ileum of undernourished mice and harbors several virulence factors, antimicrobial resistance and potential functions of utilizing aromatic amino acids critical for host nutrition. Here, we demonstrate a significant overgrowth of bacteria in the ileum in a clinically relevant murine model of undernutrition. These findings will shed light on a prevalent and consequential condition in undernourished children about which little is currently known.

Abstract 5

Protection by CD8 Tissue-Resident Memory T Cells Shapes Immunity to Repeated Respiratory Virus Exposure

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CD8 tissue-resident memory T cells (TRM) are critical mediators of immunity against respiratory viruses. Using a mouse model of respiratory virus transmission with Sendai virus (SeV), we previously showed that vaccine-induced CD8 TRM in the respiratory tract can protect against infection acquired through transmission, and the efficacy of protection correlated with abundance of SeV-specific TRM. However, respiratory TRM decline over time, resulting in reduced protection. Here, we examined how the level of protection during a primary transmission event influences the development of de novo immunity and protection against subsequent exposures. Mice were vaccinated intranasally with live-attenuated influenza virus expressing the immunodominant MHC class I–restricted SeV epitope to generate SeV-specific TRM throughout the respiratory tract. Vaccinated contact mice were co-housed with index mice infected with luciferase-expressing SeV and imaged daily by in vivo imaging (IVIS), using luminescence as a proxy for viral load. Mice were classified as having complete protection, intermediate protection, or breakthrough infection. Mice with breakthrough infections mounted robust SeV-specific IgG responses, whereas mice with no detectable viral load by IVIS exhibited significantly lower anti-SeV IgG titers and more than 50% failed to seroconvert. Furthermore, viral load strongly correlated with anti-SeV titers 30 days post-exposure. Four months later, mice were subjected to a second transmission challenge. Mice that experienced breakthrough infection during the primary exposure were protected against secondary transmission, whereas mice protected from primary infection by CD8 TRM were more susceptible upon re-exposure. These findings illustrate how CD8 TRM-mediated protection shapes immunity to respiratory viruses encountered repeatedly over time.

Abstract 6

Antigen-Specific Treg Therapy Enhances Immunomodulatory Pathways in Autoimmune Diabetes

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Despite the recent success of disease modifying therapies in type 1 diabetes (T1D), these treatments lack prolonged durability. Treg dysfunction has been readily observed in T1D, thus adoptive Treg therapy has generated interest to promote immune tolerance and prevent disease progression. Our group has previously demonstrated that NOD mice treated with a combinatorial regiment of anti-CD3 and antigen-specific BDC2.5 Tregs reversed autoimmune diabetes in recent onset mice, while treatment with polyclonal Tregs failed to achieve the same results. Mice with newly diagnosed diabetes received 50 µg of anti-CD3 for 5 days, followed by administration of congenic CD45.2 BDC2.5 or polyclonal Tregs at day 11. After 4 weeks, pancreata were collected and CD45■ cells were isolated by cell sorting, excluding B cells and donor Tregs, then analyzed using scRNA sequencing. Macrophages from mice treated with the BDC2.5 Tregs indicated an upregulation of genes related to negative signal regulation (Dusp1, Log2FC=1.53; Ubash3a, Log2FC=1.34), as well as M2 polarization (Egr1, Log2FC=1.82; Cxcr6, Log2FC=1.53). Those treated with the polyclonal Tregs upregulated genes associated with positive signal regulation (Grk3, Log2FC=-0.77; Pik3ap1, Log2FC=-0.60; Hck, Log2FC=-0.63), especially pathways related to type 1 interferon signaling (Rnf213, Log2FC=-1.02; Mx2, Log2FC=-1.58). Similarly, BDC2.5 treatment promoted a tolerogenic profile in pDCs, as evidenced by the upregulation of Fos (Log2FC=1.12), Fasl (Log2FC=1.65), and Tnfaip3 (Log2FC=0.78). These findings indicate that antigen-specific Tregs in combination with anti-CD3 treatment reprogram innate immune cells towards a regulatory and anti-inflammatory states, providing a mechanistic foundation for antigen-specific Treg therapy as an effective strategy to reverse autoimmune diabetes.

Abstract 7

Decay accelerating factor (CD55) is necessary for complement activation and T helper cell recruitment in cigarette smoke (CS)-exposed lungs

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Rationale: Complement proteins contribute to cigarette smoke (CS)-induced sterile inflammation. Increased complement deposition and reduced levels of the inhibitor CD55 have been observed in CS-exposed mice and COPD patients. Prior studies reported higher CD4■ Th1 cells in patients with emphysema, a COPD subtype. Notably, CD55 deficiency is associated with increased IFN-γ–producing T-cells, a key driver of emphysema. We hypothesized that CS-induced complement deposition promotes C3aR■ Th1 recruitment and enhances IFN-γ secretion in CD55■/■ vs. wild-type (WT) mice, driving emphysema. Methods: Lungs and primary murine lymphocytes of CD55■/■ and WT mice exposed to short- (1-month) and long-term (6-month) CS were analyzed. Complement activation (C3, C4) and deposition (C5b9) were assessed by immunofluorescence. Lung compliance was measured using FlexiVent. EL4 T lymphoblasts and naïve WT or CD55■/■ CD4■ T cells were differentiated into Th1 cells and treated with CS extract or air control (AC). Th1 polarization and activation were evaluated by FACS, qPCR, western blot, and ELISA. Results: Short- and long-term CS exposure increased lung C3 and C5b9 deposition, lung static compliance, and CD4■ T-cell infiltration in lung parenchyma of WT mice, with further enhancement in CD55■/■ mice vs. AC WT and CD55■/■ mice. Ex-vivo CS treatment (2.5%, 4 h) elevated C3aR+ expression and Th1 polarization, with increased C3aR, T-bet, and IFN-γ expression versus AC-treated cells ($p<0.005$, ANOVA 1-way). Conclusion: CS-driven sterile inflammation promotes complement activation, Th1 recruitment, and emphysema in CD55■/■ mice, highlighting a critical crosstalk between innate (CD55/complement) and adaptive (Th1) immunity that could be targeted therapeutically in COPD.

Abstract 8

Antigen-specific Regulatory T Cell Therapy Mode-of-Action in Controlling Autoimmune Type 1 Diabetes

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There are currently no curative or preventive therapies for type 1 diabetes (T1D). Regulatory T cell (Treg)-based therapies offer a promising approach to restore immune tolerance but are constrained by limited engraftment, insufficient antigen specificity, and poorly defined mechanisms of action. We previously established a combinatorial immunomodulatory regimen - anti-CD3 (αCD3), cyclophosphamide (CyP), and islet-specific Treg infusion - that reverses autoimmune diabetes in NOD mice without chronic immunosuppression. Here, we investigate how antigen-specific Tregs control islet autoimmunity compared to polyclonal Tregs. Late-prediabetic female NOD mice received αCD3 followed by islet-specific BDC2.5 or polyclonal Treg infusion; immune responses were assessed by multiparameter flow cytometry and single-cell RNA sequencing (scRNAseq). At 4-weeks, BDC2.5-Tregs preferentially homed to the pancreas, where they selectively enriched hyporesponsive CD4+ T cell states (CD44hiCD73+FR4+ anergic) without broad depletion of effector populations. scRNAseq demonstrated that BDC2.5-Treg treatment induced productive TCR-engagement signatures (ICOS, NFAT/AP-1 regulators) and controlled, self-limiting transcriptional programs (TNFAIP3, DUSP1/2, ZFP36L2), whereas polyclonal therapy triggered bystander interferon-driven inflammatory responses (IFI27L2A, RTP4, ISG15). Mice maintaining remission at six-months exhibited significantly greater pancreatic BDC2.5-Treg engraftment than relapsed mice (8% vs. 2%, p=0.012). Engrafted BDC2.5-Tregs retained stable Foxp3 with elevated Ki67, Nur77, ICOS, TIGIT, and CD103 expression compared to host-Tregs, indicating enhanced proliferative and suppressive capacity. TCRβ and CD3 expression was also elevated, suggesting higher TCR-pMHC engagement. Together, these findings demonstrate that disease-relevant Tregs mediate durable remission through targeted pancreatic engraftment, sustained TCR engagement, and induction of self-limiting immunoregulatory programs, highlighting antigen specificity as a critical determinant of effective Treg-based therapy for T1D.

Abstract 9

The Ubiquitin Ligase Cul4b Promotes Affinity Maturation and Antiviral Humoral Immunity

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High affinity neutralizing antibodies are critical for protective antiviral immunity. Vaccination triggers antibody production by inducing B cell activation and differentiation into plasma cells (PCs). High affinity plasma cells arise from GC-derived B cells (GCBs), that undergo affinity maturation by enduring multiple rounds of somatic hypermutation, proliferation, and selection. Investigating unique features of B cell biology underlying affinity maturation and plasma cell differentiation could facilitate improvements in vaccine design. We have identified a novel role for Cullin4b (Cul4b) in promoting anti-influenza antibody production and protection from infection after vaccination. Cul4b is the backbone of the Cullin Ring Ligase 4 (CRL4) complex, which ubiquitylates proteins for degradation by the proteosome. CRL4 regulates cell cycle and DNA damage responses in other cells, but it's role in B cells is unknown. Since affinity maturation requires careful coordination of both these processes, we hypothesized Cul4b promotes affinity maturation within GCBs to drive protective antiviral immunity. To investigate this, we immunized wild-type (WT) and B cell-specific Cul4b knock out (Cul4b cKO) mice with NP-Ova. We observed a marked reduction of NP-specific plasma cells and high affinity anti-NP antibodies in Cul4b cKO mice after vaccination. Immunoglobulin heavy chain sequencing of NP-specific GCBs revealed that Cul4b promoted the generation of high affinity B cell clones. In-vivo BrdU labeling after immunization showed that Cul4b promotes cell cycle progression and differentiation in GCBs. We believe this shows Cul4b supports antiviral immunity by regulating cell cycle in GCBs to promote formation of plasma cells that secrete high affinity neutralizing antibodies.

Abstract 10

A Metabolic Arms Race: Neutrophil Reprogramming and Bacterial Adaptation in Diabetic Staphylococcus aureus Infection

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Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and impaired insulin regulation. While insulin therapy has prolonged the lifespan of diabetic patients, the altered metabolic state leads to major complications, including heightened susceptibility to bacterial infections, most commonly by Staphylococcus aureus. Neutrophils are essential for controlling S. aureus infection, yet how diabetes reshapes neutrophil antibacterial effector programs remains unclear. In the streptozotocin (STZ) model of diabetes, mice are highly susceptible to bacteremia, and unlike macrophages that fail to elicit a robust oxidative burst, neutrophils exhibit a hyperinflammatory state characterized by enhanced reactive oxygen species (ROS) production and neutrophil extracellular trap release (NETosis). Surprisingly, the heightened oxidative burst and NETosis are not explained by increased glucose availability, uptake, or utilization. This suggests that alternative metabolic substrates may fuel neutrophil effector functions in diabetes, despite the traditional view that the oxidative burst in neutrophils requires NADPH derived from glucose metabolism and the pentose phosphate pathway. We identify that STZ neutrophils are adapted to use glutamine, a key substrate for the tricarboxylic acid (TCA) cycle, to support elevated ROS and NETosis. Despite enhanced neutrophil effector functions, the hyperglycemic milieu simultaneously protects S. aureus by enabling glucose-dependent adaptation that promotes resistance to oxidative stress and NET-mediated killing. Together, these findings reveal a metabolic “arms race” in diabetes in which host neutrophils rewire substrate use to sustain inflammation while S. aureus exploits excess glucose to bolster stress resistance, providing a framework to target host–pathogen metabolism to restore antibacterial defense.

Abstract 11

NF-κB RelA and Irf4 Facilitate alpha-Synuclein–Induced Disease-Associated Microglia Development

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Parkinson Disease (PD), the second most common neurodegenerative disorder, is characterized by abnormal accumulation of alpha-synuclein and progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Chronic neuroinflammation—driven by brain-resident microglia and infiltrating peripheral immune cells—is a hallmark of PD pathogenesis. The detection of alpha-synuclein–reactive T cells in prodromal patients suggests that neuroinflammation is a key driver of early disease. Furthermore, alpha-synuclein directly initiates microglial activation, and persistent microglial activation has been reported from prodromal to postmortem stages, positioning microglia as central regulators of neuroinflammation throughout disease progression. Recent single-cell transcriptomic studies have revealed diverse microglial transcriptional states, highlighting their heterogeneity and plasticity. Disease-associated microglia (DAM), a distinct microglial transcriptional state, have been consistently identified across multiple neurodegenerative disorders. Although DAM are strongly implicated in neurodegeneration, the mechanisms driving DAM development and their functional relevance remain poorly understood. Using an alpha-synuclein overexpression mouse model of PD, we demonstrated that DAM represent a hyperresponsive functional state characterized by heightened phagocytic activity and robust pro-inflammatory cytokine and chemokine production. We identified a regulatory network of NF-κB, AP-1, and IRF transcription factors—particularly the RelA/IRF4 axis—as critical drivers of DAM development. Conditional deletion of RelA or Irf4 in microglia suppressed microglial reprogramming into the DAM state, attenuating their pro-inflammatory and phagocytic programs. Taken together, our findings demonstrate that microglial transcriptional states directly dictate functional activity and identify RelA/IRF4 axis as a conserved regulatory mechanism underlying DAM development in neurodegeneration.

Abstract 12

Gut-commensal dysbiosis modulates the lung microenvironment to promote breast tumor metastasis

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This study aims to investigate the impact of the gut microbiome as a host-intrinsic factor in breast cancer metastasis to the lungs. The 5-year survival rate of metastatic breast cancer is 31%, however, little is known as to what places patients at risk for metastatic disease. We provide evidence that gut dysbiosis, an inflamed and unbalanced microbiome, modifies the lung immune environment to promote the metastatic growth of hormone receptor-positive (HR+) breast tumor cells. Single-cell RNA sequencing of the lungs from mice with gut-dysbiosis shows an enrichment of tumor-permissive pathways driven by dysbiosis. Specifically, pathways downstream of the cytokines IL-6, IL-10, and VEGFa were enriched in several immune cell types. These cytokines are validated in vivo, with the lungs of mice with gut-dysbiosis exhibiting elevated IL-6, IL-10, and VEGFa, and suggests an immune-dysregulated and angiogenic lung microenvironment. When challenged with a primary HR+ mammary tumor, the lungs of dysbiotic mice exhibit a unique pre-metastatic niche, as measured by an increase of both inflammatory and immunosuppressive cytokines including CCL2, M-CSF, VEGFa, TNFa, and IL-10. Furthermore, when challenged with intravenous injection of HR+ mammary tumors to mimic advanced metastatic disease, mice with pre-existing dysbiosis exhibit an increased susceptibility to metastatic growth, via both increased severity of tumor phenotype, and a decreased overall survival. These findings highlight the significant connection between the gut microbiome, the immune system, and tumor metastasis, and provoke investigations into the impact of gut microbiome health as a host-intrinsic factor in breast cancer outcomes.

Abstract 13

Gut dysbiosis reprograms the mammary tissue mast cells and fibroblasts to promote HR+ breast tumor dissemination

Simona Bajgai, Alkaid Feng, Audrey Putelo, Mika Poblete, Scott Dunn, Mirna Perusina Lanfranca, Cara Hatzinger, Akshita Mirani, Melanie Rutkowski

Metastatic dissemination remains a significant barrier to reducing mortality associated with HR+ Her2- breast cancer. Dissemination occurs early and is driven by immune-mediated crosstalk between the tumor microenvironment and adjacent tissue. We have demonstrated that commensal dysbiosis, an inflammatory gut microbiome with low biodiversity, promotes long-term cellular and molecular changes in normal (non-tumor-bearing) mammary tissues. When commensal dysbiosis is established in a mouse model before tumor initiation, dissemination of HR+ breast tumor cells is significantly increased, whereas primary tumor growth remains unaffected. Gut microbiome changes have been associated with relapse and metastatic disease in women with breast cancer, highlighting the importance of defining how the gut microbiome regulates breast cancer through modulation of the mammary tissue environment. Preliminary data suggest that dysbiosis activates a mast cell/fibroblast axis in the normal mammary tissue that enhances HR+ tumor dissemination. We are using a combination of methods including but not limited to untargeted proteomics, spatial profiling, high-dimensional flow cytometry, scRNAseq, coupled with various in vivo and in vitro assays to define the contribution of this axis to early metastasis. Our data show that dysbiosis causes phenotypic changes in mast cells that drive mammary tissue fibroblast activation making them sufficient to promote tumor dissemination. By uncovering mechanisms of mast cell–fibroblast crosstalk, our findings have the potential to inform the development of therapeutic and diagnostic strategies aimed at targeting tissue remodeling in patients at risk for metastatic disease.

Abstract 14

Chronic TLR5 signaling impacts myeloid cell phenotypes and function during ovarian tumor progression

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Most treatment strategies, including checkpoint blockade therapy, remain ineffective for ovarian cancer due to the immunosuppressive activity of myeloid cells in the tumor microenvironment (TME). Tumor-associated myeloid cells adapt to the hostile TME through extensive metabolic rewiring, particularly at the mitochondria and endoplasmic reticulum, that drives the acquisition of immature, immunosuppressive phenotypes. While tumor-derived cues contributing to this remodeling are characterized, the role of host-associated factors remains unclear. Our lab identified Toll-like receptor 5 (TLR5) signaling, the only known ligand for which is bacterial flagellin (FLA), as a host-intrinsic driver of myeloid metabolic stress and functional reprogramming. Ovarian tumors increase the bioavailability of commensal bacteria within the TME, resulting in chronic TLR5 engagement on myeloid precursors. Performing CITE-Seq analysis on bone marrow-derived dendritic cell cultures for 8 days using FLT3L+/- FLA as a model of chronic TLR5 signaling, we observe upregulation of MYC target genes and oxidative phosphorylation, suggesting coordinated activation of metabolic programs that drive dysfunction. Complementary Seahorse assays in wild-type mice treated with FLA confirm elevated mitochondrial respiration in TLR5-stimulated myeloid populations. Flow cytometry further reveals a reduction in XCR1■ cDC1s (non-TLR5-expressing) and a concomitant increase in cDC2s and cDC3s populations (TLR5-expressing), implicating TLR5-expressing cells as key contributors to the observed metabolic rewiring. Together, these findings identify host-derived chronic TLR5 signaling as a key regulator of myeloid metabolism and immune suppression in ovarian cancer, revealing a previously unrecognized axis that may be exploited for therapeutic intervention.

Abstract 15

Monocyte-derived border associated macrophages promote neuroinflammation in an AAV-model of Parkinson's disease

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Maladaptive immune responses characterize Parkinson's disease, as patients show increased T cell frequency in the CSF during disease and in the substantia nigra after death. Innate immune mechanisms may contribute to underlying immunopathology as well, as patients show increased frequency of monocytes that appear predisposed to present antigens and drive pro-inflammatory responses to synuclein. As clinical evidence increasingly points to monocytes and T cells as critical immune pathways in PD, a mechanism that links how these cells activate and coordinate to drive central inflammation has not yet been discovered.

Border associated macrophages (BAMs) may connect our observations in the innate and adaptive immune pathways. These cells not only turn over from monocytes within distinct niches along the meningeal and vascular borders, but also function as immune sentinels that selectively promote leukocyte invasion through brain barriers in response to inflammatory challenges. Utilizing an adeno-associated virus (AAV) model of PD, we demonstrate that BAMs robustly expand and promote pathogenicity through MHC-II restricted reactivation of CD4 T cells.

Utilizing a chimeric fate mapping approach, we demonstrate a large proportion (~50-60%) of these MHCII+ BAMs are monocyte derived (moBAMs). moBAMs can be visualized in peri-vascular areas near CD4 T cells, suggesting these subsets specifically play a role in promoting T cell reactivation and entry into the substantia nigra. These results support the notion of dynamic competition between resident BAMs and actively recruited monocytes for the niche, implying a central pathogenic mechanism for monocytes in the development and progression of PD neuroinflammation.

Abstract 16

Respiratory viral infections prime accelerated lung cancer growth

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The COVID-19 pandemic has highlighted the long-term consequences of viral pneumonia, yet its impact on cancer development remains unclear. Here, we show that patients previously hospitalized with severe COVID-19 have an increased risk of subsequent lung cancer. Across multiple murine models, severe respiratory viral infections accelerated lung cancer growth, whereas vaccination mitigated infection-enhanced tumor progression. Mechanistically, prior viral pneumonia reprogrammed the lung into a pro-tumor microenvironment marked by sustained accumulation of tumor-associated neutrophils and heightened immunosuppression. We observed persistent chromatin remodeling at key cytokine loci in immune and structural cells, linking inflammatory memory to tumor-promoting signals. Therapeutically, combined blockade of neutrophil recruitment and PD-L1 restored CD8+ T cell function and suppressed tumor growth. Together, these findings establish a causal link between prior viral pneumonia and lung tumorigenesis, underscoring the need for enhanced surveillance and targeted interventions to reduce post-COVID cancer risk.

Abstract 17

Opposing Roles of Intracellular and Secreted Osteopontin Isoforms in Microglia during EAE

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Osteopontin (OPN), a multifunctional protein encoded by the Spp1 gene, exists in two isoforms by alternative translation: secreted OPN (sOPN) and intracellular OPN (iOPN). Their distinct localizations underlie divergent functions. In microglia, OPN expression is upregulated in neuroinflammation, including multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Spp1 upregulation marks disease-associated microglia (DAM), but the roles of distinct OPN isoforms are not known.

This study elucidates isoform-specific roles in EAE using a novel mouse system that enables isoform-specific OPN expression in microglia. We found that iOPN is detrimental in EAE. iOPN interacts with the proteasome complex and promotes canonical NF-kB activity, promoting microglial activation. iOPN also interacts with TRAF3 and limits non-canonical NF-kB activation, possibly compromising tissue protection. Conversely, sOPN is beneficial in EAE. iOPN reduces Th17 and Th1 cells in the spinal cord and limits immunoproteasome formation. These results revealed opposing roles of iOPN and sOPN.

Together, these findings demonstrate that iOPN and sOPN play functionally antagonistic roles in microglial neuroinflammation, challenging the current understanding of OPN as a single pathological molecule based on studies using OPN knockout mice, which lack both iOPN and sOPN. This isoform-level resolution highlights the therapeutic potential of selectively targeting OPN isoforms in neuroinflammatory diseases such as multiple sclerosis.

Abstract 18

Combination of therapeutic vaccination, PD-1 blockade, and IL-2 for SIV functional cure

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Achieving a functional cure for HIV infection requires an effective therapeutic strategy that induces durable antiviral immunity capable of clearing viral reservoirs. Previously, we showed that Programmed Death-1 blockade (aPD-1) in chronically SIV-infected rhesus macaques (RMs) at 4 weeks post anti-retroviral therapy (ART) interruption restored CTL function and transiently controlled viremia. However, this control was observed only in RMs with a strong recall of SIV-specific CD8 T cell response. In the current study, to further enhance the therapeutic benefits of aPD-1, we combined it with a therapeutic vaccine to expand the SIV-specific CTL pool and enhance recall responses post ART interruption (ATI). We also tested a combination of aPD-1 plus IL-2 cytokine therapy administered post ATI.

A single dose of DNA-LNP vaccination induced a strong SIV-specific CD8 T cell response (GM of 4% Gag-CM9 tetramer+, as a % of CD8) that showed less than two-fold decline over 12 weeks. The MVA boost further expanded these cells by 5-fold (GM of 20%, range 6.7-52.5%). The vaccine-induced CD8 T cells in blood, lymph node, and gut were poly-functional: co-expressed perforin, granzyme B, CD28, and cytokines IFNg, TNFa and IL-2. The vaccine-induced CD8 T cell magnitude was 50-fold higher compared to unvaccinated controls at the time of ATI. These results demonstrate that DNA-LNP/MVA vaccination induces a potent, highly functional and persistent SIV-specific CD8 T cell response in blood and tissues of SIV-infected and ART-treated RMs. These results are encouraging leading up to aPD-1 and IL-2 therapy (on going).

Abstract 19

Mtb-PP3 DNA-LNP vaccines induce potent and persistent CD8 T cell and antibody response and provide protection in mice

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Mycobacterium tuberculosis (Mtb) causes tuberculosis (TB) disease and remains a leading pathogen in causing death by a single infectious agent. The Bacille Calmette–Guérin (BCG) vaccine remains the only approved and widely used vaccine and provides protection in children but varied response in adults.

Here we developed mRNA, DNA, or modified DNA (mod-DNA, patent pending) Mtb subunit vaccines encoding antigens Mtb32a, Mtb39A, and Rv1860 as a fusion protein (MTB-PP3) and formulated them as lipid nanoparticles (LNP). We immunized C57BL/6 mice with either 0.5 ug of mRNA-LNP or 0.5 ug of DNA/mod-DNA-LNP at weeks 0 and 4, and measured vaccine-specific immune response. One group of mice was challenged with Mtb Erdman (100 cfu) via the aerosol route at 4 weeks after the boost.

At 2 weeks post boost, all vaccines induced a strong Mtb32a-specific Tet+ CD8 T cell response in blood, but the responses were significantly higher in the mod-DNA-LNP compared to the other two groups (3% in DNA-LNP vs 1.5% mRNA-LNP vs 14% mod-DNA-LNP). At 10 weeks post boost, the response diminished 5-fold in mRNA-LNP immunized animals (0.6%). In contrast, the response was maintained in the mod-DNA-LNP group (10%) and increased in the DNA-LNP group (8%). Following the Mtb challenge, mod-DNA-LNP immunized animals showed protection compared to controls (CFU/mL in lungs: 10^5 in other groups vs 10^4 in mod-DNA-LNP). These data demonstrate that the mod-DNA-LNP PP3 vaccine induces a potent and durable immune response compared with mRNA-LNPs and provides protection against virulent Mtb.

Abstract 20

Subset- and stage-dependent regulatory T cell programs control inflammation and tumor immunity in metabolic liver disease

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Metabolic dysfunction–associated fatty liver disease (MAFLD) progresses along a pathological continuum from hepatic steatosis to the inflammatory and fibrotic injury characteristic of metabolic dysfunction–associated steatohepatitis (MASH), and in a subset of patients ultimately culminates in hepatocellular carcinoma (HCC). However, how immune regulatory networks adapt or become dysregulated across these disease stages remains incompletely understood. Here, we define the temporal dynamics, phenotypic heterogeneity, and mechanistic roles of hepatic regulatory T cells (Tregs) in the development of MASH and HCC. We identify a predominant T-bet■CXCR3■ Treg subset that expands during MASH, localizes within CD11c■ cell–organized intrahepatic micro-niches, and functions to restrain pathogenic type 1 inflammatory responses. Notably, Treg-specific ablation of T-bet drives the expansion of a clonally related ST2■ Treg population expressing profibrotic mediators. Despite retaining suppressive capacity, this subset fails to compensate for the loss of T-bet■ Tregs, indicating nonredundant regulatory functions among hepatic Treg populations. As disease progresses to HCC, however, the functional role of T-bet■ Tregs shifts. Rather than limiting inflammatory injury, these cells suppress anti-tumor cytotoxic T lymphocyte responses, thereby facilitating tumor progression. Collectively, these findings reveal substantial functional specialization within hepatic Treg subsets and demonstrate that their immunoregulatory roles are highly context dependent across disease stages. Our results highlight the importance of temporally and subset-specific immunomodulatory strategies when targeting Tregs in MASH and HCC.

Abstract 21

Olfactory receptor 2+ tumor-associated macrophages activate CD8+ T cells to suppress tumor growth

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Olfactory receptor 2 (Olfr2 in mice; OR6A2 in humans) has emerged as a regulator of macrophage activity. We found that Olfr2+ tumor-associated macrophages (TAMs) are required for proper CD8+ T cell activation. Using an orthotopic B16F10 melanoma model, we compared tumor growth in wild-type and Olfr2-/-mice. Bone marrow transplant experiments were performed to determine whether the phenotype was driven by loss of Olfr2 in hematopoietic cells. In vivo OT-I adoptive transfer assays were conducted to assess the ability of Olfr2+ TAMs to support CD8+ T cell priming and cytotoxicity. Additionally, RNA-seq analysis of sorted Olfr2+TAMs was performed to characterize their transcriptional programs. Gene set enrichment analysis (GSEA) was used to evaluate the transcriptional impact of Olfr2+ TAMs and their potential association with improves responses to immune checkpoint inhibitor (ICI) treatments. Olfr2-/- mice developed nearly twice the tumor burden of wild-type controls, a phenotype driven by loss of Olfr2 in hematopoietic cells. Tumors from Olfr2-/- mice exhibited reduced CCL2 and CXCL9 expression and diminished CD8+ T cell infiltration. In vivo OT-I adoptive transfer demonstrated that Olfr2+ TAMs are essential for supporting CD8+ T cell priming and cytotoxicity. RNA-seq of Olfr2+ TAMs revealed enrichment of antigen presentation, pro-inflammatory, and anti-tumor pathways, consistent with their enhanced antigen presentation capacity observed in vitro. GSEA further showed that the Olfr2+ TAM transcriptional signature was associated with improved ICI responsiveness in human melanoma datasets. Together, these findings establish Olfr2+ TAMs as central regulators of CD8+ T cell immunity in melanoma, linking olfactory receptor signaling in macrophages to anti-tumor immune activation and potentially improved responses to ICI therapy.

Abstract 22

Regulatory T cells turn into multiple pro-inflammatory effector T cells in Mice with Atherosclerosis

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Atherosclerosis is an immune-mediated disease in which regulatory T cells (Tregs) can lose lineage stability and convert into pro-inflammatory effector states (“exTregs”) that may accelerate plaque progression. However, the diversity, lineage relationships, and pathogenic roles of exTreg subsets in cardiovascular disease remain undefined. Using Foxp3 lineage-tracker Apoe-/- mice, we uncovered two distinct Treg subsets (GFP+tdTomatolow and GFP+tdTomatohi), with only the tdTomatohi population differentiating into exTregs (GFP-tdTomatohi). To comprehensively characterize these populations, we performed large-scale single-cell CITE-seq and TCR-seq across spleen, draining (axillar and cervical), and non-draining lymph nodes from Apoe-/- mice on a Western diet. Density-based clustering and trajectory inference revealed at least seven exTreg subsets, including Tfh-like (the largest population), Th1-like, Th17-like, cytotoxic and proliferating exTreg-like populations. Pseudotime analyses supported a branched rather than linear trajectory, with tdTomatohi Tregs giving rise to multiple effector-like programs. TCR analyses showed clonal enrichment predominantly within Tfh- and cytotoxic-like exTregs, suggesting antigen-driven expansion. CITE-seq identified surface markers (e.g., CXCR5/CD185 for Tfh-like and CXCR6/CD186 for cytotoxic Th1-like exTregs). Spatially, exTregs accumulated in draining lymph nodes and spleen, and were detectable in atherosclerotic plaques by intravital microscopy. Collectively, these data define a branched Treg-to-exTreg transition trajectory. Clonally expanded, antigen-experienced exTreg subsets that are expected to influence plaque biology.

Abstract 23

Talin-1 determines the direction of primary mouse neutrophils migrating in vivo

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Neutrophils rely on β2 integrins, mainly LFA-1 and Mac-1, to mediate rolling, arrest, spreading and transmigration at inflamed sites. β2 integrins activation requires talin-1 binding to the cytoplasmic tail, yet the timing and localization of talin-1 recruitment and its distribution in vivo remain unclear. To address this, we generated EGFP-talin1 (Tln1EGFP-talin1/EGFP-talin1) knock-in mice that enable real-time visualization of endogenous talin-1. EGFP-talin1 was expressed in neutrophils and supported β2 integrin activation without altering integrin surface expression or hematopoiesis. Using total internal reflection fluorescence microscopy showed that talin-1 was recruited to the plasma membrane during rolling and increased after arrest. Intravital imaging revealed that redistribution of talin-1 to endothelial contacts during luminal crawling and to the leading edge during transendothelial migration. After transendothelial migration, talin-1 polarized to the front and accumulated at emerging lamellipodia during the interstitial migration and before directional turning. These results define the spatiotemporal dynamics of talin-1 during neutrophil recruitment and highlight talin-1 polarization as a key regulator of migratory directionality in vivo.

Abstract 24

Not all Granulomas are Created Equal: Characterizing & quantifying innate immune system granulomas induced by Chromobacterium violaceum.

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A granuloma is an organized immune cell aggregation, defined by the recruitment of macrophages, around a persistent stimulus. While granulomas are typically formed by the adapative immune system, the innate immune system organizes the granuloma in our model. Chromobacterium violaceum (Cv) is an environmental pathogen with a 55% mortality rate in immunocompromised individuals. However, it is virtually harmless to immunocompetent individuals, making it an ideal model to study a pathogen that has not adapted to evade innate immunity. Shown in mice models, Cv rapidly replicates in hepatocytes during the first days of infection. Since the neutrophil swarm fails to eradicate Cv a granuloma forms to contain the bacteria and persists until the infection clears. Using the Cv infection model system in C57BL/6 and knockout mice, various components of the innate immune system can be studied. The goal of this project is to develop a histopathological scoring system to characterize and quantify granulomas. The scoring system will be based on various features of granuloma architecture, including overall area and perimeter, area of the necrotic core, presence of buds, presence of layers, size progression, percent necrosis, and total percent lesion. We will establish standard values for each feature per day post-infection in wildtype mice and compare them with those of knockout mice, testing different aspects of the innate immune system. This scoring system will minimize bias and serve as the foundation for the convolutional neural network that will automatically quantify Cv-induced granulomas from whole-slide histopathological images.

Abstract 25

Salmonella SPI2 evades detection by NAIP/NLRC4 despite utilization of a detectable needle

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Salmonella enterica serovar Typhimurium uses two type 3 secretion (T3S) systems to reprogram host cells. Whereas activity of the SPI1 T3S can be detected by the NAIP/NLRC4 inflammasome, the SPI2 T3S is not detected. In this study, we demonstrate that the SPI2 needle protein, SsaG, can be detected by the NAIP-NLRC4 inflammasome when artificially delivered to the cytosol in mouse macrophages. Surprisingly, this detection occurs primarily through NAIP2, which normally detect rod proteins. However, we found that during infection with live S. Typhimurium, this detection fails to trigger the downstream pyroptotic pathway. We investigate the hypothesis that the SPI2 effector protein SpvC inhibits NLRC4 activation. In mouse macrophages, spvC mutants were detected under SPI2-inducing conditions in an NLRC4-dependent manner. However, this masking effect of SpvC is not seen in vivo, where SpvC contributes to S. Typhimurium virulence in mice independent from NLRC4. We also hypothesized that S. Typhimurium may minimize SsaG detection by limiting protein expression. In support of this, overexpressing SsaG causes limited NLRC4-dependent clearance in vivo. Therefore, the SPI2 may evade NAIP/NLRC4 inflammasome detection by precisely controlling the quantity of detectable SsaG needle protein expressed.

Abstract 26

Investigating CXCR3 modulation of B lineage functions during anti-viral responses

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Chemokines are unsung heroes, critical for immune cell movement in homeostatic and inflammatory conditions, establishing themselves as key players in humoral immunity. Previously, our lab has shown that the chemokine receptor CXCR3 modulates protection in the olfactory mucosa by promoting plasma cell homing to the tissue. The olfactory mucosa, located in the upper respiratory tract, is not protected through systemic neutralizing antibodies due to the blood-olfactory-barrier. Interestingly, olfactory plasma cell homing is dependent upon expression of CXCR3, and its ligands, CXCL9, and CXCL10 in the draining lymph nodes not through mucosal expression of these ligands suggesting a role for CXCR3 in the priming context. Using flow cytometry and imaging approaches, this project aims to elucidate CXCR3's role in mediating protection of infected tissues by investigating its influence on activated B cell priming in secondary lymphoid organs, where CXCL9 and CXCL10 expression is highest. Using a B cell receptor transgenic mouse model, I will compare CXCR3 expressing and CXCR3 deficient B cells to assess differences in localization and activation. Finally, this project will characterize anti-viral effector B cell functionality in the presence and absence of CXCR3. Completion of the proposed project will uncover a previously unappreciated role for CXCR3 in regulating B cell processes and facilitating immune cell interactions.

Abstract 27

From mouth to brain: How Porphyromonas gingivalis infection turns microglia into lipid-loaded driver of Alzheimer's disease progression

Muhammad Shahid Riaz Rajoka, Ping Zhang

Abstract

Introduction: The key pathogen in chronic periodontitis, known as Porphyromonas gingivalis (Pg), was detected in the brains of Alzheimer's disease (AD) patients. In neurodegenerative disorders such as AD, the microglia are often characterized by increased lipid droplet (LD) accumulation, heightened activation, and impaired functionality.

Methods: Murine BV2 microglial cells were treated with Porphyromonas gingivalis (Pg; ATCC 33277), and LD accumulation was assessed by confocal microscopy and flow cytometry. Triacsin C was used to inhibit Pg-induced LD and evaluate its role in ROS production and microglial function. In vivo, APP knock-in mice were treated with Triacsin C and infected with Pg via retro-orbital injection to assess the impact of microglial LD accumulation on the progression of AD.

Results: In this study, we investigated whether Pg, a major periodontal pathogen, induces LD accumulation in microglia and alters their functional status. We observed significant LD accumulation in Pg-infected BV2 microglial cells and in the microglia from the Pg-infected App KI mouse brains, suggesting that the Pg infection promotes the LD accumulation both in in-vitro and in-vivo. Our findings demonstrate that Pg-induced LD accumulation correlates with increased reactive oxygen species (ROS) production, leading to a compromise in microglial functionality, including impairment of phagocytosis ability. Furthermore, our results indicated that the Pg-induced LD contributed to impairment of microglia functionality by downregulating TMEM119 (a homeostatic microglial marker) and upregulating MHC-II (an activation marker), indicating a loss of resting microglia identity and a transition toward an activated and dysfunctional phenotype. Importantly, treatment with triacsin C, a triglyceride synthesis inhibitor, effectively reversed Pg-induced LD accumulation, ROS production, and phagocytosis defects, highlighting the mechanistic role of lipid metabolism in microglial dysfunction.

Conclusions: Together, our results underscore the role of Pg infection in driving microglial lipid dysregulation and functionality impairment, suggesting that the lipid dysregulation contributes to the link between periodontal diseases and AD.

Future Directions: Building on our findings that Pg drives LD accumulation and microglial dysfunction, future studies will focus on defining the precise molecular mechanisms linking Pg infection to altered lipid metabolism. Specifically, we aim to investigate the signaling pathways regulating triglyceride synthesis, fatty acid oxidation, and ROS generation in Pg-infected microglia.

Keywords: Porphyromonas gingivalis, Periodontitis, Alzheimer's disease, Lipid droplet, microglia

Acknowledgement

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Abstract 28

Protective and Pathogenic Lung Immunity in Tuberculosis

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Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains one of the leading causes of morbidity worldwide, yet the immune features that separate protection from disease progression remain poorly defined. While CD4■ T cells are essential for controlling Mtb infection, the outcome of TB is unlikely governed by a single immune population. Instead, protection and pathogenesis are determined by the coordinated behavior of diverse immune and structural cell populations within the lung, where the host must simultaneously restrict bacterial growth, regulate inflammation, and preserve tissue function.

However, the field lacks a longitudinal and spatially resolved understanding of how these cellular networks evolve during infection and how vaccination reshapes them. To address this, we conducted a longitudinal study of murine Mtb infection at Days 1, 30, 60, 90, and 120 post-infection to define the pulmonary immune landscape over time. By comparing vaccinated and non-vaccinated mice, with and without Mtb infection, we aimed to identify coordinated immune trajectories associated with protection, bacterial persistence, or progressive pathology relative to uninfected controls.

Using an integrated approach combining high-throughput immune profiling, spatial transcriptomics, bacterial burden, and lung pathology, we mapped the abundance, organization, and temporal evolution of immune and non-immune cell populations across distinct disease stages. Rather than focusing on individual cell subsets, this study provides a systems-level framework for understanding how cellular crosstalk, inflammatory circuits, and spatial tissue organization shape the balance between bacterial control and immunopathology.

These findings establish a conceptual foundation for developing next-generation vaccines and host-directed therapies that enhance protective lung immunity while minimizing tissue-damaging inflammation.

Abstract 29

Antibody-Guided Discovery of a Protective Mycobacterium tuberculosis Antigen

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Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a major global health burden despite Bacille Calmette-Guérin (BCG) vaccination, whose efficacy is variable, particularly in regions with high exposure to non-tuberculous mycobacteria (NTMs). Previously, we developed a murine model that mimics BCG vaccination with continuous NTM exposure and demonstrated that BCG+NTM mice exhibit more robust and longer-term protection against aerosol Mtb challenge than BCG alone. This protection was associated with elevated anti-Mtb IgG and IgA antibodies and formation of ectopic germinal centers (eGCs) in the lung. Importantly, stage-specific eGC antibodies differentially modulated bacterial burden upon passive transfer into Mtb infected mice, supporting a functional role for humoral immunity in protection.

We hypothesized that protective immunity is driven by antibodies targeting stage-specific Mtb antigens during infection. To address this, we isolated antibodies from bronchoalveolar lavage fluid (BALF) and serum across different stages of TB and profiled their binding to Mtb fractions, including membrane, cytosolic, cell wall, and total lipid fractions. Anti-Mtb antibody binding was significantly enriched against membrane-associated antigens during the protective stages, while shifted to lipids during the chronic stage. To identify Mtb membrane-associated antigens, we performed immunoblotting along with microflow LC-MS of the membrane fraction and prioritized a panel of ~30 antigenic target proteins for downstream functional validation.

Collectively, these findings identify membrane-associated Mtb antigens as promising targets of protective humoral immunity and establish a foundation for developing antigen-directed vaccines and antibody-based immunotherapies for TB.

Abstract 30

Utility of an oncogene-driven spontaneous triple-negative breast cancer model for immunotherapeutic testing

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One in eight women will develop breast cancer, a heterogeneous disease with multiple subtypes that have distinct patient outcomes, biomarkers, and treatment options. Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that has limited treatment options. It recently received FDA approval for treatment with anti-PD1 immunotherapy, though predictors of patient success remain unclear. A major hurdle to studying TNBC is that there is a profound lack of clinically relevant animal models. Our lab has developed a spontaneous model of TNBC which utilizes CRIPSR guides targeting three commonly mutated genes in TNBC: TP53, PTEN, and BRCA1. This model, upon intraductal injection of an adenovirus expressing Cre (Ad-Cre-GFP), induces expression of Cas9, GFP, Luciferase, and the three CRISPR guide RNAs in the mammary epithelium. This allows for a more natural disease progression where the tumor develops initially in the duct before expanding into the surrounding mammary gland and gives rise to heterogenous, trackable oncogene driven tumors that have developed in an immunocompetent host. We have performed in depth characterization of these tumors' histology, gene expression, and immune profiles. Overall, we have developed both a spontaneous model of TNBC in C57BL/6 mice and, from these, a highly diverse repertoire of TNBC implantable cell lines, which will allow us to better model human breast cancer in mice. Moving forward, we will be able to characterize the responsiveness of these diverse tumor lines to immunotherapy and use them to better understand predictors if immunotherapy efficacy in patients.

Abstract 31

Obesity impairs anti-tumor responses to breast cancer vaccination

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While advances in immunotherapy have improved treatment of breast cancer, many patients do not respond to current immunotherapies, there is not a reliable predictor of immunotherapy efficacy, and responders may develop resistance to immunotherapies. Additionally, obese breast cancer patients have advanced disease at diagnosis, differential responses to therapies, and worsened prognoses. As obesity rates increase, understanding how obese patients develop breast cancer and respond to therapeutics is of critical importance. One emerging field of treatment is the use of cancer vaccines that stimulate the patient's immune system against tumor antigens. In the context of infectious diseases, obesity results in diminished vaccine-induced immune responses. However, there is a significant gap in our understanding of how obesity impacts vaccination efficacy in the context of cancer. Our group has previously published the use of a pre-clinical therapeutic vaccine targeting HER2. Using this therapeutic, we hypothesized that obesity would reduce the efficacy of therapeutic vaccination. We investigated this by using our published model of spontaneous HER2+ breast cancer. Obese mice have a longer time to both tumor formation and endpoint. Importantly, therapeutic vaccination early after tumor detection completely protects lean mice from tumor development, while only 30% of obese mice are protected. Ongoing studies are analyzing scRNAseq of cells from tumors, fat pads, and lymph nodes from vaccinated lean and obese mice to identify key differences in immune response to vaccination. These studies are critical to inform breast cancer patient care and leverage immunotherapies to most effectively treat patients.

Abstract 32

Fever as a Metabolic Regulator of Host Immunity and Staphylococcus aureus Physiology

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Fever is a conserved feature of infection, yet its impact on innate immune cell metabolism and function remains incompletely understood. During Staphylococcus aureus infection, neutrophils are essential for bacterial control through reactive oxygen species (ROS) production and neutrophil extracellular trap (NET) formation. However, most experimental models do not incorporate febrile temperature, limiting our understanding of how elevated temperature shapes immune responses in vivo. Using a murine model of invasive S. aureus infection, we compared cardiac-infiltrating neutrophils from mice housed at standard room temperature (23°C) or thermoneutral conditions (30°C), which permit a febrile response. Neutrophils from fever-permissive mice exhibited significantly increased mitochondrial biomass within infected cardiac tissue, consistent with metabolic reprogramming. This shift was accompanied by diminished oxidative burst capacity and enhanced NET release, indicating that febrile temperature alters effector prioritization at sites of infection. To determine whether temperature also directly influences bacterial physiology, S. aureus was cultured in human-like media at 37°C or 39°C. Growth at 39°C resulted in membrane hyperpolarization and reduced intracellular ATP levels, demonstrating temperature-driven perturbations in bacterial bioenergetics independent of host factors. Together, these findings identify fever as a regulator of neutrophil metabolic programming and effector deployment during bacterial infection, while simultaneously altering bacterial energy homeostasis. Incorporating physiologically relevant temperature conditions is therefore critical for understanding immune regulation and host–pathogen dynamics.

Abstract 33

You are what you eat: Short-term western diet rewires neutrophil metabolism and enables Staphylococcus aureus to suppress NETosis.

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Immunometabolism ties diet to frontline antibacterial defense. Neutrophils rely on glycolysis to power the oxidative burst and on mitochondrial metabolism to drive neutrophil extracellular trap (NET) release. We asked whether brief exposure to a high-fat, high-calorie western diet (WD), in the absence of obesity or diabetes, is sufficient to alter neutrophil function during Staphylococcus aureus infection. In a 4-day systemic S. aureus model, short-term WD alone worsened infection outcomes, with higher bacterial burdens in the liver. Ex vivo, fatty acid oxidation (FAO) supports NETosis, indicating that fatty acid availability can fuel this effector program. However, despite increased fatty acid availability in mice on a western diet, NETosis was decreased because S. aureus exploits WD-derived fatty acids to generate or modify lipoproteins that actively suppress NET release. In addition, neutrophils from WD-fed mice display a diminished oxidative burst, consistent with Randle cycle-driven substrate competition that diverts metabolism away from glycolysis-dependent NADPH oxidase activity. Together, these findings reveal a rapid, diet-driven vulnerability in innate immunity that S. aureus can leverage. Rather than fueling NETosis through FAO, the same fatty acids are used by S. aureus to blunt NET release, while the Randle cycle dampens ROS generation. Defining how diet shifts neutrophil fuel choice and how S. aureus capitalizes on that shift points to metabolic and nutritional strategies to restore NETosis and oxidative burst during infection.

Abstract 34

Investigating the role of TREM2 in lung metastatic outgrowth

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The lungs are a common metastatic site, and metastasis remains a leading contributor of cancer-related deaths. The importance of cancer metabolism has been increasingly recognized in the metastatic cascade. Evidence has shown that long chain fatty acids (LCFAs) accumulate in the lung tumor microenvironment to promote metastatic outgrowth, while limiting CD8+ T cell ant-tumor functionality. However, how intratumoral fatty acids are regulated in the lung metastatic niche is unclear. We have shown that the tumor vasculature regulates delivery of LCFAs through a VEGFB/VEGFR1/mTORC1 axis. However, knockout of Vegfb in tumor cells was not sufficient to suppress the phenotype, indicating that other sources of VEGFB may exist. Using a publicly available single cell RNA sequencing dataset, we identify a source of VEGFB in the lung TME to be macrophage-derived, and further analysis revealed co-expression of markers of lipid-associated macrophages such as TREM2 and CD36. To further investigate the role of TREM2+ macrophages in lung metastases, we injected tumor cells into Trem2-/- and wild-type (WT) control animals intravenously and found reductions in tumor burden and changes in the immune cell landscape in Trem2-/- compared to WT Trem2-/- bone marrow-derived macrophages (BMDMs) express lower levels of Vegfb following IL-4 stimulation compared to WT. To gain a broad overview of transcriptomic changes associated with loss of TREM2, we utilized RNA sequencing in BMDMs and identified changes in lipid-related pathways in WT macrophages compared to Trem2-/-. Ongoing investigations aim to further explore mechanisms and the link between lipid metabolism and TREM2+ macrophage immunosuppressive function.

Abstract 35

Spatial multiomic analyses of clinical responders with advanced triple negative breast cancer to intratumoral IL-12 therapy

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Approximately 1 in 8 women in the US will be diagnosed with one form of invasive breast cancer (BC). Roughly 10-15% of newly diagnosed BC patients develop the more aggressive, triple-negative form of breast cancer (TNBC), which lacks expression of estrogen and progesterone receptors and HER2 oncogene overexpression. Furthermore, TNBC patients have an elevated risk of post-treatment relapse and metastasis, highlighting the clear clinical importance. Here, we present preliminary analyses of patient data from a Phase II trial where patients with inoperable or metastatic TNBC were treated with intratumoral plasmid IL-12 with electroporation in combination with pembrolizumab, which was shown preclinically and in phase I trials to promote an anti-tumor CXCR3 gene signature and systemic T cell expansion.

We utilized multiple state-of-the-art, single-cell multiomic and immune profiling techniques to characterize patient responses. Spatial transcriptional profiling of formalin-fixed, paraffin-embedded (FFPE) biopsy samples was performed using 10X Genomics' Xenium platform. Pre-biopsy and post-biopsy samples were assessed using a 380-gene panel optimized for the breast tumor microenvironment. Xenium data was segmented, annotated, and superimposed on H&E-stained tissue for single-cell visualization and spatial analyses. We also evaluated systemic responses to IL-12 stimulation by leveraging an 80-plex Luminex assay to evaluate cytokine, chemokine, and growth factor induction in plasma samples; in total, eighteen cytokines, including the CXCR3 ligands, CXCL9/10/11, were significantly upregulated post-treatment. Presently, the findings in this project reveal a novel characterization of the TNBC immune landscape following IL-12 therapy that will be supplemented by incorporation of patient outcome data.

Abstract 36

Rewriting NOS2 Biology: Neutrophils and an Interferon-Independent Pathway

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Nitric oxide synthase 2 (NOS2) is widely viewed as a macrophage antimicrobial strategy. Using a murine liver granuloma-forming pathogen Chromobacterium violaceum, we show that NOS2 is required for host survival and bacterial clearance in the granuloma. Single-cell RNA sequencing revealed a striking Nos2-high population that we carefully identified not as macrophages but as neutrophils. We then confirmed the protein level expression. This was unexpected given the current view of NOS2 biology.

Confusingly, neutrophils from standard sources—including bone marrow and thioglycolate-elicited peritoneum—did not express NOS2 under any stimulation, consistent with prior reports. In contrast, neutrophils from spleens of C. violaceum-infected mice responded to stimulation and expressed NOS2, showing that this capacity is not intrinsic to all neutrophils but emerges from a distinct, yet undefined state.

Finally, interferon signaling is believed to be indispensable for NOS2 induction. However, when we examined Ifng–/– mice, NOS2 expression is still present in granulomas. To test whether type I and II interferons act redundantly, we analyzed Ifnar1–/–Ifngr1–/– mice, and NOS2 remained present. Even in Ifnar1–/–Ifngr1–/–Ifnlr1–/– mice, NOS2 expression is still clearly detectable. Thus, this discovery led us to seek a novel pathway that drives NOS2 expression independent of interferon signaling.

Together, our data identify neutrophils as a major and previously underappreciated source of NOS2, show that this capacity is restricted to a specific state, and demonstrate that NOS2 induction can occur independently of interferon signaling. These findings provide a potential explanation for the long-standing mystery of human NOS2 source and regulation.

Abstract 37

Sequestration of repressive RNA binding proteins by Neat1 and the nuclear paraspeckle is required for proper innate immune gene activation in macrophages

Kaitlyn S. Armijo, Robert O. Watson, Kristin L. Patrick

Pathogen sensing in macrophages triggers signal transduction cascades that activate de novo transcription of antimicrobial and inflammatory genes. This rapid response must be tightly regulated to ensure pathogen clearance while avoiding immunopathology. We propose that macrophages prioritize inflammatory gene expression by concentrating proteins and nucleic acids within biomolecular condensates (BCs). The paraspeckle (PS) is a stress-responsive nuclear BC that is co-transcriptionally assembled at the site of lncRNA Neat1 transcription. We found that PSs display dynamic assembly–disassembly kinetics during LPS stimulation. We hypothesized that as PSs aggregate and disassemble, they sequester and release proteins involved in activating and repressing inflammatory gene expression. Consistent with this, we found that Neat1■/■ macrophages exhibit reduced induction of inflammatory cytokines and interferon-stimulated genes (ISGs) following LPS stimulation and fail to restrict intracellular pathogen replication. However, the molecular mechanisms underpinning this phenotype remain elusive. To fill this gap, we applied oligonucleotide-directed proximity interactome mapping (O-MAP) to profile the PS proteome. We identified hundreds of proteins that are differentially enriched in resting vs. LPS-stimulated PSs. We found that several known negative regulators of inflammation are more enriched in the 0.5h LPS-induced PS proteome compared to resting PSs. Notably, an RNA binding protein called quaking (QKI), aggregates in the core of the PS following LPS treatment and loss of QKI enhances the inflammatory response to LPS. These findings support a model in which PSs promote inflammatory gene expression by sequestering inhibitory factors and provide new insight into how PSs and nuclear compartmentalization modulate innate immunity.

Abstract 38

Trastuzumab Deruxtecan Induces Proinflammatory Tumor Remodeling and Demonstrates Combinatorial Benefit with Immunotherapy in a HER2-Positive Tumor Model

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The anti-HER2 antibody-drug conjugate, trastuzumab deruxtecan (T-DXd), has transformed breast cancer treatment. However, its combination with immunotherapy in clinical trials has progressed without rigorous preclinical validation. This is largely due to trastuzumab's inability to bind to rodent HER2, limiting the availability of immunocompetent preclinical models. To address this, we developed HER2X, a novel tumor model created by editing the trastuzumab-binding region of mouse HER2 and overexpressing it in EMT6 murine mammary cancer cells. EMT6 HER2X tumors bind trastuzumab, are nonimmunogenic, and are sensitive to T-DXd, providing a robust platform to study its immunologic effects.

Using this model, we demonstrate that T-DXd elicits immunologic memory dependent on MHC-I competency and CD8+ T cells but independent of HER2 expression in rechallenged tumors. Cytometry by time-of-flight analysis revealed that T-DXd remodels the tumor microenvironment by increasing proinflammatory immune populations (T cells, NK cells, M1-like macrophages), while reducing immunosuppressive populations (M2-like macrophages, neutrophils, monocytes). Upregulated immune checkpoint expression on effector CD8+ T cells in treated tumors was also observed, suggesting the potential for immunotherapy combinations. Combining T-DXd with anti-LAG-3, anti-TIM-3, or anti-PD-1 increased complete response rates from 50% (monotherapy) to 70%, 80%, and 90%, respectively.

Mechanistically, we have demonstrated that T-DXd induces DNA damage and activates the cGAS-STING pathway in vitro and in vivo. Furthermore, STING knockout in EMT6 HER2X tumors abolished T-DXd sensitivity.

In summary, we demonstrate that T-DXd promotes a proinflammatory tumor microenvironment, provides combinatorial benefit with immune checkpoint blockade, and relies on STING for antitumor efficacy in our HER2X model.

Abstract 39

Recipient Type 2 Innate Lymphoid Cells Sustain Donor ST2■ Regulatory T Cells via Interleukin-9 to Prevent Acute Lung Injury After Hematopoietic Cell Transplantation

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Hematopoietic Cell Transplantation (HCT) is a therapy for cancers of the bone marrow but T cell reactivity to allo-antigens in normal tissues induces graft versus host disease (GVHD) including of the lung. Our laboratory has shown that soluble (s)ST2, a decoy receptor for IL33, is increased 10-fold in patients with lung GVHD vs controls while the membrane-bound ST2 expressed on regulatory T cells (Tregs) and ILC2 cells is decreased. Using murine models of HSCT and IL33 intranasal treatment or systemic blockade of sST2, we showed decreased GVHD severity, increased plasma IL33 while IFNγ and TNFα decreased in treated mice vs controls. The treated mice showed better pulmonary function, reduced infiltrating donor IFNγ+CD4+ and IFNγ+CD8+ T cells, increased frequencies of donor Tregs, and increased frequency of GATA3+ST2+ ILC2 in the lung. However, the role of ILC2s in the lung following HCT has not been explored. Here we show that IL9 specifically produced by ILC2s, but not other subsets, is required for their regulatory effects. RNA-seq analysis of sorted ILC2s from naïve GATA3 reporter mice treated with IL33 showed increased Il9 and pu1 transcripts in ILC2s which was validated in allogeneic mice treated with IL33 or ST2 blockade compared to vehicle. Using hematopoietic and nonhematopoietic IL9 KO vs WT chimeras we confirmed that recipient hematopoietic ILC2 are the source of IL9 production. Tregs particularly ST2■ Tregs proliferation and activation are decreased in IL9 KO vs WT chimera. Further, whole genome methylation sequencing on sorted Tregs from IL9 treated mice showed significant hypomethylation in the Foxp3 region vs untreated mice. IL9 deficiency (specifically in ILC2s using Nmur1iCre+/eGFP+ IL9fl/fl mice (Fu Y… Kaplan M, Sci Immunol, 2022 and Tsou AM … Artis D., Nature, 2022) reduces survival and exacerbates IPS by impairing lung ST2+ Tregs function. Together, this suggests that blockade of sST2 or administration of IL33 during HCT induce IL9-producing ILC2s that augment ST2■ Tregs to prevent lung GVHD.

Abstract 40

Impact of Opioids on Chemotherapy Induced Gastrointestinal Toxicities

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Background: Advances in chemotherapy have improved long-term cancer survival; however, many survivors require prolonged opioid use for treating cancer pain thus increasing the risk of opioid tolerance and gastrointestinal (GI) dysfunction. Opioids and chemotherapies independently disrupt the gut microbiome and intestinal function; however, combined effects remain poorly understood.
Methods: A retrospective analysis conducted at VCU evaluated the impact of prior opioid use in patients treated with the topoisomerase inhibitor, irinotecan, for gastrointestinal malignancies. A multicenter analysis conducted through the TriNetX database assessed GI dysfunction (diarrhea, abdominal pain, nausea, constipation) in colorectal and pancreatic cancer patients treated with topoisomerase inhibitors or immune checkpoint inhibitors. Preclinical studies with ICR mice receiving morphine (10 mg/kg b.i.d. for 10 days), irinotecan (75 mg/kg for four days with two days of recovery), or combined treatment. Immune cells from the lamina propria (LP) of the small intestine were analyzed by flow cytometry, and fecal samples were assessed by qPCR for Desulfovibrio abundance. Cell viability of RAW 264.7 macrophages exposed to increasing doses of hydrogen sulfide (H2S) was assessed through flow cytometry.
Results: Clinically, prior opioid use increases the risk of Irinotecan dose reduction (OR: 2.25, CI 1.27-4.07; p = 0.006) and delay (OR: 2.77, CI 1.39-5.96, p = 0.005) and increases the risk of diarrhea by 50% across cancer and treatments. In mice, combined morphine and irinotecan treatment increased immune cell infiltration in LP, particularly recently recruited monocyte-derived macrophages (F4/80■Ly6C■MHCII■), along with a threefold increase in Desulfovibrio, a hydrogen sulfide–producing bacterium, in fecal samples. H2S reduced macrophage cell counts in a dose-dependent manner with 50% cell death at 30 mM and 100% death at 100mM.
Conclusions: Prior opioid use worsens chemotherapy-associated GI outcomes, potentially through microbiome-driven immune dysregulation. Increased Desulfovibrio and hydrogen sulfide production may contribute to heightened intestinal immune responses.

Abstract 41

Exploring the Immunological Landscape in Reduced Efferocytosis

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Homeostasis in multicellular organisms is regulated by apoptosis (‘silent’ or non-inflammatory cell death). Apoptosis plays a defining role during development and in response to tissue damage and infection, leading to the resolution of inflammation and return to homeostasis. Apoptosis is linked with phagocytosis of apoptotic cells, known as ‘efferocytosis’. Dying cells display ‘eat-me’ signals, such as externalized phosphatidylserine (PtdSer), allowing phagocytes to discriminate them from live neighbor cells. Redundancy in efferocytosis mechanisms is thought to be required to maintain homeostasis. In in vitro models of failed efferocytosis, apoptotic cells undergo secondary necrosis (a non-apoptotic, inflammatory form of cell death) and release their intracellular contents, which can be recognized by the immune system and break self-tolerance. To address the impact of reduced efferocytosis on homeostasis and tolerance, we generated mice on the C57BL/6J background, globally deficient in four different PtdSer recognition receptors – BAI1, MerTK, Tim4, and CD36 (4KO mice). Using these mice, we show that: 1. Professional phagocytes (macrophages) from 4KO mice had reduced, but not ablated, efferocytosis, 2. Reduced efferocytosis did not increase the burden of apoptotic cells in most tissues during homeostasis and aging, and 3. Aged 4KO mice develop signs of spontaneous autoimmunity and low-grade tissue inflammation. We conclude that reduced efferocytosis may contribute to the loss of self-tolerance even without a dramatic increase in uncleared apoptotic cells in tissues. Identification of efferocytosis mechanisms required for homeostasis maintenance will improve understanding of triggers that promote self-reactivity and aid development of new therapeutic strategies for autoimmune diseases.

Abstract 42

Platelets initiate tissue necrosis as a beneficial innate immune defense

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Thromboinflammatory responses can remodel infected tissues in ways that may limit pathogen spread, but often at the cost of local ischemic injury and cell death. In a mouse model of liver infection with Chromobacterium violaceum (Cv), lesions develop a reproducible architecture with a neutrophil- and bacteria-rich inner core, a surrounding zone of uninfected hepatocytes undergoing coagulative necrosis, and an outer macrophage-rich ring. We hypothesized that the coagulative necrosis zone functions as a host walling-off strategy and asked whether lesion maturation is associated with focal loss of vascular perfusion. Wild-type C57BL/6 mice were infected with Cv, and livers were analyzed over time by histology and fluorescence imaging. Intravenous tomato lectin and Hoechst 33342 were administered shortly before harvest to assess perfusion. CD41 and fibrin(ogen) staining were used to evaluate platelet accumulation and fibrin(ogen) deposition, and liver histology was compared between wild-type and platelet-depleted mice. Early lesions appeared as neutrophil-rich inflammatory foci and then developed a surrounding zone of coagulative necrosis between 18 and 48 hours after infection. Perfusion assays showed sharply demarcated regions adjacent to lesions that excluded both tomato lectin and Hoechst 33342, consistent with focal loss of vascular delivery. In parallel, CD41 and fibrin(ogen) staining showed platelet accumulation and fibrin(ogen) deposition within sinusoids in the coagulative necrosis zone. Platelet depletion nearly eliminated coagulative necrosis. Together, these findings support a model in which local thromboinflammatory clotting drives focal perfusion failure and coagulative necrosis, creating a barrier that helps contain bacterial spread.

Abstract 43

Metabolic Regulation of Visceral Adipose Tissue Regulatory T Cell Homeostasis

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Obesity is a major risk factor for metabolic disease and is characterized by chronic inflammation in the visceral adipose tissue (VAT). Regulatory T cells (Tregs) residing in VAT are critical for maintaining tissue homeostasis and metabolic health. However, they are progressively lost during obesity, contributing to metabolic dysfunction. Recent evidence indicates that cellular metabolism regulates immune cell fate and function, yet the metabolic mechanisms governing VAT Treg differentiation and/or maintenance remain incompletely understood. Acetyl–CoA is a central metabolic intermediate that links nutrient metabolism to epigenetic regulation and lipid biosynthesis. Cytosolic acetyl-CoA is produced either from citrate via ATP-citrate lyase (ACLY) or from acetate via acetyl-CoA synthetase 2 (ACSS2). Transcriptomic analysis revealed elevated expression of Acly and Acss2 in VAT Tregs compared to splenic Tregs at steady state, which is reduced following long-term high-fat diet (HFD) feeding in parallel with VAT Treg loss. These findings suggest that acetyl–CoA metabolism is required for VAT Treg accumulation but is disrupted during obesity. Using CRISPR/Cas9-mediated gene editing and a VAT Treg TCR transgenic mouse model from our previous work, we demonstrate that deletion of ACLY and ACSS2 impairs VAT Treg accumulation in vivo. Together, these results identify acetyl–CoA metabolism as a key regulator of VAT Treg homeostasis and might provide a mechanistic link between metabolic dysfunction and impaired immune regulation. Targeting this pathway may provide new strategies to restore Treg function and treat obesity-associated metabolic diseases.

Abstract 44

Directional Trogocytosis by AML-Primed ST2■ Tregs Drives Parricide Killing of Bone Marrow–Resident CD8■ T Cells

Albert Dahdah, Denggang Fu, Drew Stefano, Hua Jiang, Jessica Hartman, Sophie Paczesny.

Our laboratory recently demonstrated that acute myeloid leukemia (AML) activates bone marrow ST2■ regulatory T cells (Tregs) to eliminate intratumoral CD8■ T cells through contact-dependent, granzyme B–mediated cytotoxicity (Jiang H. et al., Nature Communications, 2025). However, the mechanism enabling ST2■ Tregs to selectively target and deplete these effector populations remains undefined. We hypothesized that AML-primed ST2■ Tregs establish parricide killing through trogocytosis-mediated acquisition of antigen-specific TCR components from engaged CD8■ T cells, enabling recognition-dependent elimination. To test membrane exchange, we performed short-term coculture of bone marrow ST2■ Tregs with CD8■ T cells from AML-bearing mice and assessed trogocytosis by flow cytometry. High-resolution confocal microscopy at 60 minutes confirmed predominantly unidirectional acquisition—CD8■ membrane material transferred into Tregs at the immunological synapse. Critically, splenic Tregs from the same AML hosts exhibited minimal trogocytosis compared to bone marrow Tregs, demonstrating niche-specific conditioning by the leukemic microenvironment. Given that tumor-infiltrating CD8■ T cells exhibit altered metabolism under chronic antigen stimulation, we assessed metabolic fitness of AML-derived CD8■ cells. Real-time oxygen consumption rate (OCR) measurements over 20 hours demonstrated significantly elevated respiration in tumor microenvironment (TME) CD8■ T cells compared to non-leukemic counterparts, accompanied by increased mitochondrial mass and membrane potential. This hypermetabolic state reflects their active engagement in antitumor responses. However, despite remaining viable in isolation, these metabolically active TME CD8■ cells were preferentially killed upon contact with ST2■ Tregs compared to naïve or non-TME CD8■ populations. Rescue with the mitochondrial antioxidant MitoQ abrogated this contact-dependent killing, demonstrating that the hypermetabolic state—while enabling antitumor function—renders TME CD8■ cells selectively vulnerable to ST2■ Treg-mediated elimination, establishing the mechanistic basis for parricide killing. To determine whether shared antigen recognition underlies preferential targeting, we performed TCR sequencing on sorted bone marrow ST2■ Tregs and CD8■ T cells from leukemic mice. Both populations exhibited oligoclonal expansion, more pronounced in ST2■ Tregs. Repertoire analysis revealed up to 83 overlapping V–J gene segment combinations, with dominant usage of TRBV1 recognizing T72(Tn)—a hemoglobin-derived decapeptide (Hb67–76) O-glycosylated at threonine-72. This TCR convergence is consistent with trogocytosis-mediated acquisition of antigen-specific receptors from chronically stimulated CD8■ cells, establishing a directional parricide circuit wherein Tregs extract TCR components from their cellular targets during cognate interaction. Collectively, these findings identify directional trogocytosis as a mechanism of antigen-specific CD8■ T-cell elimination in AML. Leukemia-conditioned ST2■ Tregs extract membrane and TCR components from metabolically active, antigen-engaged CD8■ lymphocytes, promoting their granzyme B–dependent depletion. This pathway provides a cellular explanation for elective erosion of antitumor CD8■ immunity in leukemic bone marrow and positions trogocytosis as a therapeutically targetable mechanism to restore effector function in AML.

Abstract 45

Mechanisms of impaired alveolar macrophage responses to Mycobacterium tuberculosis in people with HIV

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Despite the success of antiretroviral therapy (ART), people with HIV (PWH) remain at increased risk for pulmonary tuberculosis (TB) compared to people without HIV (PWoH). The mechanisms underlying this increased risk and dysregulated lung immunity in PWH remain poorly understood. Alveolar macrophages (AMs) in the lung are crucial for shaping the early immune response to Mycobacterium tuberculosis (Mtb) infection. However, how AMs from PWH function in the context of Mtb infection is understudied. We recently reported that AMs from PWH on ART exhibit impaired TNF signaling networks in response to ex vivo Mtb infection. We identified a subset of AMs with an immunosuppressive phenotype, characterized by high levels of CD200R and IL-10, enriched in PWH. CD200R and its ligand, CD200 have been implicated in immunoregulatory functions in other diseases, but their importance in HIV or TB remains unclear. We hypothesize that CD200R+ AMs suppress pro-inflammatory responses to Mtb and promote dysfunctional T cell responses in PWH, leading to poor bacterial control. To test this, bronchoalveolar lavage (BAL) cells from PWH on ART and PWoH were infected with a mCherry-labeled strain of Mtb and processed for flow cytometry. Our preliminary data shows that AMs from PWH had higher baseline expression of CD200R than PWoH, which further increased after infection. Further characterization of CD200R+ AMs shows expression of immunoregulatory receptors like IL-10R and reduced expression of effector molecules like iNOS. Ongoing studies using blockades and agonists, are evaluating how manipulating the CD200:CD200R pathway influences AM and T cell crosstalk in BAL cells.

Abstract 46

Runx2 regulates the development and cytotoxic function of CD26high CD4+ T cells

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Novel and innovative approaches have emerged to leverage the host immune system against aggressive tumors focusing primarily on CD8 T cells. However, many patients fail to respond highlighting the need to diversify T cell-based therapies. Recent progress has demonstrated that CD4 T cells regulate antitumor immunity through orchestrating immune responses and directly killing cancer cells. We previously identified a novel population of CD4+ T cells with high expression of the molecule CD26, which are unique and distinguishable from other subsets of CD4+ T helper cells (Th1, Th2, Th17, Treg). CD4+ T cells effector functions are regulated by transcription factors and cytokine polarization. However, the polarizing cytokines and master transcription factor for CD26highCD4 T cells have yet to be identified. Here, we demonstrate that the combination of IL12, IL-7, and IL-18 drive differentiation of naïve CD4 T cells into CD26high CD4 T cells and these cells uniquely express transcription factor RUNX2. Inhibition of RUNX2 DNA binding prevents differentiation of CD4 T cells into CD26high cells. Bulk RNA sequencing and flow cytometry revealed that cytotoxic molecules (GZMB, GZMH, IFNy, Perforin) expressed by CD26high CD4 T cells requires RUNX2 transcriptional activity. RUNX2 also regulates the expression of IL12, IL-7 and IL-18 receptors on CD26high CD4+T cells. Further, CD26high polarizing cytokines can enhance killing ability of CAR-T cells. Strategies to enhance RUNX2 expression in and generate CD26highCD4+ T cells should be further investigated to improve the efficacy of T cell-based therapies for solid tumors.

Abstract 47

Investigating how host TLR5 signaling modulates FLT3L therapeutic efficacy for ovarian cancer treatment

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Immunotherapies rarely induce a therapeutic response for ovarian cancer. We have identified that toll-like receptor 5 (TLR5) signaling, the only known ligand of which is bacterial flagellin, is a host-intrinsic factor that orchestrates the failure of checkpoint therapy for ovarian cancer. Mechanistically, ovarian tumors induce chronic gut leakage, enabling dissemination of flagellin into the ovarian tumor microenvironment (TME) and disrupting the accumulation of anti-tumorigenic dendritic cells (DCs). One strategy to overcome this deficit is to promote further expansion of DCs via administration of the DC growth factor fms-like tyrosine kinase receptor 3 ligand (FLT3L). Although no benefit was observed for wild type (WT) mice with intact TLR5 signaling, an observation consistent with poor clinical efficacy of FLT3L therapy, FLT3L administration in TLR5-deficient (TLR5KO) mice resulted in prolonged and durable survival for over 80% of animals when combined with PD-L1 blockade. Current investigation aims to identify how TLR5 signaling is disrupting therapeutic efficacy. We found FLT3L therapy successfully expands cDC1s in multiple tissues of tumor bearing animals, but there is no observed significant difference between WT and TLR5KO mice. However, the signaling landscape of the tumors and TME of FLT3L treated TLR5KO mice is distinct, with increased levels of cytokines and chemokines that are suggestive of enhanced effector recruitment and function. Current studies aim to understand what cells are responsible for the production of these molecules and to disentangle how both FLT3L therapy and TLR5 signaling are involved in their ability to control ovarian tumor growth and extend survival.

Abstract 48

Peptide Repertoire Divergence Shapes T Cell Cross-Reactivity Among HLA-A*02 Second-Field Variants

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Certain second-field HLA mismatches (e.g., A*26:01 vs. A*26:02) increase GvHD risk, but the mechanism remains unclear. We hypothesize that some pathogenic second-field mismatches generate more divergent peptide repertoires; since thymic negative selection removes TCRs recognizing native self-peptidome, altered peptide presentation may trigger alloreactivity. To test this, we studied kidney transplant patients with alloreactive TCRs against A*02:01 and predicted that cross-reactivity to A02 variants (A*02:05, A*02:06, A*02:07) would correlate with peptide-repertoire overlap with A*02:01.

K562 cells-based artificial APCs expressing A*02:05, A*02:06, or A*02:07 were used to stimulate biopsy-derived T cells from A2-null recipients rejecting an A*02:01+ graft. IFN-γ ELISPOT assays quantified recall responses to A*02:01 and cross-reactivity to variants. Computationally, using the Human Ligand Atlas, Human Protein Atlas, and A*02:01-eluted peptides from K562 cells (Pyke et al.), we identified 115 A*02:01-restricted peptides as candidate targets. NetMHCpan predicted which peptides could also bind each variant, estimating peptidome overlap.

ELISPOT results showed strong recall to A*02:01 and graded cross-reactivity (A*02:06 > A*02:05 > A*02:07). Computational predictions matched this pattern: the proportion of peptides predicted to bind each variant followed the same ranking. These findings support a link between peptide-repertoire overlap and cross-reactivity, suggesting that differences in peptide presentation underlie variable alloreactivity in second-field mismatches.

Abstract 49

Dissecting Transcriptional Regulation of Macrophage Polarization in Breast Cancer

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Pro-tumor Tumor-associated macrophages (TAMs) inhibit the anti-tumor immune response and promote tumor growth in breast cancer. Efforts to deplete or repolarize pro-tumor TAMs towards an anti-tumor phenotype have met with limited clinical success. Our project aims to understand the regulatory logic governing TAM differentiation in breast cancer. To identify relevant transcriptional networks regulating TAMs differentiation in human breast cancer, we performed single-cell RNA-seq and ATAC-seq on human breast cancer biopsies. Single-cell transcriptional and epigenetic analyses of TAMs identified canonical transcription factors (TFs) known to be important for anti-tumor or pro-tumor macrophage differentiation. We also identified novel TFs that might regulate macrophage states and selected MAZ (MYC-Associated Zinc Finger Protein), which we found was enriched in pro-tumor TAMs, for further study. Using CRISPR-Cas9, we deleted MAZ from mouse bone marrow-derived macrophages (BMDMs) or human monocyte-derived macrophages. We observed no difference in macrophage survival or cell surface markers after deletion of MAZ. However, whereas negative control macrophages efficiently inhibited CD8+ T cell proliferation, MAZ KO macrophages were much less immunosuppressive. Transcriptomic profiling via bulk RNA-seq revealed that MAZ deletion leads to the downregulation of proliferation related pathways and upregulation of interferon response pathways. However, loss of MAZ did not alter the global chromatin landscape of MAZ KO macrophages. Ongoing studies will investigate the role of MAZ for TAM differentiation and function in vivo and dissect its mechanism of action. This work will hopefully allow us to identify new immunotherapeutic approaches to target TAMs in breast cancer.

Abstract 50

Determining the role of epithelial Trm during recurrent HSV-1 infection

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Herpes simplex virus type 1 (HSV-1) infects epithelial sites then establishes lifelong persistent (latent) infection in sensory neurons, affecting 67% of people globally. The greatest burden of HSV-1 is from recurrent disease (viral reactivation). CD8+T cells migrate to epithelial tissues during acute infection, where a subset differentiates into tissue resident memory T cells (Trm) that remain and provide local protection; the role of Trm in controlling HSV-1 reactivation and recurrent disease are not fully understood. We developed a murine model of HSV-1 recurrent disease, in which mice are inoculated on flank skin then viral reactivation is induced via fur plucking to produce recurrent skin lesions. This reactivation model is highly tractable, but the mechanism(s) by which fur plucking induces reactivation are not yet defined and could include local depletion of immune cells associated with hair follicles. We hypothesize that plucking removes HSV-specific Trm in the skin, allowing for viral reactivation, and that Trm control lesion severity. We inoculated WT C57BL/6 mice with HSV-1 NS, and divided mice equally based on acute lesion areas. We re-plucked/reactivated one group, then, 48 hours later, shaved the second group and harvested inoculation site and distal skin of all mice. Spectral flow immunophenotyping findings suggest that antigen-specific CD8+Trm (Ly6C/CD69/CD103+) are 1) concentrated in tissue proximal to the site of inoculation versus distal skin ($p=0.0635$) and 2) decreased in the skin of plucked/reactivated mice. Despite comparable activated CD8+T cells (CD44+) proportions, we observed a decrease in activated CD8+Trm in reactivated skin compared to latent skin ($p=0.0571$).

Abstract 51

Interrogating the role of ADRB2 on microglia phenotype and antitumor immunity in brain metastases

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Brain metastases are the most common adult intracranial tumor and carry a poor prognosis. Conventional treatment options pose a risk of cognitive impairment and efficacy is poor compared to extracranial tumors which highlights the need for improved therapeutic interventions. Previous retrospective studies have observed that patients on beta-blockers during their treatments had a decreased mortality rate across a wide variety of cancers. We have shown that a similar trend is observed in the context of brain metastases, and patients on beta-blockers have an increased overall survival compared to patients not on beta-blockers. We are interested in examining the role of beta-adrenergic signaling in the brain metastasis microenvironment. Previous publications have examined beta-adrenergic signaling in myeloid-derived suppressor cells in the context of peripheral tumors, and my work has shown that the primary immune cell expressing beta-adrenergic receptors, specifically ADRB2, in brain metastasis is the brain resident macrophage, microglia. Microglia exposed to inflammation in-vitro show signs of activation as well as an increase in ADRB2 expression which could be targeted to enhance the anti-tumor immune response. The goal of this work is to further elucidate the role that ADRB2 is playing in the activation of microglia and anti-tumor immune response and how targeting this pathway can synergize with other treatments to enhance the efficacy of immunotherapy. Future experiments will use human brain metastasis samples, in-vitro co-cultures, and genetically engineered mouse models to further assess the phenotypic and functional consequences of ADRB2 signaling in microglia that are infiltrating brain metastases.

Abstract 52

Osteopontin spatially rewires tumor-immune niches to promote colon cancer liver metastasis

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Osteopontin (OPN) is a secreted phosphoprotein that regulates tumor cells, myeloid cells, and T cells, and is a known biomarker of colorectal cancer (CRC) progression and liver metastasis. The roles of host-produced versus tumor-produced OPN in disease progression are unclear. Using a 2x2 genetic knockout mouse model, we found that loss of OPN in either compartment alone is sufficient to increase T cell infiltration and suppress liver metastasis, while dual knockout leads to further disease suppression. We applied CosMx spatial transcriptomics to mouse liver metastasis tissues, profiling 1,000 selected genes across 1,168,484 segmented cells. Tumor-derived OPN drives cancer cell proliferation via autocrine MEK/ERK signaling and sustains an immune-inactive niche enriched for highly proliferative tumor cells. Host-derived OPN licenses monocyte-to-macrophage differentiation, while OPN within the tumor microenvironment polarizes macrophages toward an immunosuppressive, M2-like state. Critically, host and tumor OPN thus cooperate to control monocyte fate and enforce an immunosuppressive microenvironment. Loss of host OPN unlocks a spatially distinct, interferon-driven anti-tumor niche characterized by cytotoxic, non-exhausted T cells and IFN-responsive monocytes. Translationally, OPN blockade immunotherapy suppressed tumor growth and enhanced immune infiltration in both syngeneic and humanized patient-derived xenograft mouse models of CRCLM. Together, these data identify tumor- and host-derived OPN as nonredundant drivers of tumor progression and support OPN as a promising target for CRC liver metastasis.

Abstract 53

IRF8 regulates T cell differentiation and anti-tumor effector function

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Interferon regulatory factor 8 (IRF8) is a myeloid cell lineage-specific transcription factor that also regulate B cell differentiation under physiological conditions. Under pathological conditions such as cancer, IRF8 acts as a negative regulator of myeloid-derived suppressor cells (MDSCs) to regulate anti-tumor immunity. However, the function of IRF8 in regulation of the adaptive immune response in the tumor microenvironment is incompletely understood. We therefore aimed at building a single cell atlas of IRF8-regulated immune cell differentiation in the tumor microenvironment using genome-wide single-cell multiomics. Unsupervised clustering in combination with maker-based annotation identified 12 major cell subpopulations in tumor-infiltrating leukocytes. IRF8 deficiency resulted in increased myeloid-derived suppressor cells (MDSCs) and memory T cells, but decreased plasmablasts, dendritic cells, and effecter T cells. Pathway enrichment analysis indicates that IRF8 primarily regulates multiple metabolic pathways in plasmablasts. In addition, IRF8 deficiency enhances TCR signaling, MTOC1 signaling, IFNγ signaling, and T cell dysfunction in tumor-infiltrating effector T cells, but not in memory T cells. This hyperactivation is correlated with increased activation of apoptosis pathways in effector T cells. Our findings determine that IRF8 regulates the plasmablasts-effector T cell axis in the tumor microenvironment, and loss of IRF8 expression leads to decreased plasmablasts and hyper activation and dysfunction of effector T cells, resulting in effector T cell elimination and tumor immune escape.

Abstract 54

RETRACTED Peroxisomes dictate anti-tumor immunity within the tumor microenvironment

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Peroxisomes are small, single-membrane bound organelles critical for balancing cellular redox homeostasis through catabolism of very-long-chain fatty acids and catalase-driven reactive oxygen species (ROS) detoxification. Recently, our group found lung macrophages require peroxisomes to dampen respiratory viral inflammation and promote alveolar regeneration following infection (Science 2025). We hypothesized that other immune cell types may also require peroxisomes for optimal functionality. While T cell peroxisomes were dispensable for respiratory anti-viral immunity, we found that loss of T cell peroxisomes impairs anti-tumor immunity and response to immune checkpoint blockade (ICB). The requirement for T cell peroxisomes is local, as peroxisomes are upregulated upon tumor microenvironment (TME) entry and promote T cell survival and redox balance. Conversely, we have found tumor cells also require peroxisomes, as peroxisome knockdown results in decreased tumor growth and increased CD8+ T cell presence within the TME. This dual-role for peroxisomes to enhance adaptive anti-tumor immunity and support tumor growth has led us to hypothesize that there is a peroxisome-mediated “competition” underpinning the magnitude of local anti-tumor immunity. Bulk RNA-seq analysis of TCGA-SKCM biopsy samples associates a peroxisome low / T cell high tumor signature with better 5-year overall survival, further suggesting a nuanced role for peroxisomes to simultaneously regulate pro- and anti-tumor function. Future work aims to understand the cellular crosstalk between tumor cells and T cells that regulates peroxisome expression in each cell type and identify methods of therapeutically targeting tumor cell peroxisome destruction and/or T cell peroxisome enhancement to improve ICB efficacy and anti-tumor immunity.

Abstract 55

Spatiotemporal Coordination of Kindlin3 and Talin1 during β2 integrin activation

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Neutrophil recruitment involves a multi-step adhesion cascade crucial for innate immunity and depends on the activation of β2 integrins. The cytoplasmic adaptor proteins, kindlin3 and talin1 regulate the β2 integrin activation by binding to its β cytoplasmic tail, yet the sequence of their recruitment under physiological conditions remain poorly understood. To address this, we generated double knock-in mice expressing fluorescently tagged mScarlet-kindlin3 and eGFP-talin1, which enabled real-time co-visualization of these adaptor proteins during β2 integrin activation. TIRF microscopy analysis of primary neutrophils revealed a novel two-stage recruitment sequence, wherein kindlin3 was recruited first during rolling and within seconds of arrest, followed by talin1 recruitment after firm adhesion. PI3K inhibition selectively reduced kindlin3 membrane recruitment without affecting talin1 recruitment, resulting in both impaired neutrophil spreading and full activation of β2 integrin. In conclusion, this study refines current paradigms of β2 integrin activation by providing real-time evidence of unexpected sequential recruitment of kindlin3 and talin1 during the leukocyte adhesion cascade.

Abstract 56

Combination CD40 and TLR3 agonism drives durable anti-tumor immunity through cDC1-mediated intracranial and peripheral CD8+ T cell stimulation in a murine model of glioblastoma.

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Glioblastoma, an aggressive primary CNS malignancy, remains uniformly fatal despite multimodal therapy. Although T cells can target CNS antigens and kill tumor cells directly, they fail to do so effectively in GBM. This is likely due to a severely immunosuppressive tumor microenvironment that impedes antigen presenting cell-mediated T cell mobilization and activation. Given the demonstrated synergy between CD40 and TLR agonists in activating dendritic cells, we hypothesized that combined CD40 and TLR3 agonism could overcome this immunosuppression to potentiate dendritic cell-mediated T cell priming. Using a syngeneic, orthotopic CT2A-ZsGreen murine GBM model, we administered agonistic anti-CD40 antibodies and/or polyIC (TLR3 agonist) on an anti-PD-1 background. Combination therapy conferred superior, durable survival with a 30–50% complete response rate. Responders were protected from rechallenge and harbored putative tissue-resident memory CD8+ T cells months after tumor resolution. Tumor control required CD8+ T cells and cDC1s, whereas CD4+ T cells and intratumoral B cells were surprisingly dispensable. Combination therapy expanded stem-like and effector CD8+ T cells within brain tumors and the meningeal dural layer. Despite comparable early DC activation across treatment arms, only combination therapy increased effector (PD1+ GranzymeB+) CD8+ T cell numbers by day 5. Notably, combination therapy increased intratumoral effector CD8+ T cell numbers and improved survival even when T cell migration was pharmacologically impeded, though unrestricted migration conferred maximal benefit. These results have shed new light and cast the intracranial cancer immunity cycle as a promising target for immunotherapies, specifically through cDC1 activation.

Abstract 57

Dendritic cells control inflammation through glutaminase-dependent metabolism

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Dendritic cells are the most potent antigen presenting cells that prime and polarize T cells to elicit their effector functions. It has been shown that dendritic cells utilize glucose and fatty acids to meet their energy demands and to induce their immune functions. Recently, glutamine has been shown to enhance antigen presentation of dendritic cells to T cells. However, the mechanisms of how metabolization of glutamine affects dendritic cell immune functions and controls disease progression remain unknown.

Glutaminase (GLS), a rate-limiting enzyme converting glutamine into glutamate inside the cells, is known to balance the polarization of naïve CD4 T cells into Th1 and Th17 subsets and to support efferocytosis of macrophages in atherosclerosis. To determine the role of GLS-mediated glutamine metabolism in dendritic cells, we employed a dendritic cell specific GLS knockout mouse model to generate bone marrow-derived dendritic cells (BMDCs) in vitro. This led us find that GLS in TLR4-activated dendritic cells increases T cell proliferation through cytokine secretion, but independent of viability, antigen processing, and expressions of co-stimulatory or co-inhibitory molecules. Interestingly, naïve CD4 T cells activated with conditioned media from LPS-activated GLS KO BMDCs under iTreg skewing condition differentiated more into regulatory T cells than CD4 T cells provided with conditioned media from GLS WT BMDCs. Aligning with this in vitro result, our preliminary data from the models of skin and lung inflammation showed increased regulatory T cells in the skin draining lymph nodes and an increasing trend of Tregs in the lungs.

Collectively, our data suggest that GLS in dendritic cells is a novel immunometabolic regulator that promotes immunogenic responses and controls the development of Tregs in inflamed tissues. We propose GLS as a new pharmacological target to modulate dendritic cell function in antigen-mediated inflammatory diseases.

Abstract 58

Chromobacterium violaceum bacterial effectors disarm neutrophil defenses igniting innate granuloma formation

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Our lab discovered that an environmental pathogen, Chromobacterium violaceum, surprisingly triggered the formation of granulomas in mice livers and eventually the bacteria is cleared by about 21 days. These C. violaceum-induced granulomas are composed of only innate immune cells. Before the granuloma forms, the initial immune response is to recruit a swarm of neutrophils, creating a microabscess within 24hrs. The granuloma forms days later. Remarkably, we see that C. violaceum survives and replicates despite this neutrophil (polymorphonuclear leukocytes; PMNs) swarm. To defeat neutrophils, the most toxic immune cell type, is something more characteristic of the causative agent of the bubonic plague (Yersinia) and is not a characteristic that would be expected from a soil microbe like C. violaceum. We hypothesize that the defeat of neutrophils is the key event that necessitates the granuloma response, which would reveal that the purpose of a granuloma is to counteract a pathogen that can survive within a neutrophil swarm. We are investigating how C. violaceum bacterial effector, CopH, inhibits intracellular ROS production in PMNs. Overall, we seek to understand how C. violaceum defeats the neutrophil swarm and that PMNs detect this failure to initiate granuloma formation.

Abstract 59

T cell–B cell interactions contribute to chronic lung immune responses after viral infection.

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Respiratory viral infections such as influenza and SARS-CoV-2 can lead to long-term lung pathology even after the acute phase resolves, particularly in aged individuals. Persistent inflammation and fibrosis contribute to impaired lung function, but the immune mechanisms driving these changes remain unclear.

Using a model of age-associated chronic lung disease following influenza infection, we observed a significant expansion of lung-resident B cells and PD-1^Hi CD4■ T resident helper cells (TRH), a population with features of both T follicular helper and tissue-resident memory cells.

To determine whether CD4■ T–B cell interactions sustain chronic immune responses, aged mice were treated with blocking antibodies against CD40L and ICOSL after resolution of acute infection. Disruption of these pathways reduced adaptive immune cell accumulation in the lungs, including decreased numbers of total B cells, CD4■ T cells, and age-associated B cells (ABCs). In addition, the frequencies of IL-21– and IFN-γ–producing CD4■ T cells were altered following blockade. Histological analysis revealed reduced dysplastic and fibrotic lung regions in treated mice. To further investigate the role of TRH cells, we used CD4-specific inducible Bcl6-deficient mice, in which TRH differentiation is impaired. These mice showed a marked reduction in total and antigen-specific TRH cells and exhibited significantly decreased inflammatory and fibrotic lung pathology compared to controls.

Together, these findings indicate that CD4■ T–B cell interactions contribute to the maintenance of chronic immune responses after viral infection. Targeting these interactions may represent a strategy to limit lung pathology, particularly in aged individuals.

Abstract 60

Identifying the correlates of protective adaptive immunity to Cryptosporidiosis

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Cryptosporidium infection causes diarrhea and is associated with growth faltering in Bangladeshi infants living in food-insecure households. We are conducting a natural history study of cryptosporidiosis in infants residing in a high–transmission-intensity urban community in Dhaka, Bangladesh, using stool surveillance and twice-weekly household visits to monitor infection and diarrheal incidence. We found that immunity following a first Cryptosporidium infection was incomplete, and reinfections were common. However, in older children with repeated infections, the disease was predominantly subclinical. The incidence of Cryptosporidium-attributed diarrhea declined from a peak of 0.19 episodes per child at 1–2 years of age to 0.05 episodes per child at 3–4 years of age. Prior work has shown that total anti-Cp23 and anti-Cp17 IgG is protective in this population. We therefore assessed whether Fc receptor–mediated antibody functions contribute to protection. Given that IgG1 is the predominant subclass mediating these responses, we measured antigen-specific IgG1 levels and FcγRIIIa binding. IgG1 responses to Cp23 and Cp17 variants (Ia and IIa) were associated with protection (Gehan-Breslow-Wilcoxon test; p=0.05, 0.05, 0.07). Although Fc receptor binding strongly correlated with IgG1 levels ($p<0.0001$), it was not associated with protection in one-year-old infants. These findings suggest that other IgG1-mediated effector functions may play a more important role. Future studies will evaluate parasite adhesion and antibody-dependent complement activation as correlates of protection.

Abstract 61

A non-redundant NFkB regulatory architecture governs CD8 T cell fate during exhaustion

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Harnessing CD8 T cell antitumor activity has become the cornerstone of cancer immunotherapy, yet the challenge of acquired T cell dysfunction, or exhaustion, remains a critical barrier to durable responses. Resolving the molecular regulators of exhaustion fate decisions offers a path toward engineering T cells that resist or reverse dysfunction in the tumor microenvironment. While the transcription factor family NFkB has been implicated in T cell activation and survival, its role in regulating exhaustion fate decisions remains poorly defined. Here, we combine high dimensional spectral flow cytometry, new molecular tools, and single-cell RNA sequencing to systematically dissect the consequences of NFkB perturbation in exhausted CD8 T cells. Using an inducible adoptive transfer system, we individually and combinatorially targeted NFkB family members (RelA, RelB, cRel, NFKB1) to define their distinct contributions to exhaustion fate specification. Using these tools, we resolved how NFkB family members regulate discrete paths of exhaustion differentiation, and found that manipulation of the NFkB pathway can be leveraged to reprogram T cell exhaustion in infection and cancer. Together, these findings establish NFkB signaling as a critical regulator of exhaustion fate progression and represent a potential target for enhancing T cell responses in cancer.

Abstract 62

Tfam-mediated metabolic perturbation in RORgt+ lymphocytes impacts intestinal tissue homeostasis and promotes GATA3+RORgt+ innate lymphoid cells

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Rorc-mediated deletion of Tfam, a mitochondrial transcription factor, causes tuft cell and type 2 immunity-driven small intestine (SI) lengthening in mice. Here, we report an indispensable role for T helper 2 (Th2) cells in this process. High-fat diet (HFD) reverts SI lengthening in Tfamf/fRorc-cre mice by suppressing IL-13-producing Th2 cells and IL-17-producing Th2 cells and group 2 innate lymphoid cells (ILC2s). HFD reduces conventional GATA3–/loRORgt+ ILC3s but promotes GATA3+RORgt+ ILCs, especially in Tfamf/fRorc-cre mice. Compared with conventional ILC3s, GATA3+RORgt+ ILCs express type 2 cytokines and increase cell proliferation but decrease cell death with metabolic re-programming. Single-cell transcriptional analyses indicate that GATA3+RORgt+ ILCs represent a distinct population, different from IL-17 natural ILC2s, IL-17+ inflammatory ILC2s, or conventional ILC3s. GATA3+RORgt+ ILCs produce IL-17 but not IL-22, resulting from competition of the aryl hydrocarbon receptor (Ahr) with GATA3 at the Il22 locus. Tfam f/fRorc-cre mice on HFD have worsened dextran sodium sulfate (DSS)-induced colitis. These data highlight the role of ILC metabolism in intestinal tissue remodeling and inflammation.

Abstract 63

H-FIRE Triggers Antigen-Dependent CD8 T-Cell Activation and Abscopal like Immune Effects in Pancreatic Cancer

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Pancreatic tumors are aggressive to treat due to their immunosuppressive tumor microenvironment. High-Frequency Irreversible Electroporation (H-FIRE) is a non-thermal ablation modality that destroys cancer cells by creating permanent nanopores in the cell membrane, disrupting ionic homeostasis and inducing cell death. Although H-FIRE has shown efficacy in local tumor control, its immune effects are not fully understood. We investigated whether and how H-FIRE induces both local and systemic anti-tumor immune responses in pancreatic cancer. C57BL/6 mice bearing bilateral subcutaneous Pan02 tumors received H-FIRE treatment to only one tumor. Tumors were harvested at 4 timepoints post-treatment for histological analysis and flow cytometric profiling of immune cells. To assess antigen presentation, bone marrow-derived dendritic cells were cultured with Pan02 cells ablated by H-FIRE and other ablation modalities, then co-cultured with CFSE-labeled naive CD8 T-cells to evaluate dendritic cell-mediated T-cell proliferation. The tumor growth curve revealed that H-FIRE-treated tumors showed complete regression within 4 weeks, while contralateral tumors initially regressed but later relapsed, suggesting an abscopal-like effect that was not sustained for long term. Histology revealed well demarcated ablation zone, hemorrhage, and vascular recovery over time. Flow cytometry showed increased infiltration of pro-inflammatory immune populations in treated and contralateral tumors, followed later by increased immunosuppressive populations corresponding with tumor relapse. In the antigen-presentation assay, H-FIRE-ablated Pan02 cells induced strong CD8 T-cell proliferation, comparable to other modalities. These findings suggest that H-FIRE induces a pro-inflammatory and antigen-presenting tumor microenvironment, but sustained systemic immunity may require combination strategies to prevent relapse and improve abscopal effect.

Abstract 64

Enhancing response in MHC-I low triple negative breast cancer with diacylglycerol kinase a/z inhibitors and STING activation

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Overcoming resistance to immune checkpoint blockade and expanding therapeutic benefit to more patients is a critical next step in advancing care in triple-negative breast cancer (TNBC). TNBCs downregulate MHC-I to suppress anti-tumor immunity and patients with decreased antigen presentation have worse outcomes to ICB. Therapeutic strategies to overcome MHC-I downregulation are of high translational relevance and interest.
Diacylglycerol kinase a/z inhibitors (DGKis) amplify T cell responses to decreased levels of antigen presentation and low affinity stimulation in vitro and enhances response to ICB in semi-responsive models. We hypothesized that DGKis will improve response to ICB in TNBC with low MHC-I expression by improving CD8 T cell response to decreased antigen presentation. This study is designed to assess how diacylglycerol kinase inhibitors can be used to improve responses to immune checkpoint blockade (ICB) in patients with downregulated MHC-I expression.
We created a novel model of MHC-I titration by utilizing microRNA-mediated silencing to create isogenic cell lines with differential levels of MHC-I expression – the miSFIT cell lines. Treatment of miSFIT cell lines with DGKi + a-PD-L1 revealed no benefit, contrasting with previous in vitro findings showing enhanced T cell response at low levels of MHC-I with the addition of DGKi. However, low-MHC-I expressing tumors treated with DGKi in combination with a nanobody STING agonist resulted in 100% complete response rates and sustained immunologic memory response. Therapeutic benefit extended to additional models of TNBC. Thus, DGKi overcomes low MHC-I expression to enhance response when combined with a treatment to increase T cell recruitment.

Abstract 65

Naturally acquired immunity to Streptococcus pneumoniae is shaped by biofilm formation during asymptomatic carriage

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Nasopharyngeal colonization by Streptococcus pneumoniae (Spn) is a critical immunizing event and a prerequisite for transmission and invasive disease. Most commensal bacteria, including Spn, asymptomatically colonize the nasopharynx as biofilms, surface-attached communities encased in self-produced matrices with distinct antigenic profiles from planktonic bacteria. How biofilm formation influences adaptive immunity remains poorly understood.

We developed the Repeated Asymptomatic Murine Pneumococcal Colonization (RAMPC■) model using Spn serotype 2 strain D39, serotype 3 strain WU2, and serotype 4 strain TIGR4, allowing sequential, non-overlapping colonization. Nasal bacterial burden, systemic and mucosal IgG/IgA, and CD4■ T cell activation were assessed over multiple exposures. Parallel analyses of antibody was performed using sera from naturally colonized human volunteers.

Initial colonization produced robust carriage, whereas subsequent exposures led to reduced burden and accelerated clearance, consistent with emergent adaptive immunity. Humoral profiling revealed strong antigenic imprinting: the first colonizing strain largely dictated IgG/IgA specificity, with limited diversification after repeated colonization. Antibody reactivity was strongest against biofilm-associated antigens and correlated with in vitro biofilm capacity. Colonization with biofilm-deficient TIGR4 mutants (ΔpsrP, ΔcbpA, ΔspxB) elicited reduced IgG responses, and 10 days post-colonization, wildtype-colonized mice exhibited higher frequencies of CD4■CD44■ helper T cells compared to biofilm-deficient mutants. Human sera similarly recognized biofilm antigens preferentially, independent of capsule.

These findings highlight biofilm formation as a key determinant of humoral and helper T cell immunity, with implications for protein-based vaccine design.

Abstract 66

Themis integrates TCR, metabolic, and cytokine cues to control T cell development and maintain peripheral homeostasis

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TCR and cytokine signaling are essential for thymocyte development and peripheral T cell homeostasis. Themis regulates both pathways in developing thymocytes and mature T cells. Consistent with this role, Themis deficient mice fail to generate mature thymocytes due to defective positive selection, and late developmental defects contribute to reduced cytokine-driven homeostatic proliferation in the periphery. Prior studies showed that phosphorylation of Themis at Y34,regulated by the kinase Lck and the phosphatase SHP-1, is required to modulate TCR signaling during CD4■ single-positive (SP) thymocyte development. However, how Themis is regulated by cytokine signaling, particularly during CD8■ SP thymocyte development and in peripheral CD8■ T cell homeostasis, remains unclear.

To identify kinases that regulate Themis phosphorylation, we cloned candidate T cell expressed kinases and co-expressed them with Themis in 293T cells. In addition to Lck, Themis was phosphorylated by multiple kinases implicated in TCR signaling and insulin receptor signaling, and notably by several Janus kinase (JAK) family members involved in cytokine signaling. To map kinase-targeted tyrosine residues, we generated a panel of Themis mutants in which each of the 18 tyrosines was individually substituted with phenylalanine (Y→F), and quantified kinase dependent phosphorylation. These kinases phosphorylated Themis at multiple residues, including Y34. To investigate Themis localization and trafficking after TCR and/or cytokine stimulation, we also engineered a series of Themis–EGFP fusion proteins, including variants incorporating circularly permuted EGFP (cpEGFP) inserted at distinct positions. We identified three constructs that were stably expressed in T cells and retained functional features, as indicated by constitutive binding to Grb2.

Together, these results suggest that Themis is regulated by diverse kinase inputs and may integrate antigen receptor, metabolic, and cytokine signals to coordinate T cell development and maintain peripheral immune homeostasis.

Abstract 67

Rational Design of a Broad Betacoronavirus Vaccine from a Cryptic Neutralizing Epitope

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Current COVID-19 vaccines incompletely induce cross-reactive neutralizing antibodies against circulating SARS-CoV-2 variants, due to extensive mutations in neutralizing epitopes. Booster vaccinations using the full SARS-CoV-2 spike ectodomain matching current circulating strains inadvertently strengthen immunity towards epitopes shared with the original strain, most of which are non-neutralizing. Different vaccination strategies are needed to induce durable neutralizing antibodies to conserved epitopes and break the cycle of reinfection.
We developed a structure-based rationally-designed vaccine that boosts antibodies to a broadly neutralizing epitope in spike: heptad repeat 1 (HR1). HR1 conformational changes provide the driving force for spike-mediated membrane fusion; disrupting them blocks viral entry. HR1 is poorly immunogenic during typical vaccination or infection, likely due to steric hindrance. Our vaccine consists of a protein fragment composed only of HR1 stabilized by a critical engineered mutation. It forms multimers consisting of 12-24 copies of HR1, and we used it as a nanoparticle-like scaffold to present many copies of additional neutralizing epitopes by adding them to the N-terminus of HR.

We vaccinated mice with the protein-subunit vaccine and induced binding titers of 1:10^6 to the 80 amino acid HR1 epitope. Surprisingly, the vaccinated animals had very weak neutralizing antibody responses despite the high binding titers, suggestive of steric hindrance of vaccine-induced antibodies. Future studies will include adjusted vaccine designs and vaccine regimens to induce antibodies that are better able to bind HR1 on full spike, and further uses of HR1 as a scaffold to present additional epitopes.

Abstract 68

Antigen-Specific Memory B Cell Responses Reveal Distinct Functional Architectures Across Spleen and Lymph Node in Coronavirus Infection

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Studying heterologous immunity to endemic and emerging coronaviruses is critical for understanding host responses. Cross-reactive humoral immunity between seasonal betacoronavirus OC43 (HCoV-OC43) and SARS-CoV-2 has been described, yet the transcriptional landscape of B cells during natural OC43 infection remains poorly defined.

Here, we profiled antigen-specific memory B cells from an organ donor with documented OC43 infection and prior SARS-CoV-2 exposure. Isotype-switched, tetramer-positive B cells binding OC43 and SARS-CoV-2 spike proteins were isolated from mediastinal lymph nodes and spleen. Single-cell RNA sequencing of 14,306 cells was analyzed. Antigen specificity was assigned by tetramer-based demultiplexing, and analyses focused on antigen-positive cells (n = 8,635).

Nine transcriptionally distinct B cell subsets were identified, with antigen specificity non-randomly distributed across clusters. OC43-specific cells dominated effector subsets (86-97%), whereas recently activated B cells were strikingly enriched for SARS-CoV-2 specificity (80.9%), suggesting recall of cross-reactive or pre-existing memory. Cross-reactive cells were primarily activated memory (58.4%) with an APC-like component (22.7%). Tissue-specific analysis revealed distinct immune architectures: the spleen exhibited T-bet-hi-dominated responses across all antigen specificities, while lymph node was enriched for activated memory and APC-like subsets, with antigen-presenting B cells localized almost exclusively at the infection site (>98%). Cross-reactive B cells were preferentially generated in lymph node and enriched in proliferating (24.5% vs. 0% in spleen) and resting (22.3% vs. 0%) subsets.

This single-cell analysis reveals that OC43 infection induces a compartmentalized memory B cell response, providing insight into how memory responses adapt to repeated endemic coronavirus exposure and newly encountered viruses across anatomical sites.

Abstract 69

Role of IL-1b signaling in preventing the progression of primary amoebic meningoencephalitis

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Naegleria fowleri is the main cause of primary amoebic meningoencephalitis (PAM), a nearly always fatal condition. Infection generally occurs by inhalation of contaminated warm freshwater. Once Naegleria fowleri comes into contact with the olfactory epithelium, it migrates along the axons of olfactory sensory neurons and reaches the olfactory bulb. Its presence in the brain induces an extensive inflammation with infiltration of monocytes and neutrophils. To this day, there is still no effective cure against PAM.
To develop effective treatments against PAM, it would be necessary to better understand the underlying pathology and the components of the host immune response that could prevent amoeba progression in the brain and/or reduce excessive immune activity.
This study shows that neutrophils and IL-1b are instrumental in counteracting Naegleria fowleri infection. In our context, this cytokine is mainly produced by hematopoietic immune cells. Moreover, it is IL-1b signaling within the non-hematopoietic compartment that limits PAM progression. Additionnally, IL-1b signaling plays an important role in the efficient recruitment of inflammatory immune cells inside the infected brain, however, it does not directly slow the final leukocyte transmigration process. Ongoing experiments show that IL-1b signaling is involved in an increase of local expression of adhesion molecules such as VCAM-1 and ICAM-1 on the endothelial compartment, enabling firm leukocyte adhesion and subsequent diapedesis, as well as “protective” BBB breakdown.

Abstract 70

Functional features of HLA-E restricted CD8 T cell responses associated with spontaneous resolution of Chlamydia trachomatis infection

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CD8 T cell responses in humans recognize peptides presented by Human Leukocyte Antigen class I (HLA-I) alleles-classical and non-classical. HLA-E is a well-studied non-classical allele and represented by two major functional alleles (E*01:01 and E*01:03) covering >99% of all human populations making this allele an attractive target for vaccine design. Prior work showed that HLA-E restricted CD8 T cells (E-CD8s) exert an important immune-regulatory role in control of several intracellular bacterial pathogens such as Mycobacterium and Salmonella. However, the role of E-CD8s in Chlamydia trachomatis (CT) persistence or spontaneous resolution remains unknown. To address this, we predicted 19 optimal HLA-E binding peptides from two immunogenic (MOMP and Pgp3) CT proteins. Most peptides tested stabilized HLA-E*01:01/03 expression on the cell surface and formed stable pHLA-E monomers in a peptide exchange-based ELISA. Tetramers made were used to test a cohort of CT persisters and resolvers (N=32). Although CD8+ T cells recognized multiple E-restricted CT epitopes ex vivo in both groups, the overall recognition frequency did not differ. Nevertheless, unique HLA-E peptide recognition repertoires were observed for each group. Specifically, 2 epitopes (one each from MOMP and Pgp3) were consistently recognized at significantly higher frequency in spontaneous resolvers ($p < 0.05$). These E-CD8s were more activated and a higher percentage of these were effector memory. In addition, polyfunctional E-CD8s were also de novo primed from healthy CT seronegative donors. Taken together, these novel data provide a role of E-CD8s in CT resolution and identifies CT antigens for a future CD8 T cell-based vaccine.

Abstract 71

Targeting NADPH Oxidase 1 to Overcome T cell Exhaustion in T cell Immunotherapy

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T cell exhaustion within the immunosuppressive tumor microenvironment remains a primary barrier to successful adoptive cell therapies. Through a genome-wide CRISPR screen, we identified NOX1 as a novel negative regulator of CD8+ T-cell function. We hypothesized that NOX1 suppresses signaling downstream of the T cell receptor (TCR) and that its inhibition could enhance the efficacy of next-generation CAR T therapies. To test this, we used CRISPR-Cas9 to generate NOX1-knockout (KO) human T cells. Analysis revealed that NOX1 KO significantly reduced basal reactive oxygen species (ROS) and resulted in a stronger, more sustained TCR-induced calcium flux. This enhanced proximal signaling translated into superior functional performance, including increased cytotoxicity against melanoma cells and elevated effector cytokine production. These results were further validated in a syngeneic in vivo mouse model, where NOX1 deficiency led to significantly improved anti-tumor efficacy and survival. Our findings demonstrate that NOX1 functions as a critical redox-dependent checkpoint that imposes a threshold on T-cell activation by limiting calcium flux. By showing that NOX1 deletion overcomes these metabolic and signaling constraints, this work establishes NOX1 as an important therapeutic target. Inhibiting NOX1 provides a promising strategy to enhance the durability and potency of T cell therapies, offering a robust method to overcome exhaustion in the fight against cancer.

Abstract 72

Pancreatic cell-intrinsic and innate immune response to acute viral infection of the mouse pancreas

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The pancreas is a complex organ comprised of exocrine and endocrine cells that aid in digestion and the regulation of blood glucose. When one or more of these compartments is dysregulated due to genetic and/or environmental triggers, diseases like type 1 diabetes and chronic pancreatitis can develop. Coxsackievirus B (CVB) is a shared environmental trigger of both endocrine type 1 diabetes and exocrine pancreatitis. Despite this, the cell-intrinsic and innate immune responses to pancreatic CVB infection are poorly understood, limiting our understanding of how infection contributes to disease. We developed a mouse model of CVB3 infection and performed high-definition spatial transcriptomics to define these responses. We found that CVB3 primarily infects pancreatic acinar cells and some peri-islet cells, whereas β-cells remain uninfected throughout the course of infection. We found that acute infection triggered upregulation of both canonical interferon (IFN) stimulated genes (ISGs) and Reg family genes in acinar cells; Regs were also enriched in β-cells despite them not being infected. These data suggest that Reg responses may act independently of IFN stimulation, which have not been previously implicated in CVB infection in the pancreas. To test these hypotheses, we are using IFN receptor knockout mice to determine which IFN induce ISGs in acinar cells, whether Reg upregulation is IFN-independent, and what role Regs are playing in the response to infection. Defining the cell-intrinsic and innate immune responses to CVB infection is crucial to understanding how they trigger the progression of chronic pancreatic diseases and inform therapeutic development for clinical intervention.

Abstract 73

TTP is a Novel Sex-Specific Regulator of NGF

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Approximately 80% of patients report mild to severe postsurgical pain, which can impede recovery, contribute to opioid dependency, and transition to chronic pain. Postsurgical pain is driven by inflammatory mediators, which are regulated at the posttranscriptional level via Adenine- and uridine-rich elements (AREs) in the 3’ UTR of their mRNA. ARE-binding proteins (AUBP) recognize these cis-acting elements and control mRNA stabilization or translation efficiency. Tristetraprolin, an AUBP, promotes mRNA decay by recruiting deadenylation factors. Nerve Growth Factor (NGF) is an inflammatory mediator that plays a major role in peripheral pain sensitivity as well as the development of chronic pain. We hypothesize that NGF mRNA is a novel target of TTP. Postsurgical incision in myeloid-specific TTP KO mice showed an increase in NGF expression and elevated mechanical sensitivity. In silico analysis of the 3’ UTR of NGF presented a putative TTP-specific ARE binding site (UAUUUAU). Additionally, NGF expression in LPS-treated bone-marrow-derived macrophages (BMDM) was more elevated in males compared to females under TTP KO conditions. In conclusion, NGF is significantly upregulated in TTP KO BMDM in a sex specific manner. With NGF being a modulator for peripheral pain development, it is crucial to understand the mechanisms of ARE-mediated regulation by TTP for developing therapeutic interventions. In future studies, using a metagenesis approach, we aim to further understand the role of AREs and the 3’UTR of NGF in TTP-mediated posttranscriptional regulation.

Abstract 74

Characterizing the role of astrocytic IL-18R signaling in neuroinflammation in Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) characterized by aberrant immune attacks on myelin and progressive neurological dysfunction. IL-18 receptor (IL-18R) been implicated in MS pathogenesis. IL-18R is broadly expressed across immune and non-immune cell types, including T cells, antigen-presenting cells, and CNS-resident astrocytes. A prior study deployed the Experimental autoimmune encephalomyelitis (EAE) model of MS and reported that while IL-18 knockout mice remain susceptible to EAE, mice lacking the IL-18Rα subunit are resistant to disease induction. This discrepancy is puzzling given that IL-18 is the only characterized ligand for IL-18R. Additionally, bone marrow chimera experiments excluded lymphocytic IL-18R deletion as the source of EAE resistance, suggesting myeloid or CNS-resident cells as the source of IL-18R responsible for this phenotype. Using immunofluorescence microscopy, we mapped IL-18R expression in naïve and EAE spinal cords. IL-18R is detectable at low levels in the naïve spinal cord but is upregulated during EAE. Among glial populations, IL-18R is preferentially expressed on GFAP■ astrocytes. In EAE, IL-18R is additionally expressed in CD45hi infiltrating immune populations. We therefore hypothesized that astrocyte-intrinsic IL-18R signaling plays a critical role in the initiation and amplification of autoimmune neuroinflammation. To test this, we generated astrocyte-specific IL-18R knockout mice using a GFAP-Cre strategy. Contrary to our expectations, astrocyte-specific deletion did not alter EAE onset, disease severity, or infiltrating immune composition in the brain or spinal cord, indicating that astrocytic IL-18R signaling is dispensable for EAE pathogenesis.

Abstract 75

Lung-Resident B Cell Immunity in Pulmonary Tuberculosis

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Protective immunity against pulmonary tuberculosis (TB) remains incompletely defined, particularly at the site of infection (the lungs), where local immune responses shape disease outcomes. We investigated how lung ectopic germinal centers (eGCs), TB stage-specific B cells, and antibody responses influence the control of Mycobacterium tuberculosis (Mtb) using a human-relevant BCG-NTM-Mtb mouse model and cross-species spatial analyses. We found that eGCs mature dynamically across TB progression: acute infection was marked by nascent B- and T-cell aggregates, early adaptive disease showed follicular dendritic cell organization, and late adaptive disease developed fully structured eGCs with light and dark zones. However, during chronic disease, eGCs were dysregulated, with excessive accumulation of B cells and follicular dendritic cells and reduced T-cell infiltration. Functionally, passive transfer of bronchoalveolar lavage fluid (BALF)-derived antibodies from early and late adaptive stages reduced pulmonary Mtb burden by 1.5-2 logs, whereas BALF antibodies from chronic disease failed to protect and instead worsened pathology. Notably, serum antibodies conferred little to no protection, highlighting the unique efficacy of lung-localized humoral immunity. Antigen profiling further showed that protective stages were associated with stronger antibody reactivity to Mtb membrane-associated proteins, whereas chronic disease remained dominated by lipid-reactive responses. Together, these findings show that progressive maturation of lung eGCs promotes the selection of protein-specific antibodies with protective effector function, whereas chronic eGC dysregulation drives the emergence of non-protective or pathogenic humoral responses. Defining these stage-specific antigen-antibody-effector interactions will provide a mechanistic framework for developing lung-targeted TB vaccines and antibody-based immunotherapies to achieve long-lasting mucosal protection.

Abstract 76

Title: Peroxisomal Control of Macrophage Senescence and Tissue aging

Xiaoqin Wei; Yueming Yuan; Jie Sun

Aging is marked by chronic inflammation, cellular senescence, and progressive loss of tissue homeostasis, but the initiating drivers of these changes remain poorly defined. Peroxisomes are critical regulators of cellular metabolic and redox balance, yet their role in mammalian aging has remained unclear. Here, using mouse models, we identify macrophage peroxisome dysfunction as an important contributor to age-related decline. Tissue-resident macrophages from aged animals exhibit impaired peroxisomal homeostasis across multiple organs, suggesting that peroxisome dysfunction is a conserved feature of macrophage aging. Moreover, macrophage-specific disruption of peroxisome function is sufficient to induce an age-dependent senescence program and accelerate systemic aging phenotypes, including increased frailty, heightened multi-organ inflammatory and senescence-associated pathology, and shortened lifespan. In contrast, pharmacologic enhancement of peroxisome biogenesis improves late-life health measures. Collectively, these findings establish macrophage peroxisomal dysfunction as a previously unrecognized driver of systemic aging and nominate restoration of peroxisomal homeostasis as a potential strategy to promote healthy aging.

Abstract 77

Non-canonical functions of PAD4 in regulating abdominal aortic aneurysm formation

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Abdominal aortic aneurysm (AAA) is characterized by abnormal dilation of the aorta, which poses a severe risk of aortic rupture with a high mortality rate. Peptidyl arginine deiminase 4 (PAD4)-mediated histone citrullination is known to regulate neutrophil extracellular trap (NET) formation. Other non-canonical functions of PAD4 independent of NET formation are less studied. Pharmacologic inhibition of the PAD family in AAA mouse models attenuates AAA rupture therefore implied a detrimental role of PAD4 in this disease. However, our preliminary results showed unexpected increase in AAA formation in PAD4 global knockout (Padi4-/-) mice. This observation is accompanied by reduced CD11b (integrin αM subunit) in circulating neutrophils from the Padi4-/- AAA mice. These results suggest a novel role of PAD4 in mediating neutrophil aggregation, and a protective role of PAD4 in AAA formation. Interestingly, neutrophil-specific PAD4 knockout mice (Mrp8CrePadi4fl/fl) showed decreased AAA formation, suggesting a detrimental role of neutrophil-expressed PAD4. Western blot analysis of the mouse aorta indicates intact PAD4 expression of non-neutrophil origin in the vascular wall. Meanwhile, confocal microscopy analysis of the en face Padi4-/- aorta revealed increased neutrophil accumulation at the AAA areas. Together, these results suggest previously unknown roles of PAD4 in different vascular cells in AAA. While PAD4 in neutrophils may promote inflammatory injury of vascular wall, loss of PAD4 in other cells may also affect other processes, such as systemic immune balance, leading to increased disease risk.

Abstract 78

Acute Fasting Suppresses Neutrophil Metabolism and Effector Function

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Acute fasting is common in perioperative care and may create a transient window of vulnerability to hospital-acquired infections. Because Staphylococcus aureus drives significant bacteremia in this setting, we asked how a short-term, 12 hours, fast alters neutrophil metabolism and antibacterial function. Human neutrophils exposed to fasting conditions displayed reduced glycolytic and mitochondrial activity and failed to mount an oxidative burst or release neutrophil extracellular traps in response to S. aureus. In mice, a single 12-hour fast markedly increased susceptibility to staphylococcal bacteremia. Notably, this was not explained by uniformly elevated tissue burdens; instead, in vivo readouts revealed tissue-specific neutrophil dysfunction consistent with impaired early containment and progression to excessive, nonproductive inflammation. Together, these data identify acute fasting as a previously underappreciated, rapidly induced state that suppresses neutrophil metabolism and effector function, thereby heightening vulnerability to severe infection in clinical contexts where patients undergo preoperative fasting.

Abstract 79

IL-26 increases Th1 effector polarization during mycobacterial infection

Danielle Lee, Christine E. Nelson

Tuberculosis (TB), a chronic infectious disease caused by Mycobacterium tuberculosis (Mtb), is the leading cause of mortality from a single infectious agent worldwide. The only licensed vaccine, Bacille Calmette-Geurin (BCG), provides limited protection in adolescents and adults. The reasons for this remain unclear but may reflect the inability of BCG-induced Th-1 type immune responses to generate durable protection against infection. As a result, there is growing interest in alternative T cell effector molecules that may offer better protection against Mtb, such as IL-26. IL-26, a member of the IL-10 cytokine family, is a candidate of interest due to its direct bactericidal and anti-mycobacterial properties. We have previously shown that IL-26 is enriched in granulomas isolated from Mtb infected rhesus macaques and negatively correlates with bacterial loads. We therefore hypothesize that IL-26 enhances bacterial control during mycobacterial infection resulting in a more protective T cell response. To test this, we have investigated the effect of IL-26 administration on CD4 polarization and bacterial loads in BCG-infected mice. The administration of IL-26 dimer did not impact the magnitude of BCG-specific T cell expansion, but did alter the differentiation phenotype of T cells in the lung. IL-26 treatment increased Th1 polarization, terminal differentiation, and dampened expression of tissue residency marker CD69. Despite these changes, IL-26 did not affect bacterial clearance. Overall, our findings indicate that IL-26 increases Th1 differentiation without enhancing protection. This suggests that IL-26 inhibition during mycobacterial infection could enhance protection by decreasing terminal differentiation and increasing tissue residency in the lung.

Abstract 80

Folate Receptor Beta Orchestrates Tumor-Associated Macrophage Immunosuppressive Functions

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The tumor microenvironment potentiates the immunosuppressive functions of tumor-associated macrophages (TAMs), dampening anti-tumor immunity and promoting resistance to immune-checkpoint blockade (ICB). The development of TAM-targeting immunotherapies is a crucially important approach to improve cancer patient outcomes. Previous studies have identified folate receptor beta (FRB) as a biomarker of pro-tumor TAMs associated with worse patient prognosis. FRB (gene: Folr2) mediates the uptake of extracellular folate. Folate is a requisite coenzyme for one-carbon (1C) metabolism, a pathway critical for the maintenance of cellular redox balance, nucleotide synthesis, and global methylation reactions. The importance of FRB-mediated folate uptake on TAM biology is unknown. Here we show FRB-mediated 1C metabolism supports pro-tumor macrophage functions through the control of reactive oxygen species (ROS). Novel Folr2-/- mice engineered by our lab exhibit decreased tumor growth, increased cytotoxicity of tumor-infiltrating CD8+ T-cells, and elevated immunostimulatory gene expression in TAMs relative to wild-type mice. Folr2-/- bone marrow-derived macrophages (BMDMs) produced more pro-inflammatory cytokines and possessed a decreased capacity to suppress T-cell activation compared to wild-type BMDMs. Interestingly, Folr2-/- BMDMs also generated increased ROS, were enriched for oxidative stress gene signatures and possessed decreased reduced glutathione metabolite pools, indicating a dysregulation of redox homeostasis. Inhibiting ROS via antioxidant supplementation reversed the pro-inflammatory phenotypes observed in Folr2-/- BMDMs. Together, these data suggest FRB-mediated 1C metabolism is critical for sustaining immunosuppressive functions of TAMs through mitigation of oxidative stress. Our studies underscore the importance of 1C metabolism as a central regulator of TAM biology and promising target for TAM-directed cancer immunotherapies.

Abstract 81

Overcoming triple-negative breast tumor suppression of MHC-II to restore sensitivity to immune checkpoint inhibitors

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Immune checkpoint inhibitors (ICI) leverage the cytotoxic capabilities of the immune system by sustaining T cell activation. However, patient responses remain heterogenous due to poorly understood mechanisms of therapeutic resistance. Although canonically associated with professional antigen presenting cells, triple-negative breast cancer (TNBC) has been shown to heterogeneously express tumor-specific MHC-II (tsMHC-II), where high tsMHC-II expression correlates with significant benefit from ICI.

ICI-resistant tumors avoid immune surveillance through epigenetically silencing critical antigen presentation genes; however, silencing of tsMHC-II in TNBC has yet to be rigorously explored. We hypothesize that epigenetic silencing drives ICI resistance through tsMHC-II loss, and that pharmacologic reversal of these mechanisms will restore tsMHC-II expression and resensitize tumors to ICI. This project will assess the role of epigenetic silencing proteins in shaping anti-tumor immunity and how combinatorial therapies could be used to restore ICI responses.

Screening of multiple human and murine TNBC cell lines for IFNγ-inducible tsMHC-II expression revealed heterogeneity in tsMHC-II induction. High-throughput screening (HTS) of epigenetic inhibitors in murine TNBC cells resistant to IFNγ-inducible tsMHC-II expression revealed histone methyltransferases (i.e. enhancer of zeste homolog-2 [EZH2]) and histone deacetylases (HDACs) most significantly restored IFNγ-inducibility of tsMHC-II. Further in vitro experiments targeting EZH2 and HDAC1/3 showed significant increases in IFNγ-inducible tsMHC-II expression in cells treated with epigenetic inhibitors. We intend to further characterize the epigenetic effects of EZH2 and HDAC1/3 inhibition in our cell lines and patient derived tissues, their effects on antigen presentation to CD4+ T cells, and their use in combination with αPD-1 in vivo.

Abstract 82

Influenza-Specific Lung-Resident Memory B cells assist CD4 Recall Responses during Challenge Infections

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In repeated influenza A virus (IAV) infections, lung-resident memory B cells (BRMs) enhance early recall responses upon re-exposure. However, in heterosubtypic infection models, antibodies produced by BRMs established during primary infection do not neutralize the challenge virus's hemagglutinin and neuraminidase surface glycoproteins, suggesting that BRMs have additional effector functions beyond neutralizing antibody secretion. In this study, we infected B cell-specific STAT1 knockout (B-STAT1 KO) mice with a sublethal dose of IAV/PR8 virus, followed by a challenge with IAV/X31 virus, and performed single-cell RNA sequencing on CD45.2+ lung immune cells. Our findings revealed that B-STAT1 KO mice, compared to wild-type C57BL/6 controls, failed to generate lung BRMs and had markedly reduced antibody-secreting cells (ASCs) post-challenge. Flow cytometry confirmed the absence of flu-specific lung BRMs in B-STAT1 KOs, along with significantly reduced flu-specific CD4+PD1hiCD11a+ and CD4+IFNγ+ effector T cells in the lungs after challenge. Additionally, ELISPOT assays showed significantly decreased flu-specific IgA and IgG ASCs post-challenge in B-STAT1 KOs. Functionally, B-STAT1 KO mice showed increased susceptibility to reinfection and delayed viral clearance compared to wild-type counterparts. These results imply that lung BRMs influence the effector functions of activated CD4 T cells during challenge infection. Interestingly, antibody-deficient B cell-specific BLIMP1 knockout mice, infected with IAV/PR8 and challenged with IAV/X31 42 days post-primary infection, maintained or even enhanced effector CD4 T cell responses following challenge, suggesting that BRMs, rather than ASCs, are involved in the early recall responses of activated CD4 T cells in the lung. To elucidate the underlying mechanisms, we used B cell-specific MHCII floxed mice with inducible MHCII deletion after tamoxifen administration. Deletion of MHCII in BRMs before IAV/X31 challenge resulted in higher peak viral titers and a significant reduction in flu-specific CD4+PD1hiCD11a+ effector T cells, along with a reduction in cytokine-producing CD4 T cells, compared to controls. Moreover, MHCII deletion in memory B cells abrogated secondary germinal center responses in the lung and mediastinal lymph nodes and diminished flu-specific IgA and IgG ASCs in the upper and lower respiratory tracts post-challenge. These findings suggest that interactions between lung BRMs and activated CD4 T cells reciprocally enhance the early B and T cell antiviral responses. Furthermore, in wild-type memory mice intranasally immunized with flu-specific B cell tetramers (NP-BV421), early antigen uptake predominantly occurred in lung memory B cells rather than lymphoid counterparts. Collectively, our results demonstrate that lung BRMs support antiviral CD4 T cell responses during secondary infection by facilitating antigen uptake and presentation. These findings underscore the importance of local BRM-CD4 T cell interactions in the lung for orchestrating robust antiviral responses.

Funding Source: NIH 1R01AI153413-01 Protective functions of influenza-specific lung-resident memory B cells

Abstract 83

Single Cell Protein Synthesis Profiling Reveals Inhibitory Receptor-Independent Metabolic Suppression of CD8+ Tumor-Infiltrating Lymphocytes

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Strategies to enhance cellular metabolism can augment T cell expansion, persistence, and effector function, all crucial for an optimal anti-tumor response. While the tumor immunometabolism field has largely focused on extrinsic barriers to tumor-infiltrating lymphocyte (TIL) metabolic function, our lab previously uncovered an intrinsic glucose metabolism defect in melanoma CD8 TIL relative to vaccine-derived acute effector T cells (Teff), driven by low enolase-1 activity. The upstream immunological and environmental drivers remain poorly understood.

PD-1 and CTLA-4 Inhibitory Receptor (IR) signaling can downregulate glycolysis-promoting signaling pathways in vitro, making IRs plausible candidates for this intrinsic suppression in melanoma TIL. We employed single-cell puromycin incorporation with flow cytometry to test this. Puromycin incorporation reflects global metabolic capacity via the high energetic cost of protein synthesis, and its single-cell resolution unveiled a previously unappreciated layer of heterogeneity in TIL metabolism. Murine melanoma TIL producing IFN-gamma and Granzyme B in situ exhibited the highest protein synthesis rates, implying a positive correlation between metabolic sufficiency and effector function. Surprisingly, these cells displayed markers of terminal exhaustion, including high TOX and IR expression, inconsistent IRs alone driving metabolic suppression. Combined CTLA-4 + PD-1 blockade enhances TIL representation in the tumor microenvironment but failed to increase per-cell protein synthesis levels or in situ effector function. These findings suggest bioenergetic capacity is a critical determinant of TIL effector functionality insufficiently restored or sustained by standard-of-care immunotherapies. Future work will define the spatiotemporal and biochemical drivers of metabolic defects to improve TIL function in solid tumors.

Abstract 84

Contributions of Neonatal-origin CD4+ T cells to the Adult Immune System

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Due to recurrent infections during early life it had long been assumed that the neonatal T cell response was immature and deficient compared to adults. Now we appreciate that the neonatal T cell response is qualitatively distinct: Neonatal T cells rapidly respond to antigen but fail to develop sufficient memory. Recent studies have demonstrated that the adult immune system is layered with immune cells from various developmental origins that contribute unique functions, including T cells of neonatal origin. Prior work focused on CD8+ and regulatory CD4+ T cells and discovered that the former maintain many of the features observed in neonates, yet none have investigated the contributions of neonatal-origin conventional CD4+ T cells. Employing a fate-mapping model that permanently genetically labels neonatal ab-T cells during their thymic development, I fate map (FM) developing thymocytes at postnatal days 1-3 (neonatal), or at postnatal days 28-30 (adult), and track the fate of these cells in adult mice. Using this model, we performed single cell RNA sequencing of CD4+ TCRb+ FM+ T cells neonatal or adult timestamped groups isolated from the spleen, lung, liver and colons. Our data demonstrates that neonatal FM CD4+ T cells skew towards an effector phenotype, including Cd44, Cxcr3, Klrg1 and Id2, and downregulate naïve-associated genes, Lef1, Sell, and Bach2, while adult FM CD4+ T cells display the opposite pattern. Utilizing spectral flow cytometry, I have validated these findings. Future work will test the functional properties of neonatal and adult FM T cells in response to infections and tumors.

Abstract 85

Radiation Therapy Remodels the Brain Immune Environment and Affects Brain Metastasis Anti-Tumor Immunity

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Brain metastases (BrM) affect approximately 20% of patients with cancer and are associated with poor prognosis. The standard of care often includes stereotactic radiosurgery (SRS), which provides effective local control of tumors. However, many patients develop new BrM in other areas of the brain that received low-dose radiation during prior treatment courses. We hypothesize that radiation alters the “soil” in which newly seeded metastases grow and affects the anti-tumor immunity for these BrM.

To model the effects of low-dose wash from SRS, we treated mice with a single dose of radiation to one hemisphere. The irradiated hemisphere had significantly fewer microglia, and the microglia that were present exhibited decreased expression of CD101. We next implanted B16-F10 melanoma tumors in the irradiated hemisphere of mouse brains 6 weeks after radiation. The BrM-infiltrating microglia in irradiated brain showed decreases in CX3CR1 expression compared to BrM growing in unirradiated brain. Additionally, tumors implanted in previously irradiated brains were infiltrated by higher proportions of Tim3+PD-1+ CD8+ T cells and lower proportions of TCF-1+PD-1+ CD8+ T cells than their counterparts in unirradiated brains.

To interrogate this phenotype, we cocultured splenocytes with irradiated microglia for 24 hours, then isolated CD8+ T cells for RNA-seq. CD8+ T cells cultured with irradiated microglia had a more exhausted-effector phenotype, whereas those cultured with unirradiated microglia displayed increased proliferative capacity. These findings suggest that irradiated microglia may promote infiltration of more terminally differentiated, exhausted CD8+ T cells in BrM, which may serve as an important target for future immunotherapies.

Abstract 86

Interrogating the immunomodulatory role of Arginase-1-expressing neutrophils in brain metastasis

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Brain metastasis (BrM) affects approximately 20% of patients with cancer and is associated with dismal clinical outcomes. Although immunotherapy has revolutionized cancer treatment, many patients with BrM either fail to respond or experience disease relapse, highlighting the urgent need for improved treatment strategies. Many immunotherapies target exhausted CD8+ T cells, restoring their ability to proliferate and kill tumor cells. However, antitumor immunity can be thwarted by additional immunosuppressive mechanisms, like depletion of key nutrients in the tumor microenvironment (TME). For example, the amino acid arginine is critical for T-cell function but is often deficient in the TME due to metabolism by Arginase-1. I've shown that neutrophils are a dominant source of Arginase-1 in the BrM TME and that patient survival worsens as neutrophils comprise a larger proportion of BrM-infiltrating immune cells. This suggests that the negative effect on survival seen with greater neutrophil infiltration may be due to elevated levels of Arginase-1 in the TME, which restricts arginine availability and constrains T cell-mediated immunity. The overall goals of my research are to elucidate the immunomodulatory role of neutrophil-derived Arginase-1 in BrM and to define how arginine depletion influences T-cell function, immunotherapy efficacy, and tumor control in the brain. Future experiments will use human samples and mouse models to determine how BrM-infiltrating neutrophils regulate arginine levels and modulate the response to immunotherapy. Ultimately, this research has the potential to identify opportunities for therapeutic intervention that counteract the immunosuppressive functions of BrM-infiltrating neutrophils to achieve improved outcomes for patients with BrM.

Abstract 87

Exhausted-like lung tissue-resident memory T cells are critical for protection against influenza reinfection

Arka Sen Chaudhuri UVA

In Su Cheon UVA
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The unique single-layer structure of the lung endows it with an antiviral immune landscape distinct from other barrier tissues. Respiratory viral infections, especially influenza, remain a major global health challenge, and circulating antibody-based vaccines are limited by frequent surface antigenic drift. In contrast, influenza-specific lung tissue-resident memory CD8+ T cells (TRMs) recognize conserved epitopes and provide both homologous and heterologous protection, serving as a rapid frontline defense during reinfection; however, the mechanisms that sustain and drive their protective functions remain poorly understood. We identified a unique endogenous respiratory TRM population in both humans and mice: PD-1HI CD103LO exhausted-like TRMs (TRM-ELs), which differ from PD-1LO CD103HI conventional TRMs (TRM-Cons) observed in most barrier tissues. Lung TRM-ELs exhibit unique transcriptional and signaling programs distinct from CD103HI TRM-Cons. Unlike reported TCR-independent TRM-Cons, we proved that local TCR signaling is dispensable for the maintenance of TRM-ELs. Importantly, the intact TRM-EL is indispensable for heterologous protection, while TRM-Cons don’t exhibit comparable efficiency. Mechanistically, the robust expansion of TRM-ELs facilitates the fast recall response and protection against secondary infection. Taken together, our research provide mechanistic insight into an unrecognized protective lung TRM population and elucidate distinct molecular pathways governing their persistence and antiviral efficacy. These results will lay the foundation for the development of universal respiratory mucosal vaccines to induce sustained local TRM immunity against influenza and related pathogens.

Abstract 88

Longitudinal Immune and Cytokine Changes Associated with Treatment Response in Multiple Myeloma

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Multiple myeloma (MM) is a plasma cell (PC) malignancy characterized by clonal PC accumulation in the bone marrow (BM) and associated immune dysregulation, which may influence response to therapy and disease progression.

Patients with newly diagnosed MM received quadruplet induction (QUAD; immunomodulatory agent, proteasome inhibitor, anti-CD38 monoclonal antibody, and corticosteroids), followed by minimal residual disease (MRD)-adapted consolidation with or without autologous stem cell transplantation (ASCT), and maintenance. Peripheral blood and BM samples were collected longitudinally at screening (pre-treatment), post-induction (QUAD ×6), post-consolidation (ASCT or QUAD ×3), and maintenance. A total of 19 patients were enrolled, with complete datasets available for 15. Treatment decisions after induction were MRD-guided: MRD-negative patients continued QUAD consolidation without ASCT, whereas MRD-positive patients proceeded to ASCT.

We performed longitudinal immune and cytokine profiling to identify biomarkers associated with treatment response across therapy phases.

At baseline, the BCMA/BAFF ratio was strongly associated with BM PC burden and outperformed either cytokine alone, supporting its role as a marker of tumor load. During therapy, the most pronounced changes were observed between post-induction and post-consolidation in transplant patients, who showed a marked increase in the CXCL9/BCMA ratio, accompanied by expansion of CD8 T cells (including EMRA subsets) and NK cells. Importantly, within this group, larger increases in the CXCL9/BCMA ratio were associated with lower MRD levels at consolidation, indicating deeper responses.

These findings suggest that dynamic changes in the balance between tumor burden and immune activation reflect treatment effectiveness and may provide insight into mechanisms of response and resistance.

Abstract 89

Assessment of the Impact of HLA-DO Expression on Endosomal pH Shifts

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Peptide loading of Major Histocompatability Complex class II molecules (MHCII) in the endosomes and lysosomes of B cells and dendritic cells is controlled by the interplay of two non-classical MHCII molecules, HLA-DM and HLA-DO. DM loads MHCII with high affinity peptides while DO is an inhibitory substrate mimic of MHCII that binds to DM and prevents peptide loading, leading to a modified cell surface presented MHCII-bound peptide repertoire. DO expression results in a slightly broader epitope repertoire that is skewed towards lower affinity MHC binding peptides. Previously, our lab identified variants of DO that bind to DM but yet do not inhibit MHCII peptide loading, suggesting DO may have other functions in the MHCII antigen presentation pathway in addition to direct DM inhibition. An unbiased screen identified the V-ATPase as a novel DM/DO interacting protein. MHCII peptide loading has an acidic pH optimum, suggesting that DO may play a role in regulating endosomal/lysosomal pH. To test this idea, endosomal/lysosomal pH was measured in HeLa-CIITA cells (MHCII+ I chain+, DM+, DO-) transfected with DO or a control plasmid using fluorescent pH probes followed by flow cytometric analyses. Results showed that endosomes/lysosomes in HeLa-CIITA cells co-expressing DO were more basic when compared to those in HeLa-CIITA cells (DO-). Further studies will be performed to confirm these results using confocal microscopy and to determine the specific mechanism by which DO interacts with V-ATPase, and how this interaction mediates an unexpected control of endosomal/lysosomal pH.

Abstract 90

A Persistent Inflammatory Effector Neutrophil State in the Inflamed Colon

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Neutrophils play a context-dependent role in Clostridioides difficile infection (CDI), balancing antimicrobial defense with immunopathology. We identified a distinct subset of neutrophils expressing the eosinophil-associated marker Siglec-F that rapidly accumulates in the colon during CDI and DSS colitis. Using murine infection models, flow cytometry, and imaging, we confirmed that Siglec-F+ cells are bona fide mature neutrophils, distinct from Siglec-F high eosinophils and other myeloid populations.

To define their functional identity, we integrated bulk RNA sequencing, single-cell RNA sequencing, and metabolic profiling using SCENITH. Siglec-F+ neutrophils formed a discrete transcriptional population enriched for genes associated with phagocytosis, neutrophil extracellular trap formation, and proinflammatory pathways, including TH1-associated and interferon-responsive programs. Pathway analysis revealed metabolic reprogramming with increased translational activity and altered bioenergetic dependencies relative to conventional neutrophils. Single-cell analysis supported a distinct activation trajectory consistent with functional specialization in the inflamed colon.

Functionally, early depletion of Siglec-F+ neutrophils in eosinophil-deficient mice worsened disease, with increased weight loss and a trend toward higher mortality, indicating a protective role during early CDI. Notably, we observed persistent colonization with toxin-producing C. difficile beyond 100 days post-infection, suggesting ongoing immune pressure. These findings raise the possibility that Siglec-F+ neutrophils contribute to long-term containment of residual bacteria. Together, our results support stage-specific therapeutic strategies that preserve early protective responses while limiting excessive inflammation.
Keywords: Siglec-F+ neutrophils, C. difficile infection, Neutrophil heterogeneity, Intestinal inflammation, Metabolic reprogramming, Extracellular traps

Abstract 91

Innate immune memory as a determinant of glioma immunotherapy

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Glioblastomas (GBM), stage IV brain tumors, lack innate immune context for productive T cell immune surveillance and only subsets appear be responsive to immunotherapy. Long-term survival of patients after polio virotherapy, an intratumor immunotherapy, associated with pre-treatment intratumoral and peripheral myeloid inflammation and post-treatment type I interferon (IFN-I) induction. Similarly, IFN-I responsiveness in patient-derived GBM ex vivo cultures associated with myeloid inflammation at baseline. Thus, the induction of IFN-I responses is linked to baseline myeloid inflammation and may determine virotherapy efficacy in patients with rGBM. Environmentally sculpted bone marrow (BM)-resident hematopoietic stem & progenitor cells (HSPCs) have been shown to predetermine antiviral responsiveness of their progeny myeloid cells. In GBM, intra-patient myeloid profiles are maintained in tumor and blood across multiple time points, implying such durable mechanisms may control systemic myeloid biology. We discovered that intramuscular (i.m.) vaccination against antigens not expressed by the tumor alters HSPC phenotype and rescues the antitumor efficacy of intratumor immunotherapy in otherwise resistant murine GBM models. I.m. vaccination caused vaccine-specific CD4+ T cell accumulation, HSPC proliferation and IFN-I signaling, and dendritic cell (DC) progenitor expansion in the BM. These changes were accompanied by higher MHC-II and IFN-I signaling at baseline and stronger IFN-I responses to virotherapy in glioma infiltrating DCs. Antitumor effects of virotherapy after i.m. vaccination depended on IFN-I, T cells, and MHC-II epitope, and were not reproduced by b-glucan—a canonical inducer of trained immunity. Thus, myeloid cell inflammation associates with GBM immunotherapy sensitivity; modulating HSPCs may improve glioma immunotherapy outcomes.

Abstract 92

Using CITE-seq to better understand the regulation of surface proteins by mRNA pathways in human immune cells from healthy PBMC donors

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Cellular Indexing of Transcriptomes and Epitopes by sequencing, or CITE-seq, is a technology that captures transcriptomic and antibody-derived tag (ADT) reads simultaneously at single-cell resolution. This technique has been applied in several studies since its invention in 2017, but the relationship between mRNA pathways and surface protein expression levels is still poorly understood. Here, we apply a novel approach to examining this relationship in human PBMCs from 12 healthy donors' blood. This is done by identifying the correlation between each ADT's expression and each RNA's expression by individual celltype, followed by gene set enrichment analysis using those gene-ADT correlations to rank the gene list. Pathway-ADT correlations are considered significant results if they are consistent through both pseudobulk and single-cell approaches to calculating these correlations. Our initial findings indicate there is a significant difference in the number and type of pathway-ADT correlations between even closely related cell-types. We found that the pathway, HALLMARK_MYC_TARGETS_V1, was responsible for most pathway-ADT correlations across all cell-types. We will validate these results using an additional cohort and flow cytometry in the wetlab. We also plan to conduct TF regulon analysis to identify whether transcription factor regulons are enriched for association with surface proteins. This research will improve our understanding of the factors that are associated with ADT expression in immune cells. It will also enable comparison of these features between healthy and disease-specific CITE-seq data, for which our early results indicate there are significant differences between healthy and CVD+ pathway-ADT correlations.

Abstract 93

ER stress mediated mitochondrial dysfunction promotes Treg instability in coronary artery disease

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In chronic inflammatory diseases such as atherosclerosis, CD4+ regulatory T cells (Tregs) can lose their stability and transition into cytotoxic exTregs. The underlying mechanisms for this shift remain poorly defined. Our findings identify persistent endoplasmic reticulum (ER) stress as a key driver of Treg instability. Human exTregs exhibit ER stress driven mitochondrial dysfunction, which remains unresolved due to defective mitophagy. Additionally, the integrated stress response (ISR), a pathway linked to inflammatory signaling, is elevated in these cells. Many exTregs express senescent marker CD57, that makes them particularly vulnerable to stress-induced dysfunction. Tregs from patients with coronary artery disease (CAD) show an exTreg-like phenotype with evident ER stress and mitochondrial depolarization. This is further exacerbated in CD4+ T cells within atherosclerotic plaques. In vitro exposure to pro-atherogenic stressors such as oxidized LDL (oxLDL) and interferon-γ induces ER stress and mitochondrial dysfunction in Tregs. We conclude that the maladaptive inflammatory environment in atherosclerosis triggers ER stress and mitochondrial dysfunction, contributing to Treg instability in CAD.

Abstract 94

Metabolic and proteomic GWAS-based dissection of genetic bases underlying preeclampsia risk in Zambian women

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Women with HIV often face a higher risk for adverse pregnancy outcomes. Yet in a Zambian cohort, the risk for preeclampsia (PE) was paradoxically lower in women with HIV (2.0%) than women without HIV (4.8%). We applied multi-omics approaches to identify PE risk signatures. Plasma samples from 2,334 pregnant women (1098 HIV+ vs. 1236 HIV-) were analyzed at four gestational age (GA) windows by high-resolution metabolomics and proteomics. Metabolite or protein associations with PE were tested using multivariable logistic regression or ANCOVA, respectively, with false discovery rate correction and adjustment for maternal age, BMI, and GA. Low-pass whole-genome sequencing was performed for 1,408 participants (831 HIV+ vs 577 HIV-). All analyses were completed for the full cohort and stratified by HIV status. Genome-wide association studies were conducted using PLINK v2.0, applying logistic regression for PE and linear regression for PE-associated metabolite and protein traits to identify loci associated with disease risk. One protein and no metabolites were associated with PE in women with HIV compared to 2,308 metabolites and 53 proteins in the full cohort. On the genomic level, DLEU7 mapped to two immunoregulatory proteins prior to 20 weeks and at ≥32 weeks TENM4 mapped to endoglin and small integral membrane protein 24. Genes PLK2, TRIB2, and CHMP7 were linked to several PE-associated metabolites, which are implicated in vascular development and innate immune signaling pathways. Integrated multi-omics identified gestational age- and HIV-specific molecular features of PE providing a framework for pathway analysis to determine how HIV may modify PE risk.

Abstract 95

A guard function of RIPK3 triggers catastrophic susceptibility to an intracellular bacterial infection

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Regulated cell death should eliminate infected host cells, thereby depriving intracellular pathogens of replicative niches. This creates evolutionary pressures that drive all host-adapted pathogens to evade apoptosis, pyroptosis, and necroptosis. In contrast, environmental pathogens exhibit the same ability to invade and replicate in host cells, but fail to evade these forms of cell death resulting in their prompt clearance. Here, we study an environmental pathogen called Francisella philomiragia. Wild type mice clear infection by 10,000,000 CFUs of this microbe within 24 hours. In contrast, immunocompromised mice that cannot generate reactive oxygen species succumb to infection by even 100 CFUs, mimicking the susceptibility of patients with chronic granulomatous disease. Because Francisella species are cytosol-invasive, we hypothesized that regulated cell death closes the intracellular niche. However, Asc KO or Casp8 Mlkl DKO mice resisted this infection. A prevailing idea in the cell death field is that distinct forms of cell death can be redundant, and in support of this, Asc Casp8 Mlkl TKO mice succumbed to low dose infection. However, replacement of the Mlkl KO with Ripk3 resulted in resistant TKO mice. Surprisingly, addition of Ripk3 mutation rescued Asc Casp8 Mlkl mice. We demonstrate that RIPK3 initiates a type I IFN response in plasmacytoid dendritic cells only triggered in a “guard” fashion by the absence of Casp8 and Mlkl. This IFN response is detrimental during F. philomiragia infection, but it is counteracted by a beneficial IL-1b response through Asc. Only when the detrimental type I IFN response is triggered simultaneously in the absence of the beneficial IL-1b response are mice susceptible to F. philomiragia. These results reveal that pyroptotic signaling and necroptotic signaling operate antagonistically, rather than working redundantly.

Abstract 96

Isotype-specific antibody secreting plasma cells harbor epigenetic and transcription factor architectures defining distinct cell lineages

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Antibody-secreting cells (ASCs) are key effectors of humoral immunity mediating specialized roles in host defense. Despite studies defining the transcriptional programs of selected ASC isotypes, specific mechanisms underlying isotype specific gene regulation are largely unaddressed. We performed an integrated multi-omics analysis (RNA-seq, ATAC-seq, and DNA methylation) of surface-sorted IgM, IgG, and IgA ASCs isolated from murine spleen and mediastinal lymph nodes following influenza infection. Integrated clustering and TF enrichment analyses revealed shared and isotype-specific transcriptional and epigenetic programs. Isotype-specific signatures mapped to TLR signaling, cell cycle, cholesterol metabolism, and cellular homing pathways. IgA ASCs showed elevated expression of cholesterol metabolism and homing-associated genes along with enrichment of RUNX and SMAD motifs emphasizing the importance of these TFs in regulatory network of IgA ASC for homing to mucosal sites. IgM ASCs exhibited higher expression of cytokines and associated receptors along with enrichment of REL/NFAT and STAT5 motifs at key loci linked to JAK/STAT signaling. Although these TF have been associated with B cell development/differentiation, these results indicate that they have an important additional role promoting IgM ASC biology. DNA methylation analysis revealed two distinct patterns: one characterized by progressive hypomethylation from IgM to IgG to IgA and the other showing more hypomethylation in IgG, suggesting alternative class-switching trajectories or proliferative histories. Collectively, our findings demonstrate that transcription factor networks establish isotype-specific epigenetic landscapes, driving functional diversification of ASCs and establish distinct ASC lineages. These findings can can ultimately help in future therapies where one isotype versus another would be beneficial.

Abstract 97

Exploring vacuolar ATPase in APCs and its interaction with the non-classical MHCII molecule H2-O

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Acidification of late endosomes and lysosomes (LE/Lyso) in antigen presenting cells is mediated by the pumping of H+ ions into the vesicles by the vacuolar ATPase (V-ATPase). Efficient MHCII antigen processing and peptide loading requires acidic pH of LE/Lyso, is catalyzed by the MHCII-like molecule H2-M, and inhibited by its binding partner, another MHCII-like molecule, H2-O. Functionally, preliminary data shows that H2-O+ LE/Lyso from B cells are less acidic, suggesting a V-ATPase/H2-O interaction downregulating V-ATPase activity. The biochemical interaction of V-ATPase with H2-O was shown in B cells by immunoprecipitation (IP) of H2-O/H2-M followed by western blotting for subunits of V-ATPase. However, reciprocal IPs targeting sectors of V-ATPase failed to immunoprecipitate V-ATPase and subsequently H2-O. Using an alternative approach, V-ATPase will be captured from B cell lysates using anti-Flag magnetic beads bound to recombinant 3xFlag tagged SidK, a L. pneumophila effector protein which binds to the V-ATPase, followed by blotting for H2-O, H2-M, and V-ATPase to confirm capture. While this approach works well to immunoprecipitate V-ATPase from mouse brain lysates, mouse B cells do not express sufficient V-ATPase to detect interaction with H2-O/H2-M. We are optimizing this approach using primary mouse B cells activated in vitro with IL-4 and LPS, which increased the amount of V-ATPase immunoprecipitated with rSidK. Reciprocal IPs of V-ATPase immunoprecipitating H2-O would further support the hypothesis that H2-O is not only a substrate mimic of MHCII, inactivating H2-M, but also directly affecting the pH of LE/Lyso to alter the peptide repertoire.

Abstract 98

Viral Microglia Reprogramming Clears Oligomeric Neurotoxic Debris

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Given their pivotal role in CNS debris clearance and homeostasis, microglia are central targets for neurodegenerative disease therapy. Intricate proximity to neurons, the inherent danger of neuroimmune toxicity, and intrinsically high plasticity and adaptability, impose grave hurdles for microglia modulation. Attenuated viruses are being tested extensively against CNS malignancies (i.e., cancer virotherapy); yet, aside from viral vector-mediated payload delivery, virotherapy for non-neoplastic CNS disease remains unexplored. Here we report disseminated targeting of microglia with the highly attenuated polio:rhinovirus chimera, PVSRIPO, that culminated in profound, durable microglia reprogramming. This phenotype, rooted in extended cytoplasmic viral (v)RNA replication, was non-cytopathogenic and did not yield virus progeny or dissemination. vRNA replication in microglia triggered selective interferon (IFN) regulatory factor (IRF) 3/IRF7 transcriptional programs in the relative absence of NFkB proinflammatory cytokine responses. It elicited robust phagocytic activity of both tumor cells and amyloid-beta (Ab), in line with broad induction of immune surveillance functions. Targeting of microglia with PVSRIPO mediates immunotherapy in a mouse glioma model and the clearance of oligomeric Ab deposits in an injectable model of neurotoxic amyloid accumulation. This work implicates attenuated virotherapy as a novel route to safely and effectively invigorate microglia function in immune surveillance and neurotoxic debris clearance and immune surveillance and neurotoxic debris clearance.

Abstract 99

Guiding antibody evolution to elicit neutralizing antibodies against HIV-1 with vaccination

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Viral spike proteins mediate entry into susceptible host cells. The spike protein of HIV-1, also known as the HIV-1 envelope glycoprotein (Env), is the sole target for neutralizing antibodies (Abs). Abs that acquire broad and potent neutralization of HIV-1 isolates are referred to as broadly neutralizing Abs (bnAbs) and develop in a rare fraction of infected individuals after ~2-3 years. The best bnAbs can neutralize more than 90% of circulating HIV-1 isolates, making them a prime target for vaccination. However, eliciting these Abs via immunization has proven challenging because they must undergo an antibody evolution process where they obtain rare amino acid substitutions to improve binding to diverse Env proteins. In this study in humanized mice, we tested a candidate vaccine regimen designed to elicit Abs against the CD4 binding site (CD4bs) of the HIV-1 Env. Our goal was to elicit and isolate Abs that acquired rare amino acid substitutions previously identified in a known CD4bs bnAb. Vaccine-induced Abs (vAbs) affinity matured to acquire the same key, rare amino acid substitutions found in the natural lineage. Interestingly, other vAbs were identified that acquired various rare mutations different than those found in the natural lineage. Both sets of vAbs displayed binding responses similar to the natural lineage, as well as neutralization of diverse viruses. Here, we provide evidence that the selection of antibodies with the desired key amino acid substitutions is feasible with immunization, and that multiple Ab maturation pathways exist that can generate neutralizing antibodies to provide protection against HIV-1.

Abstract 100

Investigating the role of germinal center B cells in the anti-tumor immune response

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B cells are found across many cancer types and are linked to either better or worse outcomes depending on context—showing favorable associations in breast, cervical, and colorectal cancers, but poorer outcomes in melanoma. Previous work has shown that germinal center (GC) like B cells correlate with favorable outcomes in head and neck squamous cell carcinoma, but their function in other cancers remains unclear. In our initial work, we focused on GC B cells, which undergo selection and differentiate into long-lived plasma cells that provide durable protection through antibodies.

We hypothesize that impaired germinal center formation in tumor-draining lymph nodes (TdLNs) leads to insufficient production of anti-tumor antibodies, thereby limiting their capacity to enhance the anti-tumor immune response. To test this hypothesis, we used mouse models of colorectal and renal adenocarcinoma and detected GC reactions in TdLNs by immunofluorescence. Additionally, we developed B-cell probes to identify tumor-specific B cells in TdLNs, which we isolated by Fluorescence-activated cell sorting (FACS) for single-cell RNA sequencing. The same probes were used to characterize tumor-specific antibodies by ELISA.

Our results show that tumor-specific B cell responses arise within 14 days in the TdLNs across multiple mouse models. Our data indicate that tumor-specific IgG antibody production is detectable, yet markedly reduced compared to levels typically observed during viral infection. These results show that tumor-bearing mice generate tumor-specific germinal center responses. Overall, our work on the development and application of B-cell probes provides a valuable approach for investigating tumor- and antigen-specific B-cell responses in cancer models.

Abstract 101

Antibody Discovery Against Unique Influenza Neuraminidase Epitopes Using BCR Repertoire Engineering

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Influenza caused 51 million illnesses and 45,000 deaths in the United States during 2024–2025, underscoring the need for broader and more durable countermeasures. Influenza neuraminidase (NA) is an important target for next-generation antibody therapeutics and vaccine design; however, most reported NA antibodies target a limited set of epitopes and often lack broad coverage. To address this gap, we developed an antibody discovery platform to identify antibodies against diverse NA epitopes. We used mice with human B cell receptor (BCR) repertoires and immunized them with cobalt porphyrin-phospholipid (CoPoP) liposomes formulated with N2-Darwin (wild-type) and an N2 mutant (M2) with a blocked active site to identify antibodies recognizing epitopes beyond the immunodominant active site. We combined antigen-specific B-cell profiling with high-throughput single-cell repertoire analysis using LIBRA-seq to recover diverse human antibody candidates against influenza NA. Using our in-house R-based analysis workflow, we identified unique clonotypes with germline divergence and strong reactivity to bait antigens. We then applied our Structure-Guided Epitope Prediction Pipeline to classify antibodies based on their sequence, into distinct binding bins, showing substantial epitope diversity. Antibodies were further evaluated by ELISA, across multiple NA antigens, and by MUNANA inhibition assays to identify active-site binders. SPR was used to confirm binding and epitope diversity. Top antibodies from our workflow were further tested in neutralization assay against H3N2 influenza strains. Ongoing cryo-EM studies will further define antibody-NA interactions and validate recognition of distinct epitopes. This BCR repertoire engineering workflow establishes a scalable platform for discovering diverse NA-targeting antibodies.

Abstract 102

Protection Against Type 1 Diabetes Development in Mice with 4E-BP2 Deletion

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Type 1 diabetes (T1D) is an autoimmune disease characterized by beta-cell destruction promoted by autoimmune responses. 4E-BP1/2 proteins are translational repressors and downstream targets of mTORC1, a key regulator of beta-cell mass and function. Activation of 4E-BP2/eIF4E pathway by 4E-BP2 deletion promotes translation initiation, which induces beta-cell expansion and proliferation and is crucial for the regulation of adaptive immunity. Using the Non-Obese Diabetic (NOD) mouse model of T1D, this study aimed to determine the role of 4E-BP2/eIF4E signaling in T1D prevention by regulation of beta-cell survival and immune modulation. To examine its role in T1D, we generated Eif4ebp2■/■ mice in the NOD background and assessed diabetes incidence, glucose regulation, pancreatic morphology, immune responses and changes in gene expression. Eif4ebp2■/■ male mice exhibited reduced diabetes incidence, preserved beta-cell mass, and enhanced glucose-stimulated insulin secretion. Immune profiling and RNA sequencing showed decreased splenic CD8■ cytotoxic T cells, increased pancreatic regulatory T cell infiltration and suppression markers. Moreover, in the pancreatic lymph nodes, we also observed a decrease in activated IGRP+ CD8+ T cells in the mice lacking 4E-BP2. Adoptive transfer studies demonstrated that Eif4ebp2■/■ lymphocytes were less diabetogenic than controls. Finally, bone marrow transplantation in females also conferred protection through 4E-BP2 deficiency. Thus, deletion of 4E-BP2 protects against T1D by modulating immune responses and preserving beta-cell mass, identifying 4E-BP2 as a promising therapeutic target in T1D.

Abstract 103

Olfactory Receptor 2 signaling Enhances Monocyte Chemotactic Migration in the Atherosclerotic Aorta Due to Dysfunctional Endothelium

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Atherosclerosis is an inflammatory disease of the arterial wall initiated by monocyte recruitment and macrophage (Mϕ) function. Human and mouse vascular macrophages, mouse bone marrow-derived macrophages (BMDMs), and monocytes express several olfactory receptors (ORs), including Olfr2 and its human orthologue OR6A2. Ligation of Olfr2 and OR6A2 with their ligand Octanal activates the NLRP3 inflammasome pathway while knocking out (KO) Olfr2 in mice reduces atherosclerosis progression in vivo. Octanal, a product of lipid peroxidation, is proposed to derive from endothelial dysfunction (ED), which leads to atherosclerosis initiation. Recently, we identified Olfr2/OR6A2 protein expression in about 20% of monocytes, with expression increasing in disease states in humans and mice. However, the role of Olfr2 in monocytes is unknown. Interestingly, competitive adoptive transfer experiments with labeled WT and Olfr2 KO bone marrow monocytes injected into CD45.1 Apoe KO mice, revealed that WT monocytes were significantly enriched in the aorta compared to Olfr2 KO monocytes and could differentiate into Olfr2+ macrophages. In vitro, BMDMs significantly migrate in response to octanal while Olfr2 KO do not. Overall, these findings reveal Olfr2 as a previously unrecognized chemotactic receptor in monocytes and macrophages, highlighting its potential role in directing immune cell migration within the atherosclerotic aorta and as a potential therapeutic target.

Abstract 104

PVSRIPO ELICITS IMMUNE PRIMING IN MYELOID CELLS

Lisbeth Disla, Michael Brown, Matthias Gromeier

Despite significant advancements in cancer immunotherapy and interventions, cancers have continued to prove difficult to treat due to baseline or acquired resistance. A particular point of resistance is the tumor microenvironment (TME) of most types of cancers, which is characterized by an enormous degree of cellular heterogeneity and a wide range of intractable immuno-suppressive traits. Our lab has developed PVSRIPO, a genetically engineered poliovirus, characterized by profound attenuation with loss of cytotoxicity, including in host cells targeted by polioviruses, eg. myeloid cells (macrophages, dendritic cells (DC), microglia). Non-cytopathogenic PVSRIPO infection engages myeloid cells (in the TME and in surrounding normal tissues) to elicit a unique, localized, pro-inflammatory antiviral response. Sublethal infection of macrophages by PVSRIPO generates a unique, sustained type-I interferon (IFN)-dominant pro-inflammatory signature that activates phagocytosis, tumor antigen-presentation, priming of antitumor CD8+ T-cell immunity, and durable tumor immune surveillance. The goal is to enhance immunotherapy efficacy of PVSRIPO by optimizing therapy with a mechanistically and rationally supported homologous prime-boost strategy. It is my hypothesis that prime-boost innate stimulation of myeloid cells may enhance pro-inflammatory activation in a manner that enhances the generation of tumor antigen-specific antitumor CD8+T cell responses.

Abstract 105

RGS16 as a Therapeutic Target to Overcome TGFβ-Induced Resistance in Adoptive T Cell Therapies

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Transforming growth factor-β (TGFβ) is a key immunosuppressive cytokine that limits effector T cell activity and remains a major barrier to effective immunotherapy, vaccine responses, and T cell activation. Identifying downstream mediators of TGFβ signaling that constrain T cell function may reveal new strategies to enhance adoptive T cell therapies. To address this, we performed transcriptomic profiling of activated human CD8■ T cells in the presence or absence of TGFβ to identify novel immunosuppressive regulators. Differential expression analysis revealed multiple TGFβ-induced genes, among which RGS16, a member of the regulator of G protein signaling (RGS) family, emerged as a prominent candidate with a previously uncharacterized role in TGFβ-driven immunosuppressive environments. Functional validation using CRISPR-Cas9–mediated gene knockout demonstrated that RGS16 functions as a critical negative regulator of T cell function. RGS16 deficiency markedly enhanced cytokine production, proliferation, and cytotoxicity, and notably rescued T cell effector function under TGFβ-mediated suppression. Importantly, adoptive transfer of RGS16-deficient T cells into tumor-bearing hosts resulted in improved tumor control and prolonged survival, establishing in vivo relevance. Collectively, these findings identify RGS16 as a key downstream effector of TGFβ signaling that constrains anti-tumor immunity. Targeting RGS16 represents a promising strategy to reprogram T cell–based immunotherapies by restoring effector function in immunosuppressive tumor environments.

Abstract 106

IL32 drives an intracellular antimicrobial defense pathway

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Chlamydia trachomatis (Ct) represents the most common sexually transmitted bacterium in the US and can cause severe sequelae in infected women. No vaccine is currently available. The ability of Ct to cause disease stems from its ability to evade sterilizing immunity in the human host, often for weeks or months. It remains poorly understood how Ct evades this sterilizing immunity.
We used a bacterial genetic screen to identify a novel virulence factor in Ct, the bacterial gene IncS, which allows Ct to survive interferon-stimulated defense within epithelial cells. When IncS is mutated, Ct becomes vulnerable to a previously undescribed defense pathway within the human epithelium. This pathway begins with the intracellular cytokine IL32, which is induced by interferon-gamma and is trafficked to Ct-containing vacuoles, rather than being secreted. IL32 is then modified by cysteine modifying enzymes which allow IL32 to recruit the autophagy adaptor p62 to the Ct vacuolar membrane. Finally, p62 recruits autophagy machinery and the bacterium is destroyed by autophagy. Additionally, we have found that IL32 can target and restrict the intracellular fungal parasite Encephalitozoon cuniculi, indicating that IL32 drives a conserved antimicrobial response against several pathogens.
This work represents the discovery of a novel IL32-mediated defense pathway, the first time that IL32 has been shown to target any pathogen within the cell, and the discovery of a novel pathway of Chlamydial immune evasion by the virulence factor IncS.

Abstract 107

Rapid IFNg production by CD8+ TRM is necessary to slow viral propagation but is insufficient to mediate clearance following respiratory virus transmission

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Tissue-resident memory CD8+ T cells (TRM) can protect against respiratory virus transmission through secretion of IFNg, however, the precise mechanisms remain poorly defined. We aimed to determine how IFNg alone, in the absence of other TRM-mediated effector mechanisms, contributes to protection. Using a mouse model of Sendai virus (SeV) transmission and bioluminescence imaging of viral dynamics, we developed an approach to activate WT or Ifng-/- influenza-specific TRM during SeV exposure to assess whether IFNg from bystander, non-SeV-specific TRM in the upper respiratory tract (URT) could protect against SeV infection. Activation of bystander TRM during SeV exposure significantly delayed viral propagation, and in some cases prevented productive SeV infection, in an IFNg-dependent manner, and this effect positively correlated with bystander TRM number. Protection was associated with enhanced immune cell recruitment from circulation and rapid activation of URT epithelial cells. Notably, the ability of bystander TRM-derived IFNg to prevent infection was decreased when the recruitment of circulating immune cells was inhibited. Additionally, we have started to exlore URT TRM interactions with the local tissue environment using Xenium spatial transcriptomics. Overall, our data suggest that the primary role for rapid IFNg production by TRM during transmission is to limit viral propagation in order to “buy time” for other effector mechanisms to mediate viral clearance. These findings deepen our understanding how different TRM effector mechanisms are coordinated to optimize viral clearance while limiting pathology.

Abstract 108

Treg infiltration in non-muscle-invasive bladder cancer is associated with progression to muscle-invasive disease following BCG therapy

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Standard of care treatment for non-muscle-invasive bladder cancer (NMIBC) includes surgical resection followed by intravesical administration of bacillus Calmette-Guérin (BCG). Despite this treatment, roughly 15% of high-risk NMIBC patients will experience progression to muscle-invasive bladder cancer (MIBC) within five years, whereupon the five-year survival rate is less than 50%. In MIBC and other solid tumors, the relative frequencies of various T cell subsets are known to influence disease control and treatment response. High infiltration of granzyme B+ CD8 T cells is associated with positive outcomes, while Foxp3+ Tregs have been shown to restrain both effector CD8 differentiation and CD4 help. However, the exact immune features that control BCG response and progression in NMIBC remain incompletely understood. We investigated how the infiltration of different T cell populations within NMIBC tumors was associated with progression rates. Using multiplex immunofluorescence on tumor samples collected from 13 NMIBC patients prior to BCG treatment, we found that total and effector-like CD8 T cell numbers did not differ between progressors and non-progressors. However, progressors were found to be highly enriched for Tregs, both as a percent of all cells and as a proportion of the CD4 T cell population. This was supported by a mouse model of bladder cancer, wherein the bladders of tumor-bearing mice showed higher Treg infiltration than those of naive mice, while there was no difference in CD8 T cells. These results suggest that Tregs may act to promote cancer progression in NMIBC independent of total CD8 T cell numbers.

Abstract 109

Antigen properties and Metabolic Stress Determine B-Cell Functional Identity in Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with limited therapeutic options and a five-year survival rate of approximately 13%. Tumor progression is largely shaped by metabolically hostile tumor microenvironment (TME) characterized by hypoxia, nutrient deprivation, and chronic antigenic stimulation. Within this environment, B cells experience significant stress, yet how antigen quality and metabolic constraints influence their differentiation into anti-tumor or immunosuppressive states remains poorly understood. Using genetically engineered PDAC mouse models with antigen-specific B-cell systems, combined with in vivo and ex vivo metabolomic approaches, we investigated how tumor antigen properties and metabolic stress regulate B-cell fate. Preliminary data reveal that membrane-tethered antigens drive robust effector B-cell function, whereas soluble antigens preferentially induce immunosuppressive B cell phenotypes. Additionally, hypoxia was found to dampen BCR Signaling and may have the potential to sustain immunosuppressive B cell phenotypes. On a nutrient perspective, in contrast to tumor cells that increase glycolytic flux under hypoxia, tumor-infiltrating B cells exhibit downregulation of glucose transporters, impaired nutrient uptake, and mitochondrial dysfunction. Therefore, targeting metabolic stress pathways or restoring nutrient uptake in B cells, in combination with modulating antigen properties, may represent a novel strategy to reprogram the PDAC immune microenvironment and enhance responses to immunotherapy.

Abstract 110

Fc engineered malaria antibody therapeutic candidates for enhanced Fc receptors binding affinities

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Globally, malaria caused 282 million cases and 610,000 deaths in 2024. Besides moderately protective vaccines, passive infusions of monoclonal antibodies (mAbs) can provide effective short-term protection. The pre-erythrocytic stage circumsporozoite protein (CSP) targeting mAbs, MAM01, L9LS and CIS43LS, were safe and protective in passive infusion clinical trials. Here, we seek to evaluate the variants of MAM01, L9LS and CIS43LS optimized to enhance functional properties by Fc modifications including, for each mAb, mutations LS (M428L/N434S), G236A and GAALIE (G236A/A330L/I332E), afucosylation and, for comparison, aglycosylation (N297G). Using high throughput surface plasmon resonance, we first confirmed that these Fc modifications did not significantly impact antigen (CSP) recognition. Then we measured mAb binding affinities to FcRn (pH 7.4 and pH 5.6), FcγRIIIa(F), FcγRIIa(H) and C1q. Consistent with the impact of Fc modifications reported in the literature, compared to corresponding mAbs without the Fc modifications, LS mutations enhanced FcRn binding (>7.6-fold); G236A mutation enhanced FcγRIIa(H) (>6.1-fold) binding and weakened C1q binding (affinities weakened >5.9-fold); GAALIE mutations enhanced binding to FcγRIIa(H) (>3.2-fold) and FcγRIIIa(F) (>8.9-fold) while abolishing C1q binding; afucosylation enhanced FcγRIIIa(F) binding (>20.8-fold); aglycosylation abolished or significantly weakened binding to FcγRIIIa(F), FcγRIIa(H) and C1q. We also found that FcγRIIb to FcγRIIa(H) binding ratios were >47% lower for mAbs with G236A containing mutations (G236A and GAALIE) than without (LS mutation and afucosylation), matching well with literature evidence that G236A enhances Fc binding to FcγRIIa but not FcγRIIb. Overall, these results showed how CSP-targeting mAbs can be further optimized for Fc receptor binding to potentially improve efficacy.

Abstract 111

Targeting Tumor-TAM Interactions to Enhance STING-Mediated Anti-Tumor Immunity in Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is an aggressive subtype with limited treatment options. Tumor-associated macrophages (TAMs) are the most abundant immune cells in the TNBC tumor microenvironment (TME) and a key driver of immunotherapy resistance, yet TAM-targeting strategies alone have yielded limited durable benefit. Here, we investigated a novel strategy exploiting the reciprocal interplay between cancer cells and TAMs: by simultaneously reprogramming TAMs toward an anti-tumor phenotype and overcoming cancer cell-intrinsic resistance to TAM-mediated killing, we aimed to establish a self-reinforcing anti-tumor circuit that drives durable immunity in advanced TNBC. We previously demonstrated that STING agonists reprogram TAMs toward an anti-tumor state. Here, we discovered that ADAR1 — an RNA-editing enzyme highly expressed in TNBC and associated with poor prognosis — suppresses the anti-tumor activity of STING-activated TAMs through tumor cell-intrinsic mechanisms (regulating fatty acid homeostasis to promote tumor cell survival) and extrinsic mechanisms (producing immunosuppressive fatty acids that dampen STING signaling in TME). Notably, ADAR1 silencing sensitizes TNBC cells to STING-activated TAMs independently of tumor cell-intrinsic STING — which is frequently silenced in advanced cancers — suggesting broad therapeutic potential. In summary, our studies identify ADAR1 as a metabolic checkpoint in TNBC that promotes tumor cell survival and suppresses TAM anti-tumor reprogramming. Targeting ADAR1 collapses these pro-tumor pathways and, combined with STING-activated TAMs, creates a therapeutically exploitable convergence of metabolic stress, RNA sensing, and TAM-driven cytotoxicity in advanced TNBC.

Abstract 112

CD4 T Cells Drive the De Novo Activation of CD8 T Cells to Promote Vascular Injury in Atherosclerosis

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Atherosclerosis is an immune-mediated disease in which chronic inflammation drives vascular injury. While CD8 T cells are known to accumulate within atherosclerotic plaques, the initiating signals that drive their activation remain unclear. Here, we identified an underappreciated pathway of direct T-T cell communication that contributes to this process, where human memory CD4 T cells (TMEM) directly activate naive CD8 T cells (TN) ex vivo, inducing an activated/memory phenotype. To address this phenomenon in vivo, we adoptively transferred OVA-specific naïve CD8 T cells (OT-I cells) along with OVA-expressing CD4 TMEM cells into congenically distinct host mice. Only mice receiving both cell types exhibit strong CD8 T cell activation compared to the transfer of naive CD8 T cells alone. To test if the TCR is involved in this process in vivo, Nur77GFP OT-I CD8 TN cells were co-adoptively transferred with CD4 TMEM loaded with Ova-peptide. Not only do we observe a strong GFP signal reflecting TCR engagement, but also upregulation of the activation marker (CD44) within CD8 TN population, suggesting that CD4 TMEM can drive direct TCR-dependent activation and differentiation in vivo. To assess disease relevance, we transferred congenically marked cells from ApoE■/■ mice on high-fat diet into early atherosclerotic ApoE■/■ hosts. The presence of CD4 activated CD8 (co-cultured) T cells, but not CD8 TN alone induced significant apoptosis in the CD45■ aortic cells. Together, these findings reveal a CD4 T cell-driven mechanism that licenses naive CD8 T cells through direct TCR signaling, ultimately promoting vascular injury during atherosclerotic progression.

Abstract 113

Hypoxic Niches Enforce Glycolytic Dependence to Sustain FOXP3 and Suppressive Function in Skin Tregs

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Regulatory T cells (Tregs) residing in the skin need to adapt to a uniquely hypoxic microenvironment, unlike lymphoid tissues such as the spleen, where oxygen and nutrient availability are higher. We found that this niche drives a tissue-specific glycolytic program in skin Tregs, with increased expression of Lactate Dehydrogenase A (Ldha), Slc2a1 (GLUT1), and other glycolytic genes, alongside elevated glycolytic flux measured by SCENITH and Seahorse assays. To define the functional relevance of glycolysis, we generated Treg-specific knockout mice for Ldha, Slc2a1, and Hif1a. Loss of any of these genes selectively reduced Treg frequencies and FOXP3 protein levels in the skin, without affecting Tregs in lymphoid tissues. Disruption of this hypoxia-glycolysis axis in Tregs impaired control of effector T cell expansion and cutaneous inflammation in a contact hypersensitivity model and inhibited hair regeneration. Metabolomic profiling revealed that LDHA-deficient skin Tregs displayed disrupted glycolytic flux, impaired NAD+ regeneration, depletion of NAD■-dependent intermediates, and reduced amino acid availability. Although Foxp3 mRNA was unchanged, FOXP3 protein levels were reduced in LDHA-deficient skin Tregs, indicating post-transcriptional regulation. Mechanistically, LDHA-deficient skin Tregs exhibited impaired protein synthesis but comparable FOXP3 protein stability, which could be partially rescued by NAD■ supplementation.

Together, these findings establish a hypoxia-driven glycolytic axis that sustains FOXP3 protein synthesis, shapes the tissue-specific program of skin Tregs, and is essential for their homeostasis and suppressive function in the skin.

Abstract 114

Early regulatory mechanisms governing stem-like CD4■ T cell differentiation in cancer

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In cancer, CD4 T cells primarily become PD1+TCF1+ stem-like CD4 T cells in the TDLNs without further differentiating into TH1 effectors, thereby undermining the anti-tumor immunity. The mechanisms underlying this process remain unclear. By providing TRAMPC1-GP tumor-bearing mice a strong activation signal (LCMV Armstrong) at different time points after tumor inoculation, we found that tumor-specific CD4 T cells can only be rescued to differentiate to TH1s up to day 7 post tumor inoculation. Beyond this point, CD4 T cells remain stem-like. We then depleted Tregs to assess whether this observed quick establishment of CD4 tolerance is attributed to Treg suppression. We found Treg depletion on day 0 does not induce Th1 differentiation, whereas depletion after day7 does, suggesting Tregs are not suppressive until day7, while an additional differentiation signal may be required at early time points. We further assessed whether insufficient antigen or suboptimal priming are the limiting factors of early CD4 differentiation in cancer. We found treating TRAMPC-GP tumor-bearing mice with LCMV gp61-80 peptide as extra antigen did not promote TH1 differentiation. We also found that even if the tumor-specific CD4 T cells (SMARTAs) were initially primed in a TH1-polarizing environment (LM-GP66 infection), they did not proceed to TH1 differentiation after switching back into tumor, but instead still became stem-like CD4. These data suggest there is an early signal—potentially determined by early dendritic cells, that decides CD4 differentiation fates in cancer. Overall, these results shed light on the mechanisms that regulate CD4 T cell differentiation in cancer.

Abstract 115

The XIST-RNP is a Novel Source of Autoantigens in Autoimmune Patients

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Despite their prevalence, autoimmune diseases are often misdiagnosed and remain challenging to effectively treat. Conditions like systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) manifest in biological females (XX) and Kleinfelter males (XXY) upwards of 14-times more than XY males, implicating X chromosome multiplicity as a key driver of autoimmunity. Located on the X chromosome, XIST lncRNA is a master regulator of X chromosome inactivation; in healthy individuals, XIST is exclusively expressed in cells with >1 X chromosome, and forms polymeric complexes called XIST-ribonucleoproteins (XIST-RNPs) to execute silencing of accessory X chromosomes.

Previously, our group discovered that ectopic expression of Xist in male mice accelerates onset and intensifies severity of SLE in vivo, highlighting the contribution of XIST itself to autoimmunity. Leveraging single-cell RNAseq datasets, we have further illustrated that expression of XIST is variable across human PBMC subpopulations, particularly memory B cells, plasmacytoid dendritic cells, and double-negative T cells.

Through analysis of serum from healthy individuals and autoimmune patients, we have also discovered that dozens of unique autoantibodies which target antigens derived from the XIST-RNP are significantly elevated in SLE and SSc cohorts versus healthy controls. Serum autoantibodies targeting XIST-RNP antigens are more accurate predictors of SLE and SSc status and severity than current clinical diagnostics. Autoantibodies reactive against RPA1 autoantigen derived from the XIST-RNP significantly outperformed clinical standards Ro60 and TRIM21. Collectively, these results suggest that XIST strongly influences onset and progression of autoimmune disease, and defines the XIST-RNP as an important biomarker and focal point for intervention.

Abstract 116

Regulatory T-cells produce neuritin to drive tumor innervation and growth

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Tumor innervation is known to promote the growth and progression of multiple cancers. However, the cellular mechanisms driving nerves into the tumor, particularly the crosstalk between immune cells and nerves in the tumor microenvironment (TME), remain largely unclear. Regulatory T-cells (Tregs) accumulate in the TME and are associated with poor prognosis in cancer. Beyond immune suppression, Tregs interact with stromal components to remodel the TME, but whether and how Tregs directly modulate nerves to drive tumor growth remain unknown.

Using a murine model of triple-negative breast cancer, we observed that Tregs were accumulated along with nerves in breast tumors compared to normal breast tissues. Both murine and human Tregs, particularly the subset - follicular regulatory Tregs (TFR), were the predominant immune producers of the neurotrophic factor neuritin. Further spatial transcriptomics analysis revealed co-enrichment of Tregs and neuritin in nerve-dense tumor regions. Ablation of Tregs or TFR led to a significant reduction in the nerve density and delayed tumor growth. Conversely, administration of neuritin restored the nerve abundance in the tumor of TFR-deficient mice. Using the PC12/E0771 coculture assay, we further discovered that neuritin-induced differentiation and neurite outgrowth of PC12 cells promoted the stem-like phenotype of cancer cells, suggesting a direct requirement of nerves in neuritin-mediated tumor growth. In vivo deletion of neuritin specifically in Tregs resulted in reduced tumor innervation and growth, further supporting the role of Treg-derived neuritin in promoting tumor innervation and growth. Together, these findings have revealed neuritin as a Treg-derived factor driving tumor innervation and growth.

Abstract 117

Role of Olfr2 in Antigen Presentation and T Cell Activation During Atherosclerosis

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Atherosclerosis is a chronic inflammatory disease driven by dysregulated lipid metabolism and maladaptive interactions between innate and adaptive immune cells. Olfactory receptors (ORs), the largest family of G protein–coupled receptors, are emerging regulators of vascular inflammation beyond their canonical sensory roles. Olfr2 is expressed in myeloid cells such as monocytes and macrophages, where it senses the lipid peroxidation product octanal to activate the NLRP3 inflammasome and promote inflammation.

Dendritic cells (DCs), professional antigen-presenting cells (APCs), coordinate adaptive immune responses. However, whether Olfr2 regulates DC-mediated T cell activation during atherogenesis remains unclear. We found that ApoE■/■ Olfr2■/■ mice fed a high-fat diet (HFD) exhibited reduced T cell activation, as assessed by CD44 and CD62L expression, compared with ApoE■/■ controls.

We detected significant Olfr2 expression in cDC2 (CD11c■CD11b■) subsets, which was associated with altered MHC-II and CD86 expression, suggesting impaired APC–T cell interactions in Olfr2-deficient DCs. Next, we performed co-culture experiments using Nur77-GFP OT-I CD8■ T cells and Flt3L-differentiated bone marrow–derived DCs (BMDCs) from wild-type or Olfr2■/■ mice under SIINFEKL peptide–pulsed and non-pulsed conditions.

While Olfr2-deficient DCs were capable of initiating TCR signaling, they failed to promote effector memory CD8■ T cell differentiation (CD62L■CD44■ cells). These findings suggest that Olfr2 signaling may enhance DC costimulatory function and downstream T cell activation rather than antigen presentation per se.

Although the precise mechanistic role of Olfr2 in antigen presentation, DC function, and downstream T cell activation during atherogenesis remains to be fully elucidated, our data identify Olfr2 as a potential regulator of DC-driven adaptive immunity in atherosclerosis and suggest that targeting this pathway may limit maladaptive APC–T cell interactions and disease progression.

Abstract 118

Macrophage diversity in response to Chlamydia muridarum infection in the upper genital tract and its impact on infection outcomes

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Inflammatory responses in the female upper genital tract (UGT) in response to Chlamydia infections result in significant tissue damage that impairs reproductive health. The cellular mechanisms underlying Chlamydia's interactions with immune cells and how these may lead to sustained inflammatory damage after bacterial clearance remain poorly understood. The UGT harbors a complex local immune network, with macrophages critical to maintaining local tissue homeostasis. Our preliminary findings in CCR2-KO mice infected with Chlamydia muridarum (Cm) indicate that the absence of macrophages leads to more severe tissue pathology. Single-cell RNA sequencing of uterine horn tissues from C57B/6J mice revealed a heterogeneous population of macrophages that accumulated from 6 to 14 days post-infection (dpi) and persisted beyond bacterial clearance at 45 dpi. These macrophages include diverse subsets with antimicrobial gene signatures, anti-inflammatory and tissue repair programs, and pathways associated with intracellular infection, such as oxidative stress and iron modulation. Pro-inflammatory macrophage subsets were enriched early during infection, while tissue-repair subsets became more predominant later. Using mCherry-expressing Cm, we also detected a substantial number of macrophages within Chlamydia-containing cells in the uterine horns at 6 dpi, specifically those with enriched expression of pro-inflammatory genes. We hypothesize that these diverse macrophage subsets are critical in balancing inflammatory signals in the UGT, impacting the resolution of infection and subsequent development of tissue pathology. This study provides insight into the influence of Cm on macrophage phenotypic diversity and their roles in pathogen clearance and repair of the UGT.

Abstract 119

Baseline Nasal sIgA Corresponds with Protection Against Influenza Infection

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Investigating the relationship between mucosal antibodies and viral shedding may provide critical insights into protective mechanisms against influenza and inform strategies for enhancing mucosal immunity. We develop a mechanistic Bayesian mixture model that combines information from baseline immunological assays, in this case mucosal IgA, and longitudinal measurements of viral load. In an H1N1 challenge study with 38 participants, we measured the secretory IgA (SIgA) against influenza antigens in serially collected nasal lavage fluid. Viral dynamics were modeled using a system of ordinary differential equations (ODE) based on RT-qPCR observations over the first week of acute infection, and parameters of the ODEs were fit using Approximate Bayesian Computation. The relationship between SIgA titer and duration of viral shedding was determined using a multi-component “hurdle mixture model”, which allows us to decouple the protective effect of SIgA into two distinct biological gates: the probability of establishing a detectable infection (Gate 1) and the probability of achieving rapid clearance once infected (Gate 2). We found that SIgA is associated with protection from infection at Gate 1 ($p < 0.02$), while it is not associated with shorter shedding durations at Gate 2, given infection. Our novel method that includes deterministic models of viral dynamics improves the statistical power for identifying biomarkers, in this case baseline SIgA association with protective immunity.

Abstract 120

Exploring the Impact of Hyperglycemia on Macrophage Antimicrobial Effector Function during MRSA Infection

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People with diabetes mellitus (DM) are more vulnerable to methicillin-resistant Staphylococcus aureus (MRSA) infections. Although hyperglycemia has been shown to impair microbial ingestion and killing, its underlying mechanisms remain unclear. We aim to identify intracellular mechanisms affecting macrophage antimicrobial functions in diabetic conditions. RNA-seq analysis of peritoneal macrophages from diabetic and non-diabetic mice revealed that macrophages from diabetic mice exhibit decreased expression of genes involved in efferocytosis (Cd36, Mertk, and Lxr) and focal adhesion responses (RhoC), indicating a genetic signature associated with reduced phagocytosis. Conversely, pathogen recognition genes such as Cd14 and Tlr2 were upregulated. Next, we performed a phagocytic kinetic assay using pHrodo-conjugated S. aureus particles to measure differences in macrophage uptake and phagosomal acidification under low- and high-glucose conditions. We observed that high glucose levels decrease macrophage particle uptake and/or phagosomal acidification compared to cells cultured in low glucose. To distinguish between microbial ingestion and killing, we employed a dual-labeled MRSA protocol, in which sGFP-expressing MRSA is stained with an Atto N-hydroxysuccinimide (NHS) ester, resulting in MRSA fluorescing both green (GFP) and red (Atto). After macrophage-mediated killing of MRSA in the phagolysosome, GFP fluorescence is lost, whereas Atto, which is resistant to intraphagosomal bleaching, remains. Our imaging and flow cytometry results suggest that high glucose impairs both ingestion and killing, as evidenced by a higher number of GFP+/Atto+ specks compared to macrophages cultured in low glucose (GFP-/Atto+). These findings indicate that hyperglycemia hampers macrophage killing of MRSA, contributing to prolonged infections observed in clinical settings.

Abstract 121

B cells protect against Klebsiella pneumoniae following oral inoculation by constraining post seeding expansion and systemic dissemination

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Klebsiella pneumoniae (Kpn) is a growing public health threat driven by multidrug resistant and hyper-encapsulated strains that disseminate systemically from the gut. Although intestinal colonization often precedes invasive disease, the immune mechanisms controlling early Kpn expansion and dissemination remain unclear, particularly the role of B cells. Using an oral infection model without antibiotic pretreatment, we investigated how B cells and antibodies shape Kpn colonization dynamics and disease outcome. B cell–deficient (μMT) mice exhibited wild type–like fecal shedding and similar initial colonization levels, yet subsequently developed uncontrolled bacterial expansion in the gut and extraintestinal sites, resulting in increased dissemination and mortality. Lineage tracking with a barcoded Kpn library and STAMPR analysis revealed that B cells do not restrict initial seeding, but instead constrain post seeding clonal expansion in both intestinal and peripheral tissues. To define the underlying antibody mechanisms, we examined mice selectively deficient in mucosal IgA transport or secreted IgM. Polymeric Ig receptor-deficient (pIgR■/■) mice exhibited elevated intestinal and systemic burdens but survived infection, whereas mice unable to secrete IgM (μS■/■) displayed normal GI colonization yet succumbed to disease. Together, these findings demonstrate that B cells protect against Kpn not by preventing colonization, but by limiting post seeding expansion and systemic spread. While mucosal IgA restricts early intestinal growth, secreted IgM is essential for survival following oral Kpn infection, establishing post seeding expansion as a key determinant of disease outcome.

Abstract 122

Sex-Specific Breaks in Tolerance in TLR7-Based Autoimmune Mouse Models

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Systemic lupus erythematosus (SLE) is a chronic autoinflammatory disease characterized by dysregulated B cell responses against self (e.g. double-stranded DNA), which predominantly affects women. Incomplete X chromosome inactivation (XCI) within immune cells is hypothesized to be one contributing factor for the high incidence of autoimmune diseases in women, and the X-linked gene TLR7 is known to be a key non-specific activator of autoreactive B cells in SLE. Due to the necessity for SLE diagnosis to occur after substantial symptoms have manifested, little is known about initial tolerance breaks and autoimmune induction. How this process might differ between males and females is unknown. To investigate the early stages of SLE-like autoreactive development across both sexes, we employed two TLR7-based mouse models: 1) a TLR7 gain-of-function model (kika) derived from pediatric human lupus, and 2) a TLR7 activation model employing regular resiquimod (R848) application to the foot of healthy B6 mice. Utilizing spatial transcriptomics and paired high-dimensional flow cytometry to analyze autoreactive immune activation in the draining popliteal lymph node, sex-specific characteristics of early breaks in tolerance could be observed. Two distinct patterns of expression were observed across X-linked genes in bulk, suggesting differential regulation across the X chromosome that underlies unique responses in male and female TLR7-based autoimmune induction. This work has the potential to increase our understanding of the impact of sex on tolerance breaks to begin to better inform both early-stage disease diagnosis and potential sex-specific intervention options.

Abstract 123

Alternative splicing regulates Z-nucleic acid sensing in Mycobacterium tuberculosis infection

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Z-form nucleic acids (Z-NAs) are left-handed conformations that arise under torsional stress (e.g., mitochondrial damage, genomic perturbation, cell death) and accumulate in the cytosol as immunostimulatory ligands. While previously described in viral infection models, their role in intracellular bacterial infection remained unexplored. The Patrick lab has identified Z-NA accumulation during Mycobacterium tuberculosis (Mtb) infection by immunofluorescence microscopy, though its immune consequences are unknown.

Z-NA-binding proteins are candidate mediators of these effects. Among them, Z-DNA binding protein 1 (Zbp1) is of particular interest, as it is regulated at both transcriptional and alternative splicing levels during Mtb infection. In mice, Zbp1 exists as two isoforms: Zbp1-L contains RHIM domains that enable RIPK1/3-mediated inflammatory cell death, whereas Zbp1-S lacks these domains and instead has an unstructured tail. Zbp1 has also been implicated in type I interferon responses through stabilization of cytosolic nucleic acids. Given that both type I IFN signaling and inflammatory cell death contribute to Mtb disease severity, Zbp1 isoform balance may represent a tunable checkpoint linking nucleic acid sensing to infection outcomes.

To define the functional consequences of Zbp1 splicing, isoform-modulated systems were developed. Zbp1 overexpression suppresses type I IFN-associated genes (Ifnb1, Rsad2) while enhancing Il1b expression, consistent with an antimycobacterial state. However, Zbp1-L overexpression also amplifies macrophage cell death during infection, potentially promoting pathogenesis. Ongoing studies aim to define Z-NA sources and determine how isoform-specific sensing shapes downstream inflammatory outcomes. Together, this work identifies Zbp1 splicing as a regulatory node linking nucleic acid sensing to macrophage responses during Mtb infection.

Abstract 124

Tissues Guide Dependence of Regulatory T cells on the Transferrin Receptor

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Activated T cells increase transferrin-bound iron uptake via the transferrin receptor, also called CD71. We previously demonstrated that targeting CD71 with an antibody to reduce iron update can modify CD4 T cell function, with different effects on TH1, TH17, and regulatory T (Treg) cells. CD71 blocking antibody-treated Tregs had no loss of viability or differentiation, and Foxp3 expression was increased. However, a genetic deletion of Tfrc (the gene for CD71) driven by Foxp3-Cre was reported to cause a lethal autoimmunity. Whether altered immune homeostasis or insufficient early developmental tolerance drive the phenotype of CD71 knockout (KO) Treg mice were unclear. Here, we examined the Foxp3-YFP-Cre KO mouse model and a tamoxifen-inducible KO model in adults to determine the role of CD71 expression in Treg cells. We hypothesized that due to a lack of iron for mitochondrial metabolism, KO Treg adapt to rely heavily on glycolysis and become unstable, promoting pro-inflammatory exTreg cells. This effect was not universal, however, and necropsy analyses revealed tissue-specific inflammation. While the colons of mice with KO Treg cells appeared healthy, skin and lung tissue were severely inflamed. Metabolically, KO Treg cells had a significant decrease in their glycolytic capacity and instead increased oxidation of amino acids and fatty acids. In inflamed skin, which that promotes increased oxidative stress, CD71 expression in Treg cells suppressed tissue inflammation in a model of atopic dermatitis-like disease. These results indicate the CD71-iron axis as a new immunometabolic regulator of Treg cell functions in immune and non-immune organs.

Abstract 125

Investigating the Role of Type I Interferon Signaling in Calcium Mobilization and PAD4 Activity During Mycobacterium tuberculosis Infection

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Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a leading cause of global mortality, accounting for 1.3 million deaths in 2023. Neutrophils are among the first responders to airway infection, deploying antimicrobial mechanisms, including neutrophil extracellular trap (NET) release through peptidylarginine deiminase 4 (PAD4)–mediated chromatin decondensation. While NETs typically contain pathogens, they fail to restrict Mtb growth. Instead, excessive NET release, in addition to neutrophil-driven inflammation and elevated type I interferon (IFN) signaling, are associated with severe immunopathology in pulmonary TB and Mtb-infected mice. Notably, type I IFN signaling promotes NET release and exacerbates lung damage.

Autophagy-related protein ATG5 promotes host survival during Mtb infection by suppressing type I IFN–driven PAD4 activity, thereby limiting tissue pathology. However, the mechanism by which ATG5 restrains type I IFN–dependent PAD4 activity remains unclear. Because neutrophils restrain responses by limiting cytosolic calcium, and PAD4 activity is calcium-dependent, we hypothesize that ATG5 suppresses PAD4 activation by limiting type I IFN–dependent calcium mobilization.

To investigate this, we monitored calcium flux in murine neutrophils by flow cytometry, assessing intracellular store release and extracellular calcium influx. Neutrophils lacking type I IFN signaling exhibited reduced calcium flux in response to the bacterial peptide N-formylmethionyl-leucyl-phenylalanine compared to wild-type cells, suggesting that type I IFN promotes calcium mobilization. Ongoing studies will test whether ATG5 regulates calcium flux and how calcium influences PAD4 activity. Together, these studies will elucidate how ATG5 limits type I IFN–mediated PAD4 activity and may inform strategies to regulate neutrophil-driven pathology in TB.

Abstract 126

Antigen-specific effector B cells differentiate in the brain during CNS viral infection

Alexander K. Merder and E. Ashley Moseman

B cells and their antibody secreting cell (ASC) progeny can potently protect against viral infections. Early after infection, B cells detect their cognate antigens and begin to proliferate, generating a wave of early ASCs that produce large quantities of antibodies – most of which funnel into circulation to provide the host protection against the invading pathogen. While circulating antibodies access and protect most tissues, the blood brain barrier (BBB) effectively blocks circulating antibody access to the central nervous system (CNS). This suggests B lineage cells must migrate into the brain to directly impact viral clearance. Using a viral-specific BCR knock-in mouse as well as fate-mapping transgenics, we tracked antigen-specific B cells and ASCs from activation in draining lymph nodes into the infected brain. While B cells are known to traffic into the inflamed CNS, how they function locally is still largely unexplored. Virus-specific B cells arrive in the brain starting five days after vesicular stomatitis virus (VSV)-driven encephalitis, localizing directly within infected regions of the brain parenchyma as IgM+ and class-switched effector cells. These effector B cells can locally proliferate and differentiate into ASCs within the CNS. Although CNS effector B cells are transcriptionally similar to previously defined early memory B cells, they also show clear evidence of local antigen- and interferon-driven activation, suggesting that antigen engagement helps tune local antibody production. Because the CNS lacks ready access to the serum antibody pool, we hypothesize that resident B cells sense available antigen and respond by generating ASCs and antibody.

Abstract 127

Intrinsic Resistance to PD-1/PD-L1 Blockade in the LVRCC67 Clear Cell Renal Cell Carcinoma (ccRCC) Model

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The programmed cell death protein 1 (PD-1) and its ligand PD-L1 form a critical immune checkpoint that tumors exploit to evade anti-tumor immunity. PD-L1 expression on tumor cells suppresses T cell function through PD-1 engagement, leading to T cell exhaustion and reduced cytotoxicity. Although PD-1/PD-L1 blockade is effective in some cancers, its efficacy often depends on a pre-existing T cell-infiltrated tumor microenvironment. In the LVRCC67 murine model of ccRCC, prior studies showed that anti-PD-1 (clone RMP1-14) monotherapy failed to control tumor growth or metastasis, consistent with limited T cell infiltration and a predominantly myeloid-driven microenvironment. Here, we evaluated the therapeutic impact of PD-L1 (clone 10F.9G2) blockade and its effects on the tumor immune landscape.

Male C57BL/6 mice were subcutaneously inoculated with LVRCC67 cells and treated with anti-PD-L1 antibody beginning 3-4 weeks after tumor establishment. Mice received five doses at 3-day intervals and were analyzed at week 6 using comprehensive immune profiling.

PD-L1 blockade increased CD44■PD-1■ antigen-experienced T cells and promoted terminal differentiation of CD8■ T cells in both primary and metastatic sites but did not affect tumor growth or metastatic burden. Dendritic cells were expanded, while Tregs, monocytes, and neutrophils remained unchanged. The tumor microenvironment exhibited a stromal-rich phenotype, with enrichment of CD44■ fibroblasts in primary tumors and increased ICAM1■ and PDPN■ fibroblasts in metastases, indicative of active ECM remodeling and inflammatory engagement.

Collectively, these findings demonstrate intrinsic resistance to PD-L1 blockade in the LVRCC67 model, highlighting the need for combination strategies targeting tumor-intrinsic and stromal resistance mechanisms.

Abstract 128

Impact of PARP inhibition on T cell dynamics and HIV persistence

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Latently-infected CD4+ T cell's proliferation and resistance to immune clearance represent the main drivers of HIV persistence during antiretroviral therapy (ART) and barrier to cure. The Poly(ADP-ribose) polymerases (PARPs) are a family of enzymes that regulate a variety of cellular processes including proliferation and apoptosis. PARP inhibitors have been developed as anti-tumor agents inhibiting cancer cell proliferation and enhancing anti-tumor immune responses. Here we evaluated the impact of the PARP inhibitor Niraparib on T-cell dynamics and HIV persistence in vitro, ex vivo, and in vivo in the rhesus macaque (RM) model.

The anti-proliferative activity of Niraparib was assessed in RM peripheral blood mononuclear cells stimulated with homeostatic cytokines or CD3/CD28 antibodies using a cell division tracking dye. Niraparib's impact on HIV persistence was evaluated in a latently-infected cell line and CD4+ T cells from RMs infected with SIV (simian immunodeficiency virus) and treated with ART by quantifying viral transcription. The immunomodulatory and safety profiles of Niraparib in vivo were also evaluated in 2 healthy RMs treated with daily oral doses at 15 mg/kg for 21 days.

Niraparib inhibited homeostatic and polyclonal proliferation of CD4+ T cells and reversed SIV latency in vitro and ex vivo. Niraparib treatment was well tolerated in RMs and resulted in increased apoptosis in memory CD4+ T cells. Our results identified Niraparib as a novel immunomodulatory latency reversing agent with a good safety profile in nonhuman primates. Further studies in ART-suppressed RMs will establish the therapeutic potential of PARP inhibition for HIV cure in vivo.

Abstract 129

PVSRIPO Immunotherapy Engages CNS Resident Border Macrophages

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The dural meninges are increasingly recognized as critical regulators of CNS immunity, antigen drainage, and antitumor responses. PVSRIPO, a profoundly attenuated, non-cytopathogenic polio:rhinovirus chimera currently in clinical trials for glioblastoma immunotherapy, triggers potent innate immune activation, yet its impact on the dural meninges remains undefined. We investigated whether PVSRIPO can infect dural myeloid cells, initiate a type-I-dominant immune response, and restructure meningeal lymphatic vessels to enhance antigen flux from the brain parenchyma to deep cervical lymph nodes in a murine model. Intratumoral PVSRIPO treatment resulted in infection of myeloid cells within the dural meninges and robust activation of border-associated macrophages (BAMs). We further observed significant expansion of meningeal lymphatic vessels along the transverse sinus, superior sagittal sinus, and confluence of sinuses up to seven days post-treatment, in both naïve mice and CT2A glioma-bearing mice. We also observed enhancement of glymphatic flux in the dural venolymphatic complex after treatment with PVSRIPO. Collectively, these findings demonstrate that PVSRIPO engages the dural immune compartment, drives meningeal lymphangiogenesis, and potentiates antigen drainage from the CNS to peripheral lymphoid tissues establishing a previously unrecognized mechanism by which viral immunotherapy may amplify adaptive antitumor immune responses across multiple CNS disease contexts.

Abstract 130

Antigen-Specific Heterogeneity of Human Lung CD8■ Tissue-Resident Memory T Cells

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Due to their position within lung tissue, CD8+ tissue resident memory T cells (Trm) act as sentinels of the respiratory tract that rapidly respond to, and mediate protection against, respiratory viruses. Trm are defined by long-term residence and are commonly identified by expression of tissue-retention markers such as CD69 and CD103. Murine studies confirm that both CD69■CD103■ and CD69■CD103■ lung CD8■ Trm populations are truly tissue resident and non-circulating. With increased access to healthy human donor tissues, studies of human CD8■ Trm have expanded, revealing that Trm heterogeneity arises from multiple factors, particularly the local tissue microenvironment and antigen specificity. While many studies compare Trm across tissues, this emerging evidence suggests that focusing analyses within a single tissue, such as the lung, may enable deeper molecular insight into Trm heterogeneity. Moreover, although viral tropism influences Trm, heterogeneity within antigen-specific human lung CD8■ Trm remains largely unexplored.

Here, we use primary human lung cells to compare heterogeneity between antigen-specific CD8■ Trm. Using tetramers, we identify lung CD8■ T cells specific for respiratory viruses, including influenza, RSV, and SARS-CoV-2, as well as systemic viruses such as CMV and EBV. We then use high dimensional flow cytometric analysis and single cell transcriptional profiling to compare the phenotypic and molecular programs associated with these distinct Trm subsets. Together, this work aims to define how viral tropism shapes human lung CD8■ Trm heterogeneity in the human lung and to provide insight into how diverse Trm populations contribute to antiviral immunity in the respiratory tract.

Abstract 131

CD8+ T cells are required for maintenance of viral suppression under antiretroviral treatment in SIV-infected rhesus macaque infants

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The antiviral role of CD8+ T-cells during HIV infection in adults includes both HIV-specific cytotoxic and non-cytolytic activities that promote latency during antiretroviral treatment (ART). In infants cytotoxic responses are suboptimal, failing to control viremia. Notwithstanding, it is unknown whether the pro-latency activities of CD8+ T-cells described in adults are present in infants and how they may be influenced by the tolerogenic immune environment in early life.

To interrogate the role of CD8+ T cells in pediatric HIV persistence, we performed experimental CD8+ T-cell depletions in infant rhesus macaque (RMs) infected with a simian immunodeficiency virus (SIV) and treated with ART. 10 RMs received the anti-CD8α-depleting antibody MT807R1, including 5 RMs that additionally received the latency reversing agent AZD5582. Six control RMs were maintained on ART only.

All RMs experienced >99% of peripheral CD8+ T-cells without adverse events resulting in virus reactivation evidenced by on-ART viremia. Interestingly, combined treatment with AZD5582 induced higher levels of on-ART viremia (P=0.0001) and accelerated early CD8+ T-cell reconstitution (p=0.0090). This reconstitution was driven by the memory cell population with a lasting shift from naïve to memory phenotypes. CD8+ T-cell reconstitution was also slower as compared to adults (p=0.0341).
Our study demonstrates that CD8+ T cells contribute to the maintenance of viral suppression on ART in the pediatric population and suggest key differences in CD8+ T cell dynamics as compared to adults. Further analyses are warranted to elucidate the mechanisms regulating the pro-latency activity of the CD8+ T cells during pediatric HIV/SIV infection

Abstract 132

Development of a Protective Cellular Therapy to Prevent Graft Versus Host Disease Post Allogenic Hematopoietic Stem Cell Transplant

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Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT), driven by donor T cells that mount a dysregulated inflammatory response against recipient tissues. This limits post-transplant survival and underscores the need for immunomodulatory strategies that mitigate GVHD while preserving the graft-versus-tumor (GVT) effect. Emerging evidence highlights innate-like T cell subsets, defined by semi-invariant T cell receptors, rapid effector function, and tissue residency, as critical regulators of alloimmunity.

Mucosal-associated invariant T (MAIT) cells, natural killer T (NKT) cells, and Vδ2 γδ T cells have been associated with reduced GVHD severity and improved outcomes. Despite distinct antigen specificities, these populations share expression of the transcription factor PLZF (Zbtb16), a key regulator of innate-like T cell programming that enables rapid cytokine production and regulatory function.

Building on prior work demonstrating that ectopic PLZF expression imparts innate-like properties to conventional αβ T cells, we hypothesized that adoptive transfer of PLZF-engineered donor T cells could suppress GVHD. In murine acute GVHD models (B6→BALB/c), transfer of PLZF-engineered conventional T cells significantly delayed disease onset and improved survival compared to controls. Mechanistically, these cells promoted expansion of regulatory T cells, shifting the immune response toward tolerance.

These findings support PLZF engineered overexpression as a programmable strategy for GVHD prophylaxis, with potential to preserve GVT activity. Ongoing studies are evaluating antitumor efficacy and translation to humanized models.

Abstract 133

Dynamic Regulation of MLKL in Macrophages Drives Stage-Specific Processes in Atherosclerotic Plaques

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During early atherosclerotic cardiovascular disease (ASCVD), macrophages metabolize lipids to limit their accumulation in the arterial wall. However, as ASCVD progresses, metabolism becomes overwhelmed, leading to foam cell formation. Necrotic core, a hallmark of advanced ASCVD causing plaque rupture and fatality, occurs as foam cells undergo necroptosis, which is a pro-inflammatory form of cell death. In addition to necroptosis, mixed lineage kinase domain-like protein (MLKL) also engages non-necroptotic roles, including endocytosis. We previously reported that Mlkl knockdown in Apoe-knockout mice reduces plaque lipids, while attenuating necrotic core formation. We therefore hypothesize that MLKL promotes macrophage lipid metabolism in early ASCVD but shifts towards necroptosis and necrotic core formation in advanced ASCVD. Treatment of mouse bone marrow-derived macrophages with the atherogenic lipoprotein oxidized low density lipoprotein (oxLDL, 100ug/ml) for 48 hours induces phosphorylated MLKL (pMLKL) and necroptosis as expected, whereas 24-hour treatment induces pMLKL in the absence of necroptosis. Additionally, 24-hour oxLDL treatment induces polyubiquitination of MLKL, as revealed by tandem ubiquitin binding entities (TUBE) pulldown, consistent with reports of its endocytic functions. To study differential MLKL expression in plaque macrophage subsets, we created a novel HA-tagged MLKL transgenic mouse (MlklHA/+) and induced atherosclerosis via intraperitoneal administration of AAV8-PCSK9 and feeding a Western diet for 8 weeks. Flow cytometry analysis of aortic plaques reveal increased MLKL in foamy vs non-foamy macrophages (11.2-fold, $P<0.01$). Together these data underscore a dynamic regulation of MLKL during atherogenesis and suggest its dysregulation as a driving force in ASCVD progression.

Abstract 134

HDAC9 Constrains Antibody-Secreting Cell Commitment Through Repression of MEF2 Target Genes

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Activated B cells make an early commitment to the antibody-secreting cell (ASC) fate, yet the epigenetic regulators governing this decision remain poorly defined. Using an in vivo CRISPR/Cas9 screen targeting epigenetic and transcriptional regulators at B cell fate branch point, we identified the class IIa histone deacetylase Hdac9 as a negative regulator of ASC commitment. Loss of Hdac9 significantly increased ASC differentiation at the expense of memory B cell precursor populations. To define the transcriptional mechanism, we performed in vivo Perturb-seq, which validated the Hdac9 phenotype at single-cell resolution. Hdac9 knockout cells displayed population shifts toward ASC and plasmablast fates with corresponding depletion of memory precursors. Per-cluster gene set enrichment analysis revealed coordinate enrichment of ASC gene expression in Hdac9 KO plasmablasts. MEF2B ChIP-seq targets, Xbp1 secretory pathway genes, Prdm1-activated and repressed targets, plasma cell signature genes, and Irf4 targets were all significantly positively enriched. These transcriptional changes were strongest in the transitional plasmablast population, suggesting that Hdac9 gates the commitment decision rather than terminal plasma cell differentiation. The enrichment of MEF2B ChIP-seq targets, many of which are plasma cell program genes, supports previous literature in which Hdac9 functions with MEF2 as a corepressor, maintaining repression of the ASC differentiation program until appropriate signals are received. We further validated the Hdac9 phenotype using a single-guide retrogenic knockout system, confirming enhanced ASC output in an independent in vivo experiment. Together, these findings establish Hdac9 as an epigenetic gatekeeper of ASC fate commitment.

Abstract 135

Innate sensor responses set a supra-unitary threshold for viruses to successfully infect cells

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Cells resist viral infection by triggering rapid and sensitive innate immune responses, initiated by cytoplasmic viral sensor proteins (CyVSPs), RIG-I, MDA5, and PKR. Our single-cell analysis revealed that viral replication requires a supra-unitary viral genome input, significantly more than one, which we defined as the virion input threshold for replication (VITREP). This threshold varies depending on viruses and cell types, independently of viral protein synthesis. Through mathematical modeling and experimental testing, we uncovered that supra-unitary viral genome quantities activate negative feedback regulators of CyVSPs including Death-Associated Protein Kinase 1 (DAPK1), Rio Kinase 3 (RIOK3) and Protein Phosphatase 2 Regulatory Subunit Alpha (PPP2R2A), that suppress innate immune responses and allow replication. We validated key predictions of our model, namely downregulating negative feedback regulators or coinfecting cells, reduced or promoted replication respectively. Together, these findings reveal a fundamental mechanism governing viral infectivity and tissue tropism.

Abstract 136

Galactose Accentuates RLR-mediated Interferon Response in Human Monocyte-Derived Macrophages

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A defining property of cancers that lack intrinsic immune surveillance is the absence of costimulatory context in myeloid cells—macrophages and dendritic cells (DC)—preventing tumor antigen cross presentation and antitumor CD8+T cell priming. Overwhelming empirical evidence shows that tumor antigen-specific antitumor immunity requires endogenous, type-I interferon (IFN-I) signaling in myeloid cells. To this end, we utilize PVSRIPO, a profoundly attenuated rhino:poliovirus chimera for cancer immunotherapy. PVSRIPO, like its poliovirus parent, has natural tropism for myeloid cells due to expression of the poliovirus receptor CD155, yet is non-cytopathogenic. Instead, upon infection, PVSRIPO establishes viral RNA (vRNA) replication that elicits an endogenous, sustained IFN-I response, due to activation of the pattern recognition receptor, MDA5. MDA5/RIG-I-mediated IFN-I responses in myeloid cells are highly susceptible to metabolic conditions, in particular glycolytic metabolism/nucleotide production. Our premise is that unraveling the nexus linking vRNA replication and MDA5:TBK1:IRF3 innate signaling to glycolytic metabolism will enable new immunotherapy approaches that enhance innate IFN-I responses with metabolic modifiers. Using primary human monocyte-derived macrophages, we demonstrated that galactose, a naturally occurring epimer of glucose, augments the ensuing IFN-I responses post infection. Mechanistically, we hypothesize that galactose increases nucleotide availability to the virus by exploiting glycolytic shunting through the pentose phosphate pathway (PPP), supporting vRNA replication and dsRNA production.
Our data demonstrate that understanding mechanisms of myeloid cell immune programming through elucidation and exploitation of interconnected metabolic pathways is key to further understanding the myeloid immuno-metabolic axis; which may accentuate virotherapy clinical outcomes.

Abstract 137

TLR7/9–IFNα–LDHB Checkpoint Rewires NET Subtypes in Lupus

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Excessive NET deposition inflames systemic lupus erythematosus (SLE) pathology, yet patients remain unusually susceptible to invasive bacterial infection—a paradox suggesting qualitative defects in neutrophil effector programs. Building on our prior work showing that mitochondrial dysfunction in SLE neutrophils impairs suicidal NETosis and host defense, and that neutrophil mitochondria sense staphylococcal lactate to trigger bactericidal NET release, we identify a TLR7/9–IFNα–LDHB checkpoint that rewires NET subtype output and undermines antibacterial immunity in murine models of SLE (MRL/lpr) and human SLE neutrophils. Chronic nucleic-acid sensing by TLR7 and TLR9 represses mitochondrial LDHB expression thereby blocking lactate-triggered mitochondrial ROS and NETosis. Instead, staphylococcal α-hemolysin induces calcium influx and rapid degranulation that liberates preformed IFNα and drives vital NET release. These programs are antagonistic: inducing vital NETs suppresses subsequent suicidal NETosis and yields NETs with reduced antibacterial proteins and poor bactericidal activity. Consequently, NETs are not monolithic—suicidal and vital NETs arise via distinct triggers, carry different proteomic cargo, and diverge in bactericidal capacity. In vivo, dual hydroxychloroquine and IFNAR1 blockade restores LDHB expression, rebalances NET subtype composition, and lowers organ bacterial burdens, while ex vivo treatment of human SLE neutrophils similarly rescues LDHB, mitochondrial ROS, and suicidal NETosis and suppresses IFN-amplified vital NETs. Thus, persistent TLR7/9–IFNα signaling enforces an LDHB-low, vital-NET-biased state that impairs host defense, providing a mechanistic rationale and biomarker framework for therapeutic normalization of neutrophil function in SLE.

Abstract 138

IL-5 signaling protects from acute lung injury

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Acute Lung Injury (ALI) is characterized by alveolar damage caused by a variety of etiologies that ultimately result in barrier dysfunction, edema, and hypoxia. Previously, the Sperling lab demonstrated that IL-5 signaling protects from lung injury-induced mortality and edema in the bleomycin mouse model of ALI. While eosinophils are primarily involved in IL-5 signaling, we observed the protective effects were independent of eosinophils, suggesting a role for non-canonical IL-5 responding cells such as B cells or epithelial cells.
We discovered that epithelial cells increased their expression of IL-5Rα seven days post-bleomycin and that these IL-5Rα+ epithelial cells were highly proliferative by expression of Ki-67. Conditional depletion of IL-5Rα in lung epithelium led to more weight loss during bleomycin-induced ALI suggesting that IL-5 signaling in lung epithelial cells protect against lung-injury. In support of these findings, primary human lung epithelial cells, from control donors express IL-5Rα. Additionally, when grown in an air liquid interface culture, these cells responded to IL-5 treatment by activating Erk and Akt. Interestingly, IL-5Rα-positive B cells were also increased in the lung tissue seven days post bleomycin administration, and depletion of IL-5Rα in B cells led to a trend in increased weight lost during bleomycin-induced ALI. Collectively, these data suggests that IL-5 signaling axis synergizes the mucosal barrier and the immune system to confer protection during bleomycin-induced ALI.

Abstract 139

Targeting ADAR1 to harness the anti-tumor potential of PARP inhibitor-elicited RNA sensing

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Triple-Negative Breast Cancer (TNBC) is the second most common subtype of breast cancer but has the highest mortality rate. PARP inhibitors (PARPis) are the first FDA-approved targeted therapy to treat TNBC by exploiting deficiencies in homologous recombination repair to achieve synthetic lethality. However, their clinical benefit is limited to the ~15% of patients harboring pathogenic BRCA1/2 mutations. Whether PARPis engage additional mechanisms with therapeutic relevance in BRCA1/2-wild-type TNBC remains unclear.

We have recently identified a previously underexplored mechanism of PARPi. In TNBC cells, we found that PARPi induces cytosolic accumulation of double stranded RNA independent of BRCA1/2 mutational status. This indicates that PARPi can engage RNA-sensing pathways associated with cytotoxicity and immunogenicity. Our preliminary findings further implicate ADAR1, an RNA-editing enzyme highly expressed in TNBC, in suppressing PARPi-induced RNA sensing. Concurrent targeting of ADAR1 and PARPi significantly slows tumor growth in an aggressive and PARPi-resistant genetically engineered mouse model of TNBC. Initial mechanistic studies found that te combination treatment not only increased the tumor cell cytotoxicity but expanded effector CD8+ T cell population in the tumors.

Taken together, these findings represent a paradigm shift in our understanding of PARPis, expanding their therapeutic utility beyond cancer patients with pathogenic BRCA variants. Because PARPi has been approved for the treatment of breast, ovarian, prostate, and pancreatic cancers, our work may redefine patient eligibility criteria, rationalize novel PARPi-based combination strategies, and enhance the immunogenic potential of PARPi across a spectrum of cancer types to include those that are currently ineligible for PARPi therapy.

Abstract 140

Nr4a1E2 Deficiency Disrupts Vascular Wall Homeostasis Under Tumor-Associated Stress Conditions

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Vascular wall homeostasis depends on coordinated interactions between endothelial cells, smooth muscle cells, extracellular matrix, and circulating immune cells. Non-classical monocytes (Ly6Clow nMo) mediate immune surveillance of the vascular wall and are essential for maintaining vessel integrity during physiological and pathological stress. An orphan nuclear receptor Nr4a1 is a lineage-defining regulator of nMo survival and patrolling. We have previously shown that the E2 sub-domain within the Nr4a1 super-enhancer region is essential for regulating Ly6Clow nMo functions. Here, we hypothesized that disruption of Nr4a1-E2-dependent nMo surveillance compromises vascular structure and adaptive remodeling under acute stress. To test this, we examined vascular homeostasis in Nr4a1-E2-deficient mice bearing B16F10 melanoma. Loss of Nr4a1-E2 resulted in a marked reduction of circulating nMo, increased systemic inflammatory and soluble adhesion marker profiles in serum, including IL1a (p-val=0.02), ICAM-1 (p-val=0.008), E-Selectin (p-val=0.009), PECAM-1 (p-val=0.0003), and L-Selectin (p-val=0.006) in Nr4a1-E2-KO compared to wild-type mice. At baseline, mason's trichrome analysis of the aortic wall revealed a significant reduction in fibrosis in E2-deficient mice, indicating altered extracellular matrix organization. E2-deficient mice showed increased endothelium-independent vasorelaxation in mesenteric arteries, indicating altered smooth muscle function. Under B16F10 tumor-induced vascular stress, bulk RNA sequencing of flow-sorted CD31+ lung endothelial cells revealed extensive transcriptional reprogramming, characterized by altered expression of genes governing vascular tone. Specifically, Col6a2, Tagln, Tgfa, Itgb5, Itgal, and Col3a1 were significantly down-regulated, while endothelial junction-associated genes including Pecam1, Cdh5, Ctnna1, and Cldn5 were up-regulated. Pathway analysis identified disruptions in integrin-mediated cell interactions, collagen biosynthesis and degradation, and ECM organization processes critical for vascular stability and stress adaptation. Together, our findings demonstrate that loss of Nr4a1-E2-dependent nMo surveillance establishes a structurally fragile and functionally dysregulated vascular homeostasis that is poorly adapted to tumor-associated stress.

Abstract 141

SARS-CoV-2 Alphavirus Replicon Particle Vaccine Induced Mucosal Immunity

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Vaccines against SARS-CoV-2 are highly efficacious against severe disease and have prevented over 2.5 million deaths worldwide. However, existing vaccines fail to induce durable immune responses within the respiratory tract. Consequently, breakthrough infections remain pervasive, accelerating the evolution of new viral variants that escape vaccine immunity. We have previously described an alphavirus replicon particle (VRP) vaccine platform that induces mucosal immunity following peripheral administration. Here, we demonstrate that intranasal (IN) vaccination with a SARS-CoV-2 spike VRP significantly reduced nasal SARS-CoV-2 titers in a mouse adapted challenge compared to peripheral vaccination. Given the ability of VRPs to protect nasal epithelium we next evaluated cross-protection in an Omicron challenge model. The Omicron lineages are the most divergent SARS-CoV-2 variants with over 30 mutations in the spike alone. Collectively, these mutations alter antigenicity by driving antibody escape while increasing transmissibility through increased replication efficiency in the nasal epithelia. We demonstrate that IN VRP vaccination significantly enhances vaccine cross-protection against mouse adapted Omicron BA.5 for up to 3 months post vaccination, as shown by reduced viral titers, abrogation of weight loss, and diminished lung pathology. Our data suggests cross-neutralizing mucosal IgA is the primary driver of protection against breakthrough infection whereas tissue resident memory T cells (Trms) mitigate Omicron disease pathology by reshaping the local cytokine milieu. However, Trms alone are insufficient to protect against severe disease. Collectively, our data highlights the role of vaccine administration route in shaping the quality and breadth of immunity at barrier tissues, informing future pan-coronavirus vaccine development.

Abstract 142

Merkel cell carcinoma-derived macrophage migration inhibitory factor (MIF) may promote persistence of Chronic Lymphocytic Leukemia

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While concurrent diagnoses of Merkel cell carcinoma (MCC) and other cancers, like Chronic lymphocytic leukemia (CLL), are rare, patients with MCC have a 30-fold higher incidence of CLL. While these increases have been attributed to the ability of CLL to suppress immune responses allowing for the emergence of MCC, here we found evidence that MCC could support the persistence of CLL. Using single cell sequencing approaches and computational analyses of MCC and CLL from a patient where both cancers were present in the same lymph node, we found that production of macrophage migration inhibitory factor (MIF) by MCC could promote the persistence of CLL through stimulation of CD74 and CXCR4. These results may explain why blood cell counts rapidly normalized after treatment for MCC and were maintained at normal levels despite the absence of treatment for CLL.

Abstract 143

Sniffing Out Inflammation: Olfr2 Fuels Lipid Uptake and Foam Cell Formation in Atherosclerosis

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Atherosclerosis is driven by chronic inflammation and lipid accumulation in arterial wall macrophages; however, the mechanisms coupling oxidized LDL (oxLDL) uptake to pro-inflammatory macrophage activation remain incompletely defined. Here, we identify Olfr2/OR6A2 as a critical regulator linking lipid uptake to inflammatory reprogramming in macrophages. Genetic ablation of Olfr2 markedly reduced atherosclerotic lesion burden and lipid accumulation in plaque macrophages in Apoe-/- mice fed a high-fat diet. Pharmacologic inhibition with the Olfr2 antagonist citral recapitulated this protective effect. Consistent with in vivo findings, Olfr2 deficiency attenuated lipid accumulation in bone marrow-derived macrophages (BMDMs), as demonstrated by reduced BODIPY and Oil Red O staining, specifically in response to oxLDL. Mechanistically, Olfr2 deletion reduced oxLDL uptake without altering oxLDL binding or CD36 expression, indicating regulation at a post-binding internalization step. Immunofluorescence revealed co-localization of Olfr2 with CD36, and blockade of Olfr2-dependent Ca2+ influx with diltiazem impaired oxLDL internalization. These findings suggest that CD36 engagement cooperates with Olfr2 signaling to drive Ca2+ dependent uptake. Transcriptomic profiling revealed that Olfr2 deletion promotes an anti-inflammatory macrophage phenotype, with increased reparative markers and suppression of inflammatory genes. Olfr2 inhibition also reduced cellular and mitochondrial ROS while improving mitochondrial membrane potential. Targeting OR6A2, the human ortholog of Olfr2, similarly reduced lipid accumulation and inflammatory genes in human macrophages. Spatial transcriptomic analysis of human atherosclerotic plaques revealed OR6A2-high areas are enriched for proinflammatory macrophages compared to OR6A2-low areas. Together, these findings identify Olfr2/OR6A2 as a key regulator of macrophage driven atherosclerosis and a potential therapeutic target.

Abstract 144

Regulatory T cell inspired engineering of CAR-T cells enhances anti-tumor efficacy in solid tumors

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Chimeric antigen receptor (CAR) T cells have revolutionized treatment for hematologic malignancies. Yet, their efficacy against solid tumors remains limited due to poor infiltration and the immunosuppressive tumor microenvironment (TME). Regulatory T cells (Tregs), however, thrive under these conditions due to expression of the transcription factor FOXP3, which enhances tumor homing chemokine receptor expression and intratumoral fitness. Leveraging this biology, we engineered effector TRP1 CAR-T cells to overexpress FOXP3 (CAR-FOXP3-T) and evaluated their therapeutic potential in solid tumors. In vitro, CAR-FOXP3-T cells were comparable to CAR-T cells in killing B16F10 melanoma cells. In vivo, CAR-FOXP3-T cells displayed superior tumor infiltration, persistence, and control of B16F10 melanomas in immunocompetent syngeneic C57BL/6 mice compared to CAR-T cells. FOXP3 overexpression did not alter CAR-induced T cell activation but did protect CAR-T cells from activation-induced cell death. Interestingly, coding exon 2 of FOXP3 was required for these enhancements. RNA-seq revealed increased expression of tumor-homing chemokine receptors, but reduction in some inflammatory and cytotoxic molecules in CAR-FOXP3-T cells compared to CAR-T cells. We hypothesized that combining CD4■ CAR-T cells with CAR-FOXP3-T cells enhances anti-tumor efficacy. RNA-seq showed that CD4■ CAR-T cells induced CD8■ CAR-FOXP3 T cells to upregulate anti-tumor cytokines, notably tumoricidal IL-24, and reduce exhaustion markers. Co-administering CD4■ CAR-T cells with CAR-FOXP3 T cells significantly improved control of melanoma and triple-negative breast cancer in vivo, highlighting a synergistic strategy to enhance CAR-T efficacy against solid tumors. Our findings provide proof-of-concept for a novel CAR-T cell therapy designed to overcome barriers in solid tumor immunotherapy.

Abstract 145

Atherosclerotic Risk in Humans is Associated with Coordinated Monocyte-Neutrophil Crosstalk

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Atherosclerosis is a progressive inflammatory disease in which immune cell activation contributes to vascular injury and plaque development. Monocytes and neutrophils are key drivers of vascular inflammation, yet how their crosstalk contributes to early atherosclerosis remains poorly understood. We hypothesize that nonclassical monocyte-neutrophil crosstalk contributes to early atherosclerosis by reshaping circulating innate immune cell states.

Using fresh peripheral blood from 14 patients in the CAVA cohort at the University of Virginia, we performed CyTOF analysis with a 39-marker panel to define myeloid phenotypes associated with cardiovascular risk. Samples were batch-corrected with CyCombine and analyzed with a focus on neutrophil and monocyte populations. Patients were stratified into high-risk and low-risk groups based on coronary computed tomography angiography (CTA) scores obtained approximately 3 years before blood collection, with high risk defined as >20 and low risk as <10. High-risk patients showed increased frequencies of intermediate monocytes (CD14+CD16+), nonclassical monocytes (CD14neg/dimCD16hi), and a 3-fold expansion of early precursor neutrophils (CD15+CD11bdimCD62Ldim), together with a 2-fold reduction in CD177-negative neutrophils. To further examine neutrophil states associated with atherosclerotic risk, we mined a public scRNA-seq dataset of neutrophils from ApoE knockout mice fed chow or high-fat diet for 6 weeks. Neutrophils from high-fat diet mice upregulated genes including Nr4a1 and Cxcr4, and Gene Ontology analysis identified pathways related to leukocyte migration and cell adhesion.

These findings suggest that early cardiovascular risk is associated with circulating myeloid remodeling and altered neutrophil transcriptional programs consistent with a pro-atherogenic innate immune state, potentially driven in part by monocyte-neutrophil crosstalk.

Abstract 146

Treg-Specific Iron Acquisition is Required for Colitis Inflammation

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Regulatory T cells (Tregs) require metabolic adaptation to maintain immunological tolerance, though the influence of iron metabolism on Treg regulation of intestinal inflammation remains underexplored. We hypothesized that transferrin receptor (Tfrc)-dependent iron uptake is required for Treg suppression during colitis.

To investigate this, we used tamoxifen-inducible Foxp3-ERT2-Cre/Tfrc floxed mice for Treg-specific Tfrc deletion and CD4-Cre/Tfrc mice for pan-T cell Tfrc deletion. Colitis was induced for 5 days using 3% Dextran Sodium Sulfate (DSS), followed by evaluation on day 7.

Surprisingly, Treg-specific Tfrc deletion significantly protected against DSS-induced colitis, evidenced by decreased intestinal bleeding, lower Disease Activity Index (DAI) scores, intact colon architecture, and reduced histological inflammation. This protection was accompanied by decreased CD45■ immune cell infiltration in the lamina propria, including neutrophils, macrophages, Treg, and Th17 cells. Conversely, this protective phenotype was not recapitulated by CD4-Cre-mediated Tfrc deletion, suggesting that iron uptake in effector CD4■ T cells is dispensable for disease regulation. Tfrc-deficient Tregs had increased T-bet expression, suggesting a plastic Th1/Treg phenotype which may promote disease pathogenesis.

Collectively, our findings show that Intestinal homeostasis selectively depends on iron absorption in Tregs, but not CD4■ T cells. These findings identify iron-dependent immunometabolism as a therapeutic target for inflammatory bowel disease and context-specific T cell regulator.

Abstract 147

Impact of diet on host defense during Staphylococcus aureus skin infection

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While standard diets (Chow) are typically made from agricultural by-products, purified low-fat (LF) diets contain a higher proportion of easily metabolizable carbohydrates, along with lower levels of polyunsaturated fatty acids, micronutrients, and fiber, all of which are linked to metabolic and inflammatory dysfunction. Whether dietary composition influences susceptibility to skin infections remains unclear. We hypothesize that the LF diet, commonly used as control, could promote adverse systemic inflammatory effects that alter localized immune responses. To investigate this, male C57BL/6J mice were fed either a chow diet (28.9% protein, 13.6% fat, 57.5% carbohydrate and 5.3% fiber) or a low-fat (LF) diet (20% protein, 10% fat, 70% carbohydrate and 4.7% fiber) for 5 weeks. Mice were then subcutaneously infected with methicillin-resistant Staphylococcus aureus (MRSA), and lesion sizes were measured on days 1, 3, 6, and 9. Tissue biopsies were collected on days 1 and 9 for histological analysis and assessment of bacterial burden. Our results show that animals fed a LF-diet exhibit impaired infection control, as evidenced by larger skin lesions and higher bacterial burdens at both early (day 1) and later (day 9) time points. Histological analysis revealed that LF-diet mice develop larger abscesses and greater inflammatory infiltrates compared to chow- fed controls. Collectively, these findings suggest that reduced fat intake is associated with compromised immune function. Future studies are underway to explore how micro- and macronutrients, fiber and fatty acids affect host defense and whether diet-induced changes in gut microbiota influence skin immune responses.

Abstract 148

Characterizing cellular drivers of cytokine release during initial anti-CD20 therapy

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Monoclonal antibodies (mAbs) treat cancer, autoimmunity, and infectious diseases. However, initial mAb administration in treatment-naïve patients triggers a rapid spike in circulating cytokines, termed cytokine release syndrome (CRS). Some patients progress to a first-dose infusion reaction (FDIR) characterized by fever, hypotension, rash, chills, and dyspnea. In chronic lymphocytic leukemia (CLL), initial mAb administration causes CRS in 64% of patients, 77% of whom have FDIRs. At low doses, rituximab, an anti-CD20 mAb for CLL, rapidly elevates inflammatory mediators (e.g. IL-6, IL-8, CXCL10). Despite this, the mechanism of anti-CD20 cytokine release is unknown. Within minutes after anti-CD20 infusion, liver and spleen macrophages rapidly engulf opsonized circulating B cells via antibody-dependent cellular phagocytosis (ADCP), the key cytotoxic mechanism of anti-CD20 mAbs. Rapid ADCP following initial anti-CD20 infusion correlates with CRS, highlighting ADCP by tissue-resident macrophages as a potential driver of anti-CD20-induced cytokine release. We hypothesize anti-CD20-mediated ADCP causes macrophages to release inflammatory cytokines contributing to CRS. To test this, we established a co-culture system where M-CSF-differentiated human monocyte-derived macrophages (hMDMs) from healthy donors were cultured with Ramos B cells opsonized with anti-CD20 or IgG control mAb, and CRS-associated cytokines were quantified by ELISA. Live-cell imaging confirmed efficient hMDM ADCP of anti-CD20-opsonized Ramos cells. Preliminary studies demonstrate elevated CRS-associated cytokines in cultures with anti-CD20-opsonized Ramos cells compared to controls, indicating macrophage ADCP as a contributor to cytokine release during initial mAb administration. These studies will improve our understanding of mAb-induced CRS and guide strategies to make first-dose infusions safer and more widely accessible.

Abstract 149

Overcoming macrophage hypophagia in monoclonal antibody therapies.

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Immunotherapies such as monoclonal antibodies (mAbs) have revolutionized cancer treatment. However, the efficacy of some mAbs is constrained by the finite capacity of the patient immune system. For example, the anti-CD20 mAb rituximab robustly depletes B cells during initial treatment of leukemia but loses potency upon repeated administrations. This acquired resistance is associated with a reduced ability of macrophages to perform antibody-dependent cellular phagocytosis (ADCP), the primary cytotoxic mechanism of rituximab. Mechanistically, sustained ADCP drives macrophages into hypophagia, an exhausted state characterized by significantly attenuated ADCP capacity and loss of Fc gamma receptor 1 (Fcgr1), a key mediator of ADCP. Notably, we have shown that this exhaustion is not a global defect of phagocytosis, as hypophagic macrophages retain their capacity to clear apoptotic cells. This distinction suggests that ADCP-specific components, such as Fcgr1, may represent key regulatory nodes of hypophagia. Based on these observations, we hypothesize that loss of surface Fcgr1 is the principal driver of macrophage hypophagia. Supporting this, our published data demonstrate that IFN-gamma-induced upregulation of Fcgr1 restores ADCP in hypophagic macrophages. But because IFN-gamma exerts broad pleiotropic effects, a more targeted strategy is required to isolate the role of Fcgr1. To address this, we are generating inducible Fcgr1-overexpression systems in primary mouse macrophages to enable precise temporal control of receptor expression, allowing us to directly test its contribution to hypophagia. Collectively, this work aims to enhance the efficacy of mAb therapies by establishing a foundation for targeted adjuvant strategies to bypass macrophage hypophagia.

Abstract 150

Antigen Presenting Cells Shape Regulatory T Cell Heterogeneity in Visceral Adipose Tissue

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Regulatory T cells (Tregs) in visceral adipose tissue (VAT) maintain local immune homeostasis and systemic metabolic health. Distinct VAT Treg subsets (ST2■ and CXCR3■) perform non-redundant functions, suggesting a division of labor in controlling adipose homeostasis. In obesity, ST2■ Tregs are lost, exacerbating insulin resistance, while CXCR3■ Tregs persist but fail to compensate. Notably, CXCR3+ Tregs are critical for limiting viral infection-induced VAT immunopathology. Additionally, both subsets exhibit clonal expansion with largely non-overlapping TCR repertoires, consistent with distinct antigen specificity. However, the antigen-presenting cell (APC) populations that support VAT Treg maintenance and subset specialization remain poorly defined.

Here, using conditional MHCII deletion in specific APC populations, we investigated APC requirements for VAT Treg subsets at steady state. Analysis of H2-Ab1flox/flox CD11c-Cre■ and Lysm-Cre■ mice revealed that ST2■ Tregs depend on CD11c■ and LysM■ APCs, suggesting macrophage or monocyte support, whereas CXCR3■ Tregs persist in the absence of CD11c■ antigen presentation, indicating alternative APC dependence. Importantly, splenic ST2lo/int precursor populations remain unchanged in mutant mice, suggesting these effects reflect local regulation rather than developmental defects.

Ongoing studies aim to identify the relevant VAT APCs at steady state and during obesity, including distinguishing tissue-resident macrophages from monocyte-derived subsets and identifying CD11c■ populations supporting CXCR3■ Tregs. Complementary co-culture assays will test whether specific APC–Treg interactions are sufficient to drive subset differentiation, while defining the role of cytokine and costimulatory signals. Together, this work seeks to characterize cellular mechanisms governing VAT Treg regulation and may identify targets to restore immune and metabolic homeostasis.

Abstract 151

Functional T cell Imaging to Enhance Tumor Infiltrating Lymphocyte Therapy

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Patients with advanced solid tumors face poor outcomes despite intensive multimodal treatments. Tumor-infiltrating lymphocyte (TIL) therapy, first approved in 2024, offers curative potential, however its broader success is constrained by the inability to determine whether surgically resected tumor tissues contain functional, cancer-reactive TILs prior to manufacturing. Current methods, including immunohistochemistry and flow cytometry, are destructive, labor-intensive, low-throughput and primarily assess phenotype rather than cytotoxic function. Here, we present TILight, an intact tissue imaging strategy that detects functional cytotoxic T lymphocytes (CTLs) by visualizing granzyme B (gzmB) release during tumor cell killing. TILight is enabled by AGB-CyP, a near-infrared fluorogenic single-chain antibody probe that is selectively activated by gzmB and binds to the enzyme for signal retention at the immune attack site. AGB-CyP is non-T-cell-permeable but penetrates perforin pores into cancer cells, enabling sensitive detection of extracellular and cancer-cell-associated gzmB while minimizing background from terminally exhausted T cells which express and carry high-level gzmB. Using engineered human CAR T cells, TILight showed gzmB-dependent fluorescence confined to engaged cancer cells. In a 3D lymphoma spheroid model, it distinguished functional from terminally exhausted T cells within two hours. Application to surgical resections from a cohort of 13 patients demonstrates the feasibility of high-throughput functional evaluation of TILs in intact tissues while preserving the integrity for downstream manufacturing. Together, this approach offers a broadly applicable imaging strategy for functional immune profiling, biomarker discovery, and the acceleration of adaptive cell immunotherapy.

Abstract 152

Leveraging CD4 T cells to enhance antitumor immune responses in ovarian cancer

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Ovarian cancer is the deadliest gynecological malignancy, with a five-year survival rate of just 30% for patients with advanced disease, highlighting the urgent need for more effective therapies. Our lab has engineered CD8 T cells with a high-affinity T cell receptor (TCR) targeting the tumor-associated antigen mesothelin (TCRMsln) that extends survival in the ID8VEGF preclinical ovarian cancer model. Despite prolonged survival, treated mice ultimately succumb to the disease, suggesting TCRMsln T cells lose effectiveness against the tumor. Thus, overcoming immune-resistance in the tumor microenvironment may be critical for durable therapeutic efficacy. Emerging evidence reveals that intratumoral CD4 T cells can both support and suppress antitumor immunity, and distinct subsets correlate with clinical outcomes in solid tumors. We hypothesized that CD4 T cells in ovarian tumors acquire features that limit therapeutic efficacy but can be reprogrammed to enhance antitumor immunity. Using single-cell RNA sequencing, we found that treatment with TCRMsln T cells altered the endogenous CD4 T cell phenotype, promoting both proinflammatory TH1-like CD4 cells and suppressive TH2-like cells. Notably, combining CD8 TCRMsln T cells with three checkpoint inhibitors (PD-1, Tim-3, and Lag-3) induced a robust TH1-like gene signature in CD4 T cells and led to significantly greater median overall survival and long-term tumor control in a subset of mice. These results highlight a possible role for CD4 T cells in ovarian cancer and suggest that therapeutic intervention can reprogram CD4 differentiation toward antitumor states, which may be critical for enhancing the efficacy of engineered T cell therapy in ovarian cancer.

Abstract 153

Differentiation of tolerogenic Fgl2+ CD8+ T cells is driven by CEBP/α and BASP1

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The mechanisms by which transplant rejection is precipitated are incompletely understood. Here, we found that fibrinogen-like protein 2 (Fgl2), an immunosuppressive cytokine, was elevated in transplant recipients with stable graft function relative to those undergoing rejection ($p<0.01$) or to healthy controls ($p<0.0001$), but the source and function of Fgl2 was unknown. Human Fgl2+ CD8+ T cells were enriched for PD-1, TIGIT, and FcγRIIB vs. Fgl2- CD8+ T cells ($p=0.0049$, $p=0.0015$, and $p=0.0078$). To determine if Fgl2 expression by alloreactive CD8+ T cells impacts rejection, we employed a graft-specific T cell conditional knockout mouse model. Results indicated that the absence of Fgl2 production by graft-specific CD8+ T cells significantly accelerated allograft rejection ($p=0.043$), increased alloreactive CD8+ T cell accumulation ($p=0.019$), and increased IL-2 production ($p=0.004$). Transcription factor analysis revealed increased expression of CEBPα in human Fgl2+ CD8+ T cells ($p=0.0273$). Conditional deletion of CEBPα in CD8+ T cells resulted in a loss of Fgl2 production, demonstrating that CEBPα drives the production of tolerogenic Fgl2. Transcriptomic analysis of Fgl2+ vs Fgl2- human CD8+ T cells revealed that Fgl2+ CD8+ T cells exhibit a ~32-fold increase in expression of BASP1, a protein known to inhibit calmodulin ($p=0.0079$). Co-culture with a BASP1 mimotope resulted in significantly increased expression of Fgl2 and decreased proliferation by CD8+ T cells. Fgl2 production in CD8+ T cells promotes allograft acceptance, is driven by CEBPα, and is associated with expression of the calmodulin inhibitor BASP1. Pharmacologic manipulation of BASP1 and/or Fgl2 may be an effective therapeutic approach in transplantation.

Abstract 154

Mechanisms of local regulation of alloimmunity by GM2A

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Durable, donor-specific tolerance remains an unmet goal in transplantation. Transcriptomic analysis of kidney transplant recipients following calcineurin inhibitor withdrawal revealed higher expression of GM2 activator protein (GM2A), a lysosomal glycosphingolipid-processing protein, in stable patients compared with those who rejected their allografts. Although GM2A is well characterized in neuronal metabolism, its role in transplant immunity remains unknown.

To define the role of Gm2a within the graft, Gm2a-/- or WT male skin was transplanted onto WT female recipients. Gm2a-sufficient grafts demonstrated significantly prolonged survival compared to Gm2a-deficient grafts ($p = 0.0003$). Mechanistically, loss of graft-derived Gm2a induced early activation of graft-infiltrating MHCII+ myeloid cells by D3, followed by increased myeloid cell accumulation by D7. Interestingly, loss of graft derived Gm2a also altered the phenotype of recipient derived APC in the graft-draining lymph node (dLN). Furthermore, the magnitude of alloreactive CD8 T-Cell response was increased in both the graft, and excitingly in the dLN, in response to the Gm2a deficient graft as compared to the WT. These data suggest the effect of the lack of Gm2a within the graft was propagated into recipient cells, both APC and T-Cells.

Together, these findings identify GM2A as a previously unrecognized regulator of the graft microenvironment that restrains both local and systemic early innate and late adaptive immune activation. Emerging technologies, including ex-vivo organ perfusion and gene-editing approaches, could enable targeted enhancement of GM2A expression in donor tissue and represent a clinically tractable strategy to promote durable transplant tolerance.

Abstract 155

Hemochromatosis Protein and Transferrin Receptor Endocytosis in T cells Regulates Autoimmunity

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The balance of T helper (Th) cell subsets, such as Th1, Th17, and regulatory T cells (Treg), plays a key role in the regulation of inflammation and preventing autoimmunity. We have shown that manipulating iron metabolism can restore T cell subset homeostasis in the autoimmune disease systemic lupus erythematosus (SLE). Blocking the transferrin receptor (CD71) with an antibody negatively impacted Th1 and Th17 populations but spared Treg function, lowering disease pathology in mouse models of SLE. T cells from patients with SLE and lupus mice had high levels of intracellular iron driven by endosomal recycling of CD71. The Hemochromatosis (HFE) protein, which regulates CD71 endocytosis, was elevated compared to healthy controls. However, it is not known whether CD71 is dysregulated in other autoimmune diseases or if high HFE levels drives high intracellular iron in T cells. Our preliminary data shows that activated T cells produce HFE, and Tregs produce less HFE compared to other subsets. Therefore, we hypothesized that chronically activated T cells in autoimmunity produce high HFE which increases CD71 recycling on T cells and intracellular iron. Indeed, activated Tregs from WT mice had significantly lower CD71 expression compared to other Th cells. We found that soluble HFE (sHFE) was significantly higher in plasma samples from patients with SLE, rheumatoid arthritis (RA) and psoriatic arthritis (PsA) compared to healthy controls at the University of Virginia Rheumatology Clinic. Interestingly, labile iron was increased in patient Tregs with SLE, but not RA, suggesting disease-specific perturbations in iron metabolism in T cells.

Abstract 156

Subviral particles produced following immunization with a ZIKV M-E DNA vaccine encoding a truncated form of the viral structural protein prM elicited a higher quality neutralizing antibody response compared to a vaccine encoding the full-length prM-E sequence

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Flavivirus assembly at the endoplasmic reticulum (ER) is driven by the structural proteins envelope (E) and premembrane (prM). Both proteins have essential functions during the formation of virions, which initially incorporate numerous prM-E heterodimers. The pH-dependent cleavage of prM by host furin proteases is thought to be a requisite step for the generation of infectious, mature virions. Here, contrary to the established paradigm for flavivirus assembly, we demonstrate that the biogenesis of infectious flavivirus particles does not require an intact prM nor host proteolytic activation. The expression of E preceded by a truncated version of prM (M-E) was sufficient to drive the formation of non-infectious Zika virus (ZIKV) subviral particles and pseudo-infectious reporter virions displaying an antigenic structure similar to mature virions. M-E particles accumulated within cells, suggesting a role for prM in escape from the ER rather than virion budding or assembly. Applying this knowledge, we demonstrate that plasmids encoding ZIKV M-E elicit a neutralizing antibody response that is insensitive to the virion maturation state, a feature of flavivirus humoral immunity shown to correlate with protection. Thus, M-E vaccines that present antigenic features associated with mature virions, a qualitatively superior neutralizing antibody response, and the absence of infection-enhancing prM epitopes offer a promising and broadly applicable approach to flavivirus vaccination.

Abstract 157

Machine Learning Analysis of Spectral Flow Cytometry in Uveitis Vitreous Reveals Disease-Associated Monocyte Signatures

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Uveitis is a leading cause of blindness in the western world. Accurate diagnosis of uveitis etiology can take up to a year, with delayed or incorrect diagnosis increasing risk of vision loss. Novel diagnostic approaches providing cellular-level insights could address this challenge. Here, we applied high-parameter flow cytometry and machine learning to identify vitreous-infiltrating leukocyte phenotypes across uveitis etiologies.

We utilized 36-color spectral flow cytometry to immunophenotype leukocytes from diagnostic vitrectomy samples. Non-uveitic peripheral blood samples served as baseline controls. Phenotypic shifts in vitreous-infiltrating versus peripheral blood leukocytes were analyzed using dimensionality reduction and standard gating strategies. Pseudotime analysis tracked developmental trajectories toward infiltrating populations.

Vitrectomy samples contained all major leukocyte populations found in peripheral blood, but exhibited notable phenotypic shifts. Monocytes showed a striking transition from predominantly classical phenotype (MHC-II+CD14+CD16-) in peripheral blood to intermediate phenotype (MHC-II+CD14+CD16+) in vitreous, suggesting disease-relevant functional changes. This phenotypic shift correlated with distinct uveitis etiologies, indicating potential diagnostic utility.

Abstract 158

Colonic Macrophage Dynamics During Clostridioides difficile Infection Are Reshaped by IL-33

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Antibiotic exposure predisposes to Clostridioides difficile infection (CDI) by disrupting intestinal immune homeostasis. In the colon, embryonically derived tissue-resident macrophages (TRMs), defined as CD11b■ CD64■ Ly6C■ MHCII■ CD4■ TIM4■ cells, constitute a long-lived population. Clodronate-mediated depletion of TRMs led to increased mortality and worsened clinical disease scores following CDI, underscoring their protective role. We previously showed that IL-33 promotes a protective type 2 immune response during CDI by activating colonic ILC2s, thereby limiting epithelial damage and mortality. In this context, IL-33 restored the CX3CR1■ population within the CD11b■ CD64■ Ly6C■ MHCII■ macrophage compartment as early as day 2 post-infection. Moreover, IL-33 replenished embryonically derived TRMs (CD4■ TIM4■) and selectively expanded a CD4■ TIM4■ macrophage subset, representing an intermediate macrophage (iMac) population with features of both TRMs and monocyte-derived cells. IL-33 treatment further increased CD206 expression, consistent with a reparative and regulatory macrophage phenotype, and enhanced F4/80 expression compared to PBS-treated infected controls, indicating preservation of mature, tissue-adapted macrophages. In contrast, CDI induced a marked accumulation of Ly6Chi inflammatory monocytes in PBS-treated mice, which was significantly reduced following IL-33 administration. Collectively, these findings demonstrate that antibiotics and CDI disrupt defined TRM populations, whereas IL-33 reshapes the macrophage compartment toward regulatory and tissue-repair phenotypes, thereby promoting restoration of intestinal immune homeostasis.

Abstract 159

Fine-tuning CD8+ T cell sensitivity: GM2A role in TCR expression and CD8+ repertoire

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T cell sensitivity to antigen is a critical parameter in controlling T cell-mediated immunity. However, the molecular mechanisms are incompletely understood. We identified a novel regulator of TCR expression that limits antigen sensitivity of CD8+ T cells and controls the magnitude of the allogeneic response. In a transcriptomic screen of renal transplant recipients who were weaned from immunosuppression, we found increased expression of glycosphingolipid-catabolizing protein (Gm2a) in CD8+ T cells from stable patients vs. those that went on to reject. Using a skin graft model to investigate the role of Gm2a in regulating CD8+ T cell immunity, we found that Gm2a-/- CD8+ T cells exhibited increased accumulation and mediated accelerated allograft rejection vs WT, demonstrating a CD8+ T cell-intrinsic role for Gm2a. Analysis of an in vivo mixed-lymphocyte reaction revealed increased proliferation and a 50% augmentation in the frequency of alloreactive precursors among Gm2a-/- vs CD8+ T cells. TCR sequencing analysis demonstrated that Gm2a deficiency increased the number and diversity of unique antigen-specific CD8+ T cell clones. Mechanistically, Gm2a-/- CD8+ T cells exhibited sustained TCR expression upon activation compared to WT cells, conferring increased responsiveness to low-affinity antigens in vitro and in a melanoma model. Finally, treating human PBMCs with exogenous GM2A reduced CD8+ T cell proliferation vs. control. Therefore, we show that Gm2a limits the CD8+ T cell spectrum of reactivity by reducing surface TCR expression and attenuating CD8+ T cell sensitivity to antigen. Moreover, this work highlights the therapeutic potential of targeting GM2a to mitigate T-cell-mediated disease.

Abstract 160

Microbiome Drives Allergic Inflammation Independent of IL-33/ST2 Signaling

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IL-33 is an innate cytokine implicated in many inflammatory diseases, and a coveted clinical target in type-2 immunity. In controlled SPF settings, it has been shown that mice lacking IL-33 (IL-33-/-) or its receptor ST2 (ST2-/-) exhibit reduced inflammatory responses to allergen, demonstrating the necessity of IL-33/ST2 signaling for Th2 responses in vivo. Despite this strong preclinical evidence, no human ■-IL-33 therapeutics have been sufficiently efficacious for regulatory approval. This disconnect suggests that, in humans, IL-33 is not an obligate driver of allergic responses, but its role is shaped by environmental contexts missing in SPF studies. Intriguingly, we have observed in our mice that IL-33/ST2 genetic deletion is not sufficient to inhibit allergic inflammation, modeling the immunological context of IL-33 signaling in humans. Concurrently, ST2-/- mice generated in another independent SPF facility exhibited a reduced response to allergen that recapitulates the phenotype observed in the literature. We hypothesize that microbial colonization from a non-SPF environment generates crosstalk with immune cells, resulting in the activation of compensatory, IL-33-independent allergic pathways. To assess facility-specific microbiome differences, we performed 16S sequencing on fecal samples from WT and ST2-/- mice. Our mice exhibited features consistent with gut dysbiosis, including lower ■-diversity overall and decreased genotype-associated microbiome variance. Antibiotic treatment significantly decreased allergic responses of ST2-/- mice from our facility only, confirming that IL-33/ST2-independent pathways are microbiome-dependent. These data highlight that environmental context can fundamentally shape the immune repertoire in allergic asthma, with important implications for evaluating clinically actionable targets.

Abstract 161

Modulation of regulatory T cell biology by GM2A in transplantation

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Durable, donor-specific tolerance remains an unmet goal in transplantation. Transcriptomic analysis of kidney transplant recipients following calcineurin inhibitor withdrawal revealed higher expression of GM2 activator protein (GM2A), a lysosomal glycosphingolipid-processing protein, in stable patients compared with those who rejected their allografts. Although GM2A is well characterized in neuronal metabolism, its role in transplant immunity remains unknown.

Our lab previously identified a cell-intrinsic role for Gm2a in regulating alloreactive CD8■ T cells, where deficiency lowered the threshold for TCR activation and enhanced responsiveness to low-affinity antigen. Despite this heightened sensitivity, Gm2a-/- mice do not develop spontaneous autoimmunity, suggesting compensatory regulatory mechanisms.

Here, we investigated the role of Gm2a in Foxp3■ regulatory T cells (Tregs). In a murine skin transplant model, Gm2a-/- recipients exhibited accelerated allograft rejection accompanied by reduced Treg frequencies in the blood, draining lymph nodes, and graft compared to WT controls. In contrast, steady-state analysis revealed increased frequencies of thymic Tregs in Gm2a-/- mice, with no significant differences in peripheral Treg number or activation status. Functionally, Gm2a-deficient Tregs demonstrated enhanced suppressive capacity in vitro, reducing activation marker expression and proliferation of conventional CD4■ T cells compared to WT Tregs.

Together, these findings suggest that Gm2a regulates Treg development and function, potentially by expanding the thymic selection window and generating a more suppressive peripheral Treg repertoire. Collectively, Gm2a represents a promising target for strategies aimed at promoting transplant tolerance and reducing the need for lifelong immunosuppression.

Abstract 162

Understanding the T-cell Subsets and Mechanisms of Interaction with Epithelial Cells in Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a severe and fatal interstitial lung disease (ILD), characterized by progressive scarring and respiratory failure, with a median survival of 3–5 years. Both CD4 and CD8 T-cells are increased in the severe fibrotic regions of IPF lungs, indicating their involvement even in the advanced stages of the disease. We hypothesized that certain subsets of T-cells may favor epithelial injury, and identifying these populations could advance our understanding of this disease. Here, we utilized multiplex immunofluorescence by CODEX (Co-Detection by indexing) of lung sections together with Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) of lung-isolated T-cells to evaluate the subsets with relevant activation in the lungs of IPF patients compared to controls. Additionally, we compared the activation profile of these subsets in IPF and other forms of ILD. Our single-cell RNA and protein profiling on ~90,000 CD3■ T-cells from control (n=10) and fibrotic lungs (n=19) revealed eleven distinct subsets of CD4■ and CD8■ T-cells in the lungs. Among these, we identified a rare CD56■ regulatory T-cell (TREG) subset that is highly activated in fibrosis and exhibits an immunosuppressive phenotype comparable to CD4 TREG. We observed that CXCR4/MIF signaling emerged as a central axis mediating T-cell–epithelial interactions, and epidermal growth factor receptor (EGFR) and TGFβ pathways dominated in multiple T-cell subsets. Together, these studies provide a deeper characterization of T-cells in fibrosis and reveal how proximity and interactions with the epithelium are central to shaping T-cell activation in IPF lungs. (Support: K12AR084232, ALA1456724, P01HL172729)

Abstract 163

ArborMap: A Randomized Ensemble Tree-based Single-Cell Analysis framework

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Accurately identifying cell types is a pivotal step in single-cell analysis. Several widely adopted tools, including the Seurat package, utilize unsupervised cluster-based frameworks, and face significant limitations: poor performance in predicting rare cell types, extensive parameter tuning to achieve optimal results, and unsatisfactory outputs when scaled to large or incomplete datasets. To address these limitations, we introduce ArborMap, a single-cell cluster identification pipeline utilizing an ensemble of totally random trees for unsupervised data transformation.

ArborMap is applicable to single or multiple modalities. For multimodal datasets, we developed a weighted integration framework implemented prior to the ArborMap workflow. We evaluated ArborMap against Seurat using diverse datasets spanning three biological sources across scRNA-seq and CITE-seq platforms. Without requiring iterative parameter adjustments, ArborMap accurately identified biologically relevant populations, including rare cell types missed by Seurat. UMAP comparisons demonstrated that ArborMap produces cleaner, denser, and more distinctly separated clusters.

We further validated ArborMap's ability to extract biological insights using a CITE-seq whole blood study of healthy and melanoma samples. We observed an expansion of classical monocyte proportions in melanoma versus healthy samples, consistent with existing literature. Notably, this expansion was not present when differential abundance analysis was performed on the Seurat-defined cluster. These results demonstrate that ArborMap provides a robust, computationally efficient, and high-fidelity alternative for analyzing complex, large-scale single-cell datasets.

Abstract 164

IgA Sialic Acid–Dependent Suppression of antiviral Fc Effector Function

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Abstract: HIV envelope–specific serum immunoglobulin A (IgA) antibodies were associated with reduced HIV vaccine efficacy across multiple clinical trials, suggesting that IgA may counteract protective vaccine-induced immune responses. However, the mechanisms underlying this detrimental association remain poorly defined. Given that IgA carries abundant sialylated glycans, and sialic acids are known to regulate immune cell functions, we hypothesized that IgA dampens vaccine efficacy by suppressing Fc mediated antiviral functions through its sialic acid–containing glycan structures.

We evaluated the ability of monoclonal HIV-specific antibodies treated with the sialic acid–cleaving enzyme Sialidase A to trigger immune cell signaling via a multiplex assay. Desialylation enhanced SYK and ERK phosphorylation, indicating that IgA sialylation dampens both proximal and downstream Fc receptor–mediated signaling. Functional profiling further revealed that sialidase treated antibodies exhibited increased antibody dependent phagocytosis. This enhancement was observed for IgA1 (~25-33% increase), and IgA2 (up to 54% increase) but absent for IgG1 and IgG3, which naturally carry fewer sialic acids. These findings support a model in which sialic acid rich IgA glycans attenuate antiviral effector functions.

Together, this work provides mechanistic insight into how IgA may interfere with the protective efficacy of HIV vaccines. Ongoing studies are probing the Siglec–sialic acid axis as a potential pathway through which IgA glycosylation modulates immune signaling and antiviral activity.

Abstract 165

Identification of TLR-based adjuvants suitable for polysaccharide antigen vaccines in humans

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The TLR4 agonist, monophosphoryl lipid A, significantly increases antibody (Ab) responses to T cell independent type 2 antigens (TI-2) in mice in a manner dependent on B cell-intrinsic TLR4 expression and MyD88 signaling. Given the poor responsiveness of human B cells to TLR4 agonists, we sought to identify alternative MyD88-activating TLR agonists that could potentially function as suitable adjuvants to enhance humoral responses to polysaccharide antigens in humans. We activated human PBMC and purified B cells with TLR agonists, strong B cell receptor crosslinking, or both and assessed activation and Ab secretion in vitro. Agonists that augmented Ab secretion in conjunction with BCR crosslinking over that achieved with either stimulation alone were further tested in mice. Pam3Csk4, a TLR1/2 agonist, in combination with squalene, significantly increased primary and secondary IgM and IgG responses to pneumococcal polysaccharide (PPS) and provided increased protection against pneumococcal infection in wild type mice. Furthermore, Pam3Csk4-squalene significantly increased the production of PPS-specific Abs in huPBMC-reconstituted NSG mice and these Abs provided significantly increased protection in a lethal pneumococcal challenge model. Collectively, this work reveals the promise TLR1/2 agonists as adjuvants for polysaccharide vaccines in humans.

Abstract 166

The effect of aging on glioblastoma immunobiology and immunotherapy

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Background: Glioblastoma is a universally fatal brain tumor that commonly presents in the elderly (median onset of 65 years), yet all preclinical studies are conducted in 6-8 week old mice; the equivalent of a teenager. Dendritic cell vaccine therapy (DCV) has some success, with a third of patients surviving long-term. However, patients > 60 years receiving DCV have significantly worse survival compared to younger patients. This project aims to determine how aging affects glioblastoma immunity, DCV, and outcomes. Methods: 6-8 or 85-90 week mice were intracranially implanted with orthotopic glioblastoma tumor, and the effects of age on endogenous immune responses as well as DCV were compared. Results: Aged mice have shorter survival in two orthotopic glioblastoma models. In aged mice, neutrophils and eosinophils increase in the brains and, when given fluorescent beads directly into the brain tumor, bead+ neutrophils and eosinophils accumulate in the meninges. Surprisingly, aged DCV were phenotypically similar to young in vitro (CD40, CD80, CD86, CD83, and PD-L1) and SIINFEKL peptide-pulsed DCVs efficiently activate OT-I T cells. When transferred into either young or aged hosts, recovered DCV maintain their similar phenotypes. During aging, however, there is an increase in Treg and decrease in naïve T cells. Conclusion: Although no in vitro DCV phenotypic or T cell activation differences were observed, effective meningeal lymphatic network drainage and endogenous T cells were compromised. Future studies will determine whether meningeal lymphatic drainage homing molecule expression and DCV-induced T cell activation are affected in aged mice, resulting in poorer survival outcomes.

Abstract 167

A novel CAR-T therapy targeting tumor testis antigen LY6K against Solid tumors

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Introduction:
lymphocyte antigen 6 complex locus K(LY6K) is a protein belonging to the lymphocyte antigen-6 (LY6)/urokinase-type plasminogen activator receptor (uPAR) superfamily. It is known as the tumor testis antigen and is normally expressed only in testis and is upregulated up to several folds in human cancers, including breast, bladder, cervical, colorectal, head and neck, and lung carcinomas. High LY6K expression is often clinically associated with poor prognosis in cancers like, non-small cell lung cancer (NSCLC).
Hypothesis:
Here, we hypothesis to generate and test the efficacy of CART cells expressing anti-LY6K scfv against a panel of human cancer cell lines.
Methodology:
We have developed a novel CART platform targeting lymphocyte antigen 6 complex locus K(LY6K). We aim to test the efficacy of different versions of this CAR-T against a variety of tumors overexpressing LY6K.
Results and conclusions:
Preliminary data demonstrates that LY6K directed CART cells effectively recognize and eliminate LY6K+ tumor cells, confirming the feasibility of the approach.

Abstract 168

Pancreas-derived lymphocytes exhibit increased glycolytic dependance in non-obese diabetic mice

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In type 1 diabetes (T1D), heterogeneous immune cell populations including T cells infiltrate the islets of the pancreas, driving insulitis. Immune cell activation and effector functions are intricately regulated by metabolic reprogramming, which is further influenced by the metabolites available in different tissue microenvironments. As such, our lab has shown that regulatory T cells (Tregs) require iron to suppress autoimmunity in some tissues, but not others. However, the metabolic demands of pathogenic immune cells in the pancreas tissue during T1D has not been elucidated. We utilized Single Cell Energetic Metabolism by Profiling Translation Inhibition (SCENITH) to test metabolic dependencies of immune cells from the pancreas, lymph nodes (LN), and spleens of non-obese diabetic (NOD) mice. We hypothesized that cells in the pancreas tissue would require more glycolysis and less mitochondrial oxidative phosphorylation (OXPHOS) to support pro-inflammatory potential compared to the same cell types in secondary lymphoid organs.
We found that pancreas-derived CD4+ Treg, macrophages, and CD8 T cells had lower mitochondrial dependence and higher glycolytic capacity than cells derived from LN and splenic tissues. Interestingly, we also found that pancreatic-derived CD8+ T cells were more iron-dependent than CD8+ T cells from LN and spleens. Conversely, macrophages and neutrophils in the pancreas had lower iron dependency than LN or spleen-resident cells.
Overall, immune cells within the pancreas of NOD mice exhibit more pro-inflammatory metabolic profiles than those from LN or spleen, characterized by increased glycolysis and iron utilization. These tissue-specific metabolic shifts may contribute to localized immune activation and progression of autoimmune diabetes.

Abstract 169

CRISPR-based functional genomics uncovers novel transcriptional regulators controlling PDCD1 (PD-1) expression in human T cells

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Programmed cell death protein 1 (PD-1), encoded by PDCD1, is a critical inhibitory receptor that regulates T cell activation, exhaustion, and response to immunotherapy. While PD-1 blockade has transformed cancer treatment, many patients fail to respond or develop resistance, underscoring the need for novel strategies to modulate PD-1 function. A promising approach involves direct modulation of PDCD1 gene expression, yet the transcriptional mechanisms controlling PDCD1 in human T cells remain incompletely defined. Although transcription factors such as NFATc1, NFATc2, TOX, NF-kB, STAT, cFos, Helios, Nur77, Blimp-1, and T-bet have been identified as regulators of murine Pdcd1, it remains unclear whether the same regulatory networks govern human PDCD1, given interspecies differences in promoter architecture and chromatin landscape. Recent ATAC-seq and H3K27Ac profiling of human T cells from our laboratory has identified potential novel regulatory elements within the PDCD1 locus, suggesting the involvement of previously uncharacterized transcription factors. To systematically identify these regulators, we developed a robust targeted CRISPR-Cas9 screening platform in primary human T cells. Candidate transcription factors (~100) were prioritized based on chromatin accessibility, histone activation marks, and motif enrichment across potential PDCD1 regulatory regions, and targeted using a custom sgRNA library delivered via retroviral transduction. Following CD3/CD28 stimulation, cells were sorted into PD-1-high and PD-1-low populations by flow cytometry, and sgRNA representation was quantified by next-generation sequencing. Differential enrichment analysis using MAGeCK identified IRF and NFAT family members with strong regulatory effects on PD-1 expression. Ongoing individual CRISPR perturbations, transcriptional profiling, and chromatin accessibility assays are confirming the functional roles of these and other select candidates. We hypothesize that these uncharacterized transcription factors shape the degree of T cell exhaustion and responsiveness to immunotherapy. By systematically identifying and validating these regulators through integrative multi-omics analyses, this work will reveal novel regulatory pathways controlling PDCD1 expression and offer novel targets for gene-targeted therapeutic strategies in cancer and chronic infection.

Abstract 170

Persistent Lung-Resident CCR8■ST2■ Regulatory T Cells Are Essential for Restraining Allergic Airway Inflammation

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Dysregulated type 2 immune responses to environmental antigens in the lung represent a central pathogenic mechanism underlying allergic asthma. CD4+Foxp3+ regulatory T (Treg) cells play a key role in maintaining immune homeostasis; however, their specific contribution to controlling allergen-induced type 2 immunity in the lung remains incompletely understood. We found that Treg cells within lung tissue exposed to fungal allergen, Alternaria alternata, which is implicated in human asthma, expressed higher levels of Il2ra, Pdcd1, Ctla4, Il10 and Gzmb than Treg cells in draining lymph nodes. Mass cytometry revealed multiple lung Treg cell subsets following Alternaria exposure, including a population characterized by robust expression of GATA-3, ST2 (IL-33R) and CCR8. These CCR8+ST2+ Treg cells exhibited a Th2-like transcriptional profile, while highly upregulated immunoregulatory molecules and potently suppressed effector CD4+ T cell proliferation. Upon repeated allergen exposure in vivo, antigen-specific CCR8+ST2+ Treg cells preferentially accumulated within lung tissue after 4-week of Alternaria exposure and exhibited tissue persistence for at least 8 additional weeks without allergen re-exposure; importantly, their tissue residency was not affected by FTY720 treatment. Both conditional and global Ccr8 deficiency rendered mice susceptible to low-dose allergen exposure that was tolerated by wild-type mice, resulting in lung pathology resembling human asthma. Conversely, IL-2-complex treatment expanded lung-resident CCR8+ST2+ Treg cells following chronic Alternaria exposure in vivo, highlighting a potential therapeutic strategy for allergic asthma. Together, these findings identified a lung tissue-resident CCR8+ST2+ Treg cell population that plays a pivotal role in restraining type 2 immunity and preventing allergen-induced immunopathology in the lung.

Abstract 171

POU2F2 regulates abundance of nonclassical monocytes and their transcriptional programs

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Nonclassical monocytes (nMo) are a patrolling monocyte subset derived from classical monocytes (cMo) that supports endothelial health. Unlike cMo, which can differentiate into macrophages in atherosclerotic plaques and immunosuppressive tumor-associated macrophages (TAMs), nMo can limit atherosclerotic lesion development and consume tumor material, making them an attractive target in cardio-oncology. However, the transcriptional mechanisms governing the classical-to-nonclassical monocyte transition remain incompletely understood.
Using single-cell RNA-seq and gene regulatory network analysis (SCENIC) of human PBMCs, we identified high expression and regulon activity of the transcription factor POU2F2 in nMo. Using co-expression network analysis (hdWGCNA), we further identified two gene co-expression modules in human monocytes, with module#1 enriched in nMo and POU2F2 identified as a top hub gene. In addition, POU2F2 expression correlated with genes important for cMo-to-nMo differentiation, including NR4A1 (NUR77), C/EBPβ, IRF2, and KLF2.
To validate these findings, we confirmed high POU2F2 expression in mouse nMo and generated monocyte-specific Pou2f2 knockout mice (Cx3cr1-Cre; Pou2f2fl/fl). We found that Pou2f2 deficiency significantly reduced nMo abundance in blood, bone marrow, and spleen without altering myeloid progenitor frequencies. In addition, residual nMo in POU2F2-deficient mice upregulated surface CD11c across tissues. Using mixed bone marrow chimeras, we found that the effect of POU2F2 deficiency on nMo frequency is cell-intrinsic. Annexin V and Ki-67 staining revealed that the reduced nMo abundance observed in POU2F2-deficient mice was not due to impaired survival or proliferation. Together, these data suggest that POU2F2 is a novel transcription factor for regulation of nMo abundance and phenotypic states.

Abstract 172

T Cell Acly deficiency reduces atherosclerosis in female mice

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Atherosclerosis, the most common form of cardiovascular disease, is characterized by lipid accumulation and chronic inflammation in the artery wall. T cells are known to comprise at least 50% of infiltrating immune cells in carotid plaques. Among these, CD4+ T cells influence atherosclerosis in important ways, with T-bet+ Th1 cells contributing to lesion development while regulatory T cells (Tregs) protect against the disease. However, Tregs become dysfunctional as the disease progresses and adopt a Th1-like, T-bet+ IFNg+ phenotype. Uncovering regulators of CD4+ T cell function in atherosclerosis can help inform future therapies for the disease. In an atherosclerotic mouse model, we found that T cell deficiency of ATP Citrate Lyase (ACLY), a metabolic enzyme that converts citrate to acetyl-CoA, reduces frequencies of inflammatory CD4+ effector cells including T-bet+ IFNg+ Th1 and PD1+ CXCR5+ T follicular helpers. Additionally, Tregs from T cell Acly-deficient mice have decreased expression of Th1 markers T-bet and CXCR3, suggesting Acly deficiency can decrease Treg plasticity. T cell Acly deficiency reduced total atherosclerotic lesion area in female mice. In order to determine whether loss of Acly in the Treg compartment alone would be sufficient to confer disease protection, we conducted atherosclerosis experiments using mice with Treg-specific loss of Acly (FoxP3Cre+Aclyfl/fl) and found that while FoxP3Cre+Aclyfl/fl had reduced splenic frequencies of T-bet+ CXCR3+ Tregs, they did not have reductions in lesion area compared to FoxP3Cre+ controls. Overall, we conclude that loss of T cell Acly is protective in atherosclerosis through reducing inflammatory CD4+ effector populations.

Abstract 173

G6PD deficiency decreases B cell production of IgM to oxidation specific neoepitopes and increases atherosclerosis.

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PDdef) is the most common enzymatic disorder worldwide, resulting in disrupted redox homeostasis and increased oxidative stress. To date, few studies have specifically examined the role of G6PDdef in lymphocytes. As redox homeostasis is critical for B cell function and survival, this study aimed to explore how disrupted endogenous redox systems by G6PDdef influences B cell activity in atherosclerosis. To address this, we engineered novel humanized mice harboring the most common variant of G6PDdef in the US, the African variant (hG6PDA-) or the nondeficient human G6PD allele (hG6PDND) and induced oxidative stress through hyperlipidemia. Our findings indicate that hG6PDA- affects the humoral immune response as hyperlipidemic hG6PDA- mice exhibited significantly reduced levels of IgM antibodies to oxidation specific neoepitopes (IgMOSE) compared to hG6PDND controls and increased atherosclerosis. Importantly, B cell G6PD enzymatic activity levels directly correlated with the number of IgMOSE antibody secreting cells and total lesion area. To determine if the reduction in IgMOSE observed in the hG6PDA- mice were intrinsic to the B cell, we utilized a G6PD specific inhibitor (G6PDi-1) in-vitro on cultured peritoneal B cells, finding that in-vitro G6PD inhibition of B cells resulted in reduced LPS-induced production of IgMOSE. In parallel, treatment of human B cells with G6PDi-1 resulted in a similar reduction in IgMOSE production, supporting the translational relevance of these findings. Together, these data suggest that G6PD may act as a metabolic regulator of antibody production by B cells and are the first to demonstrate that hG6PDA- promotes atherosclerosis.

Abstract 174

Challenging Serological Assumptions: Antibody Responses to Influenza A Virus Are Not Driven by Viral Burden or Clinical Symptoms in a Controlled Human Infection Model

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Serological assays measuring antibodies to influenza A virus (IAV) are widely used to assess seasonal epidemic burden and vaccine efficacy, yet the relationship between viral replication, clinical disease, and antibody production remains unclear. We employed a controlled human infection model to quantify respiratory viral shedding, clinical symptoms, and serum antibody responses following challenge with influenza A/Perth/16/2009 (H3N2). Thirty-two adults with low pre-existing immunity (HAI ≤40) were inoculated intranasally (n=18) or by inhalation of virus-laden aerosols (n=14). Nasopharyngeal swabs and saliva were taken to assess viral shedding by RT-qPCR and plaque assay. Clinical symptoms were assessed by a daily FLU-PRO survey. Antibodies were measured by hemagglutination inhibition (HAI) and microneutralization assays on days 0, 15, 30, and 60 post-inoculation. Twenty-one participants (65%) shed viral RNA, and 18 shed infectious virus. Among those shedding infectious virus, 55% (n=10) exhibited a significant rise in HAI titer and 61% (n=11) in microneutralization titer. Antibody fold-change did not correlate with total viral load, saliva shedding, or symptom burden. Multivariate analyses and assessment of antigen-specific binding to HA, NA, and NP by ELISA are ongoing. These findings indicate that systemic antibody induction following IAV infection is not solely determined by viral burden or clinical disease severity, challenging assumptions underlying serology-based assessments of infection and immunity.

Abstract 175

The Role of Iron Metabolism in T-Cells in Celiac Disease

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Imbalances among CD4■ T cell subsets, including Th1, Th17, T follicular helper (Tfh), and regulatory T cells (Tregs) drive pathology in many autoimmune diseases. These subsets possess distinct metabolic requirements for iron. We previously showed that in systemic lupus erythematosus (SLE), increased Transferrin Receptor expression elevates intracellular iron in T cells, promoting metabolic dysfunction and immune imbalance. However, whether similar iron-dependent mechanisms occur in Celiac disease (CeD) remains unknown.

CeD is unique among autoimmune disorders as it results from a defined interaction between dietary gluten and genetic predisposition. Gluten-derived peptides activate pathogenic T cells, leading to cytokine production, villous atrophy, and increased intraepithelial lymphocytes. Iron deficiency anemia is a common early manifestation, particularly in young children, raising the question of whether altered iron availability also directly influences systemic T cell responses.

We hypothesize that iron dysregulation contributes to altered metabolic processes and imbalance between pro-inflammatory Th1 cells and Tregs in CeD. To test this, we will analyze peripheral blood mononuclear cells (PBMCs) from adult CeD patients and healthy controls. 30 patients with CeD and 17 healthy controls were enrolled in a prospective observational study to monitor iron phenotypes over time. We will utilize spectral flow cytometry to phenotype T cell subsets including Th1, Th17, Th2, Tfh, Tregs, MAIT, γ/δ, and CD8 T cells. Using Ferritin as a surrogate marker of iron storage, and a BioTracker dye for intracellular labile iron, we will quantify changes in iron utilization in these cell types during disease onset and ongoing treatments.

Abstract 176

Omental microenvironment shapes memory properties of antigen specific CD8 T cells.

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Adipose tissues have long been identified as reservoirs of antigen specific CD8 T cells during systemic infections. However, we found that during influenza A virus infection in the lung flu antigen specific (NP+) CD8 T cells are not only detected but persist in the omental visceral adipose tissue. Furthermore, post resolution of infection in the lung, we see the highest accumulation of memory NP+ CD8 T cells in omental VAT compared to other VAT depots and secondary lymphoid organs. In the omental VAT, they seem to be a quiescent non-circulating memory population with a phenotype not typical of long-lived memory cells. In addition, we found the omental-derived memory NP+ CD8 T cells, but not those from the conventional memory reservoir, i.e., the spleen demonstrated superior viral clearance, were more cytotoxic and expanded much better during recall responses to flu infection in the lung. The omental derived memory NP+ CD8 T cells also exhibited unique metabolic properties during primary infection compared to those in the SLOs. We suspect the local omental microenvironment shapes the memory properties of NP+ CD8 T cells and we are currently elucidating the unique drivers that confer memory properties to them.

Abstract 177

Understanding the role of tumor-intrinsic MHC-II in the anti-tumor immune response of NSCLC brain metastasis

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Amongst non-small-cell lung cancer (NSCLC) patients, those who develop brain metastasis have a higher mortality rate than those who do not, indicating a need for investigation into the anti-tumor immune responses associated with brain metastasis. We and others have observed an association between the expression of tumor intrinsic major histocompatibility complex class-II (tiMHC-II) with improved survival in NSCLC. However, the role of tiMHC-II and its interactions with the tumor microenvironment in brain metastasis remains poorly understood.

To bridge this gap in understanding, we analyzed resected tissue from 22 NSCLC primary tumor and 8 NSCLC brain metastasis samples through multiplex immunofluorescence histology (mIFH) staining and image acquisition using the Vectra3 imaging system. We then quantified the presence of immune cells in conjunction with MHC-II. Two mIFH panels were designed and used. The first panel stained for markers of lymphocytes, namely CD3, CD8, and CD56, alongside major histocompatibility complex class-I (MHC-I), MHC-II, and pancytokeratin (PanCK), a tumor marker. The second panel stained markers of professional antigen presenting cells, namely CD68, CD20, and CD208, alongside MHC-II and PanCK. We hypothesized that tiMHC-II is linked with anti-tumor immune responses in the tumor microenvironment (TME).

Early analysis of these data reveals a correlation between immune cell abundances and tiMHC-II expression. We will report on our analysis of immune cell presence and co-localization with tiMHC-II expression in these primary and metastatic samples. Understanding the specific mechanisms by which tiMHC-II interacts with the TME would assist in establishing biomarkers of positive outcomes and developing more effective immunotherapies.

Abstract 178

An innovative robust assay platform to assess ADCC responses against distinct conformations of the HIV-1 Env trimer

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Elicitation of antibody responses mediating antibody-dependent cellular cytotoxicity (ADCC) against HIV-1 in the Thai RV144 vaccine trial correlated with decreased virus acquisition risk. However, there are significant limitations with existing in vitro ADCC assays that preclude their utility as robust, cost-effective, high-throughput assay platforms amenable to standardization. We sought to develop an ADCC assay that addresses these challenges to facilitate evaluation of antibody-based vaccines and cure strategies.

Firefly luciferase- (ffLuc) expressing CEM.NKR T-cells comprising stably integrated replication defective doxycycline-inducible HIV-1 proviruses were derived and characterized. Proviruses included wild-type (wt), vpu and/or nef mutants representative of different HIV-1 strains. ADCC was assessed by measuring ffLuc in co-cultures of target and effector (KHYG1) cells with and without broadly neutralizing (bnAb) or non-neutralizing antibodies (nnAb). Augmented by a 96-well format and the live-cell ffLuc reporter, our approach enabled high throughput and automated data collection and analysis, including ADCC quantified using serial dilutions of antibodies for IC50 and IC80 calculations. HIV-expressing target cell lines, including wt, nef ,and nef/vpu mutants of transmitted/founder viruses, exhibited antibody binding properties similar to published results using primary T cells infected with HIV-1. bnAbs mediated ADCC against wt viruses, nef, and nef/vpu mutants, while nnAbs mediated ADCC only against nef/vpu mutants.

Our findings demonstrate the development of a robust high-throughput assay platform capable of quantifying ADCC mediated by antibodies that target distinct conformations of the HIV-1 Env trimer. We anticipate this innovative approach will be useful to elucidate ADCC correlates of immune protection and cure strategies.

Abstract 179

Functional impact of the atherosclerosis-associated ID3 genetic variation on primary human B cell responses

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The helix-loop-helix transcription factor ID3 is a key regulator of B cell development and immune homeostasis. We previously demonstrated that a single nucleotide polymorphism in the human ID3 gene, rs11574, is associated with increased risk of atherosclerotic disease burden in numerous clinical cohorts. Whether disruption of ID3 function impairs the functional properties of primary human B cells remains unknown. To address this, we designed a CRISPR–Cas9-based genome editing approach to either knockdown ID3 or introduce the rs11574 risk allele into primary human B cells. Using this system, we will assess whether ID3 deficiency impairs B cell effector functions implicated in the regulation of atherosclerosis, including proliferation, migration, and antibody production. We hypothesize that ID3-deficient human B cells will have impaired mitogen-stimulated proliferation and IgM production compared to controls. Together, these studies may determine how disruption of ID3 levels in human B cells impacts their atheroprotective functions and may reveal a potential mechanism linking ID3 genetic variation to impaired B cell function and increased cardiovascular disease risk.

Abstract 180

F5446 Epigenetically Reprograms T cells to Boost CEA CAR T Cell Immunotherapy Efficacy in Human Colorectal Cancer Metastasis.

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Chimeric Antigen Receptor (CAR) T cell therapy emerges as a promising cell-based immunotherapy for human cancer; however, it has shown little efficacy in solid tumors. Recent studies have shown that SUV39H1 decreases CAR T cell persistence and knocking out SUV39H1 increased CAR T efficacy in tumor-bearing mice. Analysis of human colon cancer liver metastases scRNA-seq datasets revealed that histone methyltransferases of H3K9me3 are upregulated in colon tumor and expressed in subsets of T cells in liver metastases of human colon cancer patients. To overcome human colon cancer resistance to CAR T cell immunotherapy, we have developed a SUV39H1-specific small molecule inhibitor F5446. Previously, we found that H3K9me3 promotes differentiation of Tex cells and targeting H3K9me3 is an effective approach to increase IFNghi Tex-int cells to reinvigorate CTL functionality to suppress colon cancer liver metastasis. Based on this data, we hypothesize that F5446 is effective in increasing persistence of CAR T cells to suppress metastatic human colon cancer growth in NSG mice. Utilizing an experimental liver metastasis model in NSG mice, we treat the mice with one dose of α-CEA CAR T cells and treat every three days with F5446 until endpoint. We then identify the CAR T cell phenotypes (memory, effector, naïve, or exhausted) via flow cytometry. We anticipate that F5446 will increase the persistence of CAR T cells in the liver metastases compared to CAR T therapy alone. F5446 is a promising conjunctive treatment with CAR T cell therapy to increase efficacy in solid tumor colon cancer.

Abstract 181

A Unified Human Immune Cell Atlas Spanning Cancer and Cardiovascular Disease Reveals Both Shared and Disease-Specific Immune Programs

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Background:
Immune cells play critical roles in cancer progression and cardiovascular disease (CVD), yet the shared and disease-specific mechanisms that shape their functional states across these conditions remain poorly defined. Our goal is to generate a unified human immune cell atlas spanning cancer and CVD that can identify shared and disease-specific cellular programs, uncover key regulators of pathogenic immune activation, and serve as a resource for future immunotherapy development.
Methods and Results:
To address this, we performed large-scale integrative single-cell RNA-seq analyses across multiple human cohorts and tissue compartments, including peripheral blood, primary tumors, and atherosclerotic plaques. Our dataset comprises more than 1.3 million cells from 243 samples across 19 scRNA-seq datasets and includes both PBMC and tissue samples from multiple cancer types, including head and neck squamous cell carcinoma (HNSCC), breast cancer (BC), kidney renal clear cell carcinoma (KRCC), endometrial cancer (EC), and melanoma. Cardiovascular samples included both carotid and coronary plaques. Datasets were harmonized through batch integration, iterative subclustering, and manual annotation using canonical cell type markers. As a result, we constructed a comprehensive atlas of immune populations, including T/NK, B-cell, and myeloid subsets, as well as non-immune populations across disease contexts. In total, we identified 63 immune and non-immune cell types. Differential abundance (DA) analysis suggested condition-associated differences in subset composition between Athero.PBMC and Cancer.PBMC, as well as between plaque and tumor samples. However, the magnitude and breadth of these shifts suggest that technical or dataset-level confounding may also contribute to the observed patterns.

Abstract 182

CTLA-4Ig Immunosuppression Inhibits Alloimmunity but Maintains Anti-Tumor Immunity in Murine Transplant Recipients with Melanoma

Hongmin Yao (Emory University), Mandy Ford (Emory University)

Transplantation is a curative treatment for end-stage organ failure. However, this success comes at a cost of significantly increased risk of malignancy as a result of the life-long immunosuppression required to prevent allograft rejection. Therefore, the goal of our research is to identify immunomodulatory agents that can effectively suppress alloreactive T cell responses, but maintain immune competence against tumors.

We have established a pre-clinical mouse model in recipients receive an allogeneic skin transplant, begin immunosuppression, and then are inoculated with melanoma. The magnitude and functionality of both graft-specific and tumor-specific CD8+ T cell responses are quantified by flow cytometry analysis. In this series of experiments, animals were immunosuppressed with an analog of belatacept, a CTLA-4Ig fusion protein that blocks both CD28 and CTLA-4. Results indicated that treatment with CTLA-4Ig significantly reduced the frequency and absolute number of graft-specific CD8+ T cells in the spleen as compared to vehicle ($p<0.05$). Unexpectedly, the frequency and absolute number of tumor-specific CD8+ T cells in the spleen was not reduced following treatment with CTLA-4Ig. Moreover, while CTLA-4Ig significantly reduced the frequency of IFN-■-, IL-2- and TNF-producing cells among graft-specific CD8+ T cells, it exhibited no negative impact on the frequency of IFN-■-, IL-2- and TNF-producing cells among tumor-specific CD8+ T cells. Finally, animals treated with CTLA-4Ig maintained allograft survival while simultaneously exhibiting a significant decrease in tumor volume ($p<0.05$).

These results suggest that immunosuppression with belatacept, which blocks CTLA-4 similar to ipilimumab, may be advantageous for the immunosuppression of transplant patients with malignancy.

Abstract 183

Chemotherapy induced AML persister cells undergo immune remodeling and are sensitized to CD70 directed CAR-T therapy.

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Relapse in acute myeloid leukemia (AML) remains an unmet clinical challenge and is driven by drug-tolerant persister cells, yet the immune biology of this adaptive state remains poorly defined. Whether these persister states enforce immune silence or create exploitable immune vulnerabilities is a critical unresolved question. We investigated tumor-intrinsic immune phenotypes that emerge following drug-induced persister formation, with a focus on antigen presentation and immune modulatory surface molecules relevant to immune-based therapies. Using AML tumor models treated with chemotherapy (cytarabine, Ara-C) or targeted therapy (venetoclax), we characterized tumor-intrinsic immune remodeling associated with persister formation using single-cell multiomics coupled with lineage tracing. Single cell transcriptional profiling of Ara-C and venetoclax derived persisters revealed extensive immune reprogramming, marked by enrichment of interferon-gamma and inflammatory signaling, upregulation of MHC class I antigen presentation machinery (HLA-A, B2M, TAP1), and induction of immune interaction ligands (CD86, TNFSF9, MICB). Interestingly, Ara■C and venetoclax■derived persisters upregulated CD70, an immunostimulatory TNF-alpha family ligand, and were preferentially targeted by CD70■directed CAR■T and TCR■T cells in a dose■dependent, antigen■specific manner compared with naïve cells. To delineate CD70 upregulation in persister cells, we performed an unbiased genome-wide CRISPR knockout screen and identified TP53 as a top positive regulator of CD70. Consistent with this, p53 activation by chemotherapy or Nutlin was sufficient to upregulate CD70, providing a mechanistic basis for the enhanced CD70 expression observed in therapy-induced persister cells. Together, these findings indicate that persister cells adopt an immune-responsive and antigenically remodeled state, enabling CD70 specific immunotherapeutic vulnerabilities to overcome relapse.

Abstract 184

CD11b: A novel marker for CD8+ T cell activation and potentially costimulation blockade-resistant CD8+ T cells

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Costimulation blockade therapies are a promising alternative to standard-of-care calcineurin inhibitors that are associated with significant toxicities and side effects in transplant recipients. However, costimulation blockade, such as belatacept (CTLA4-Ig), confers increased rates of acute rejection often associated with specific memory T cell subsets with a reduced requirement for costimulation. Identifying potential therapeutic targets to better control these “costimulation blockade-resistant” T cells remains an important goal. We analyzed murine graft-infiltrating CD8+ T cells in the context of costimulation blockade-resistant rejection in a full MHC mismatch model and found that, unexpectedly, up to 30% of graft-infiltrating CD8+ T cells expressed the myeloid cell marker CD11b. Similarly, non-human primate CD8+ T cells stimulated in an in vitro allogeneic MLR revealed that ~5-25% of alloreactive CD8+ T cells expressed CD11b. Bulk RNASeq of MLR-stimulated CD11b+ versus CD11b- alloreactive CD8+ T cells identifies CD11b expression is associated with increased transcripts related to trafficking and effector function. Moreover, characterization of anti-CD3/28-stimulated healthy human PBMCs found that known “risky memory” subsets, CCR7-CD45RA+ TEMRA and PD-1-CD57+ CD8+ T cells, were most enriched for CD11b unstimulated (20%) with the highest expression 1 day post-stimulation (30-45%). Overall, CD11b is a novel early activation marker on a subset of alloreactive CD8+ T cells known to be associated with costimulation blockade-resistant rejection. This suggests CD11b could provide an early marker for detection of potentially alloreactive CD8+ T cells and be a target for adjunctive therapy to directly target multiple “risky memory” CD8+ T cell subsets and limit costimulation blockade-resistant rejection.

Abstract 185

Stem cell memory T cells and how they interact with glioblastoma

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Glioblastoma (GBM) is the most common primary brain tumor with a poor patient prognosis of less than 7% surviving five years after diagnosis despite surgical resection, radiation therapy, chemotherapy, and tumor treating fields treatments. To address the disparity in glioblastoma outcomes, our group pioneered a novel platform called polyclonal Adoptive Cellular Therapy (pACT). This platform includes host conditioning and transfer of: hematopoietic stem cells, dendritic cells that have been electroporated with tumor RNA for tumor-specific antigen presentation, and T cells that have been activated and expanded against tumor RNA-loaded dendritic cells. Stem cell memory T (TSCM) cells are a minimally differentiated T cell subset with self-renewal capacity, multipotency, and enhanced persistence, making them attractive for adoptive cellular therapy (ACT). Understanding the role TSCM play in pACT for treatment of GBM is critical for understanding the immune cell interactions and will enhance knowledge of immune cell dynamics. Here, I examine how TSCM interact with and eliminate tumor cells by coculturing tumor-specific stem cell memory T cells with their targets. I intend to examine proliferative potential, killing capacity, and differentiation capacity of TSCM once in response to target stimulation. These results will be the first step in understanding how TSCM respond to tumor challenge and can protect against future rechallenge.

Abstract 186

Mitochondrial respiration is a critical vulnerability node in the immune response to Mtb

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Genome wide association studies have linked mitochondrial dysfunction to susceptibility to mycobacterial infection, implicating diverse processes such as mitochondrial genome maintenance, organelle dynamics, and quality control. However, how these defects manifest within immune cells during infection remains unclear. Using scRNA-seq of CD45+ lung immune cells from Mtb-infected mice isolated at day 21 and 77, we defined the myeloid landscape across disease progression and uncovered a striking collapse of mitochondrial electron transport chain (ETC) gene expression within these populations. We hypothesized that this transcriptional collapse functionally constrains macrophage effector responses. To test this, we stably knocked down in Ndufa13, Sdhb, Uqcrc1 and Cox4i1, ETC complexes I-IV respectively, in iBMDMs and assessed downstream phenotypes. ETC knock down broadly impaired macrophage effector function, including inflammatory responses and antigen presentation capacity. Together, these findings support a model in which loss of mitochondrial respiratory programs contributes to myeloid cell dysfunction during Mtb infection. We propose that impaired mitochondrial respiration limits macrophage effector function and may represent an important feature of progressive tuberculosis, with potential implications for understanding immune failure in TB patients.

Abstract 187

Alternative splicing of RUNX transcription factors is an additional layer of transcriptional control of CD8 T cell differentiation.

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CD8 T cells differentiate into effector and memory populations with distinct functional roles following acute viral infection. While transcription factors (TFs) are well-established regulators of these decisions, how alternative splicing of TF mRNA shapes differentiation remains largely unexplored. We have previously shown that the RUNX-family TF RUNX3 is required for memory CD8 T cell formation, but the roles of its paralogs are poorly understood. Using shRNAmir-mediated depletion in P14 TCR-transgenic CD8 T cells during acute LCMV infection, we show that RUNX1 compensates for RUNX3 loss, evidenced by residual chromatin accessibility at RUNT motifs in RUNX3-depleted cells. RUNX1 and RUNX3 exert opposing influences on differentiation: RUNX1 drives commitment to CX3CR1^hi KLRG1^hi terminal effectors, whereas RUNX3 counteracts this program to maintain a precursor state competent for tissue-resident and central memory formation. Cloning of Runx1 identified two splice isoforms differing in inclusion of an exon encoding an ETS-family protein interaction domain (EID). Overexpression suggests that these isoforms differentially bias CD8 differentiation outcomes, and epistasis experiments confirm that ETS1 is required for the phenotype conferred by the isoform containing the EID. De novo transcriptome assembly of bulk RNA-sequencing data revealed analogous isoform variation in Runx2, but not Runx3, and suggested differential usage across subsets, extending this regulatory logic across the RUNX family. This approach to resolving unannotated TF isoforms in reveals a layer of regulation in which alternative splicing tunes RUNX–ETS interactions to control CD8 T cell fate, with direct implications for enhancing memory formation in chronic infection and cancer.

Abstract 188

Redistribution of Kindlin3 during neutrophil recruitment in vivo

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Neutrophils respond rapidly during inflammation and are highly migratory cells. Their ability to reach sites of injury and infection depends on integrin-ligand binding, which provides the mechanical attachment required for crawling and transmigration. Integrin activation is tightly regulated by Kindlin-3 (K3); its deficiency causes Leukocyte Adhesion Deficiency III (LAD3), characterized by impaired neutrophil migration and life-threatening infections. The mechanisms by which K3 regulates integrin activation in vivo remain unclear. We hypothesized that K3 is recruited to the membrane prior to integrin activation to mediate conformational changes. We generated a knock-in mouse fusing the fluorescent protein mScarlet-I to K3 (KI-K3) which enables subcellular tracking of K3 within neutrophils via intravital imaging (IVM). Although hematopoietic cells expressed KI-K3 and mice were viable, the fusion induced allelic hypomorphicity, reducing total K3 mRNA and protein levels and causing compensatory neutrophilia. Using IVM in the cremaster muscle and skin, we observed that KI-K3 is distributed homogeneously in circulating neutrophils but concentrates at the neutrophil-endothelial interface during luminal adherence, crawling, and transmigration. Post-diapedesis, KI-K3 polarizes to the lamellipodia and uropod during interstitial migration. By breeding KI-K3 mice with those expressing human beta2-integrin (hITGB2), we visualized KI-K3 and active integrin using the mAb24 antibody, which binds to high-affinity beta2-integrins. K3 colocalized with mAb24 at the neutrophil rear and uropod. Our real-time in vivo observations demonstrate that K3 localization is highly dynamic and undergoes differential spatial reorganization throughout the recruitment cascade, suggesting a specific spatiotemporal role in mediating integrin activation.

Abstract 189

NPY-Driven Fibrotic Signatures Across Skin Diseases: A Multi-Model Transcriptomic Analysis

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Neuropeptide Y (NPY) plays a pivotal role in various biological processes, including immune response and cell differentiation. Previous studies in our lab identified a premature hair graying phenotype in Npytet/tet mice at 25 weeks of age delineating a role for NPY in dysfunction of the melanocyte lineage. The Npytet/tet mouse has a knock-in mutation that induces entopic overexpression of Npy. Utilizing transcriptomics comparing Npytet/tet and Npy+/+ mice, we also observed a significant shift in skin homeostatic signatures and the presence of inflammatory disease signatures between 22 and 35 weeks of age. These findings suggest that the Npytet/tet model could serve as a valuable tool for studying chronic, inflammatory, fibrotic skin responses. To evaluate this, we initiated a comparative analysis by aligning our 35-week-old mouse dataset with six publicly available systemic sclerosis models from NCBI. Utilizing Cloud computing and Bioconductor's R packages, we preprocessed raw count data, computed the intersection of common genes across all datasets, aligned the gene identifiers, and combined all datasets into single matrix, applied batch effect correction using the sva package in R. To identify gene expression patterns across datasets, we performed k-means clustering analysis on this data matrix using the ClusterR package. Preliminary results indicate that the Npytet/tet model closely resembles the bleomycin-induced mouse model of the inflammatory subtype of systemic sclerosis. Extending this analysis to models of atopic dermatitis and psoriasis and further to human patient datasets, we aim to identify disease-specific gene modules and shared molecular signatures. Our long-term objective is to establish the Npytet/tet model as a comprehensive resource for fibrotic disorders and to pinpoint common key regulatory genes.

Abstract 190

The crucial role of VEGFR3 mediated lymphangiogenesis in colitis resistance, and new insights into the role of VEGFR2 in colitis

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Inflammatory bowel disease (IBD) is exacerbated by lymphatic abnormalities through the loss of lymphatic vessel (LV) density, LV occlusion, and intestinal lymphangiectasia. While IBD has been characterized by a loss of intestinal epithelial barrier integrity, immune dysregulation, and microbiome dysbiosis, considering the lymphatics role is crucial to further explore this multifactorial disease.

The vascular endothelial growth factor (VEGF) family is critical to angiogenesis, lymphangiogenesis, and vascular permeability, all of which are highly implicated in IBD. VEGFR2 signaling induces zippering of lymphatic endothelial cell junctions, decreasing the permeability of LVs, and hindering lymphatic drainage. Across IBD patient populations, increases in the VEGFR3 ligand, VEGF-C, have been commonly found as well as an inverse correlation between LV density and disease severity. The manipulation of LV density and lymphatic permeability provide new avenues to target chronic inflammation.

In a murine acute colitis model, the blockage of VEGFR3 via a sVEGFR3 AAV increased the severity and lethality of the inflammatory response. The increase in severity after VEGFR3 blockage elucidates the critical role expanded lymphatic vasculature plays in resistance to intestinal inflammation. Conversely, VEGFR2 mediated lymphatic permeability failed to support resistance to both acute and chronic DSS-induced colitis in anti-VEGFR2 treated mice.

Modulating lymphatic drainage continues to show potential in treating chronic inflammation and IBD, with VEGFR3 mediated lymphangiogenesis playing a key role in disease resistance. The failure of VEGFR2 blockage to reduce the severity of inflammation in colitis highlights the unique properties governing lymphatic vessel permeability in the colon.

Abstract 191

NK Cells Drive Granuloma Formation against Chromobacterium violaceum in the Absence of Adaptive Lymphocytes

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Granulomas are organized immune structures that contain and facilitate clearance of persistent pathogens. We previously identified Chromobacterium violaceum as an environmental pathogen that induces formation of an innate granuloma. Here, we show that natural killer (NK) cells are sufficient to drive granuloma formation in the absence of adaptive lymphocytes. Consistent with this, mice lacking all lymphocytes rapidly succumb to C. violaceum infection. Mechanistically, NK cells are a significant source of IFN-γ during C. violaceum infection, and IFN-γ signaling is required for bacterial clearance. Using cell Ifngr1fl/fl LysMcre mice, we further demonstrate that IFN-γ signaling to macrophages and neutrophils is required for resolution of infection. Together, these findings identify a central role for NK cells in orchestrating innate granuloma formation and host defense against C. violaceum.

Abstract 192

Engineered IgM and IgG cleaving enzymes for immunomodulation

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Immune■modulatory regimens (IMRs) are used across diverse clinical settings to control undesirable immune responses, but many still rely on broad immunosuppression. While enzymatic strategies to selectively cleave IgG have advanced, comparable methods to target IgM remain largely underdeveloped. As a model to evaluate targeted enzymatic strategies, we focused on adeno■associated virus (AAV) gene therapy, where systemic dosing can trigger adverse events, including complement activation driven by anti■capsid IgM and IgG responses. We identified a novel endopeptidase, IceM, that selectively degrades human IgM, a key initiator of the anti■AAV immune cascade. We then engineered IceMG, a dual■specificity fusion enzyme that cleaves both IgM and IgG. IceMG removes B cell surface immunoglobulin, disrupts downstream phospholipase Cγ signaling in vitro, and inhibits complement activation more effectively than IgG■specific cleavage alone. In macaques, intravenous administration of IceMG induces rapid and reversible depletion of circulating IgM and IgG. Antisera from treated animals show markedly reduced AAV neutralization and complement activation. Consistent with these findings, preconditioning with IceMG restores AAV transduction in mice passively immunized with human anti■AAV antisera. These studies have implications for improving safety of AAV gene therapies and possibly broader applications including organ transplantation and autoimmune diseases.

Abstract 193

Molecular titration of heterogeneous CD4 epitopes tunes target B cell fate dynamics

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Antigen-dependent determinants of immunogenicity are crucial to modern vaccine design. Multiple factors contribute to the antigenicity of a protein: professional antigen-presenting cells (APC) take up, process, and present protein antigen as short linear peptide fragments mounted on major histocompatibility complexes (pMHC). CD4+ T cells with receptors (TCRs) specific for these unique pMHC class II molecules bind in addition to costimulatory molecules resulting in their activation and fate determination. Additionally, antigen-specific B cells bind target epitopes also causing them to engulf, process, and present antigen on pMHC-II as well. These pMHC-II-TCR interactions are critical for determining B and T cell selection, proliferation, and differentiation—defining aspects of adaptive immunity. In theory, this knowledge could be leveraged with computational methods to control and optimize nuanced outcomes of an adaptive immune response to vaccination. However, much remains unknown as to how to apply protein design in a way that can predictably alter B and T cell fates. To better understand how designable features of a protein could shape an immune response, we have developed a T cell antigen packaging system to independently manipulate T cell help provided to a target antigen-specific B cell population. This reductive model system allows us to better understand how basic parameters of helper T cell epitope redundancy and heterogeneity influence the fate of target antigen-specific B cells. These parameters are easily represented with newly developed techniques in computational protein design, and by incorporating our findings into these new methodologies, this work will inform the next generation of computationally designed protein nanoparticles capable of fine-tuning a desired adaptive immune response.

Abstract 194

Human Apolipoprotein B Specific CD8 T Cells in Atherosclerosis

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Atherosclerosis is a chronic inflammatory disease with autoimmune features. CD8 T cell infiltration, activation, and oligoclonal expansion have been observed in human atherosclerotic lesions; however, their antigen specificity and functional states remain poorly defined. Prior work in mice identified apolipoprotein B (APOB) as a relevant autoantigen, but whether APOB-specific CD8 T cells exist in humans has not been established. Here, we investigated HLA-A*02:01–restricted APOB epitopes and characterized APOB-specific CD8 T cell responses in healthy individuals and patients with atherosclerosis. Using in silico prediction, we selected ~0.1% top-ranked APOB peptides that bind HLA-A02:01 and evaluated their immunogenicity through expansion-based restimulation and IFN■ ELISpot assays. APOB peptide pools induced robust CD8 T responses marked by expression of activation markers (4-1BB, CD69, and CD25), proinflammatory cytokines (IFN■ and TNF■), and cytotoxic molecules (granzyme B and perforin). Top 6 responding APOB peptides were used to generate tetramers. These tetramers detected circulating APOB-specific CD8 T cells in both healthy donors and individuals with atherosclerosis. To further define their phenotype, APOB tetramer-positive CD8 T cells from patients were analyzed by single-cell RNA sequencing and paired TCR sequencing. APOB-specific cells exhibited increased clonal expansion and transcriptional programs associated with NK-like, terminally differentiated, and regulatory states. They also expressed high levels of CD45RO and PD■1, consistent with antigen experience and activation. These findings demonstrate that human APOB contains dominant HLA-I–restricted epitopes capable of eliciting autoreactive CD8 T cell responses, providing new insight into antigen-specific immunity in atherosclerosis.

Abstract 195

Memory Regulatory T Cells Reprogram into Protective Tfh-like Effectors in Recurrent Malaria

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

Abstract 196

Early-life exposure to antibiotics disrupts the development of the small intestine microbiota, suppressing intestinal epithelial-immune crosstalk, and resulting in increased lipid absorption and adiposity.

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The mechanisms by which the infant gut microbiota protects the host against metabolic dysfunction remain largely unknown. Previous research into the infant gut microbiota has predominantly relied on data from 16S rRNA sequencing of fecal samples. Yet, the small intestine (SI) microbiota, a distinct community from the fecal microbiota, modulates intestinal lipid metabolism and host adiposity. To investigate the composition of the early-life SI microbiota, we performed metagenomic sequencing of distal SI content from 2- and 3-week -old mice. Microbial abundance increased during the 2-to-3-week period as Lactobacillus intestinalis and segmented filamentous bacteria (SFB) expanded in the SI. Exposure to low dose penicillin (LDP) blunted the development of the SI microbiota and led to significantly greater abdominal fat than mock-treated mice. Single-cell RNA sequencing of intestinal epithelial cells (IECs) from young mice exposed to LDP revealed increased expression of lipid transporters in absorptive IECs, which was associated with higher lipid absorption by SI IECs in these mice. PPARa, a regulator of intestinal lipid absorption, was upregulated in the epithelium of LDP-treated mice. Significantly, perturbation to PPARa abundance was required to increase adiposity in antibiotic-exposed mice, as LDP treatment no longer increased fat accumulation in PPARa knockout mice. We determined that early-life microbiota expansion suppressed epithelial PPARa abundance by inducing IL-22 production by immune cells. Indeed, restoration of IL-22 protected antibiotic-treated mice against increased adiposity. Together, our data reveal a previously underdetermined mechanism by which the development of the infant SI microbiota protects against early-life fat accumulation by regulating epithelial-immune interactions.

Abstract 197

A human multi-omic atherosclerosis atlas reveals distinct p300-associated regulatory programs in T-cell subsets

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Atherosclerosis is the main cause of cardiovascular morbidity and mortality worldwide. The pathogenic role of T cells in atherosclerosis is well known, but the regulatory mechanisms that control their activity are still not clear. p300 is an important transcriptional co-regulator in several cell types that are involved in atherosclerosis, including endothelial cells, macrophages and foam cells, and vascular smooth muscle cells. However, how p300 shapes pathogenic T-cell states and inflammatory programs in human atherosclerosis is still unknown. To addressed these gap, we used a large human atherosclerosis single cell atlas. We found that p300 showed distinct patterns across T-cell subsets and disease states. Specifically, p300 was enriched in exhausted CD8 T cells, while p300 expression in CD4 and CD8 effector memory T cells (CD4Tem and CD8Tem) was negatively associated with disease severity measured by GeniScore. We then combined tissue-level and p300-positive single-cell differential gene expression analyses to define the possible functions of p300 in these T-cell subsets. In circulating CD4Tem and CD8Tem cells, NFATC2-regulated p300 may increase TNFSF12 expression, which may act on stromal cells in plaques. In circulating CD8Tex cells, p300 may regulate CD247 expression and exert an inhibitory role. In plaque CD4Tem, CD8Tem, and CD8Tex cells, EGR1-regulated p300 may increase IFN-γ expression and promoting apoptosis and inflammation that worsen necrotic core formation. These findings were also supported by scATAC-seq and spatial transcriptomics data integrated with the atlas. Together, these findings suggest that p300 marks distinct regulatory programs across T-cell subsets and tissue contexts in human atherosclerosis.

Abstract 198

Epithelial Cell Presentation of Injected Bacterial Antigens Drives Tissue-Resident CD4+ T Cell Memory at Mucosal Barriers

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Conventional vaccine design emphasizes antigen dose and adjuvants but we find that intracellular antigen localization is the dominant determinant of CD4+ tissue-resident memory T (Trm) cell programming at mucosal barriers.

Using Citrobacter rodentium—a model for human enteropathogenic E. coli—engineered to express the same epitope fused to bacterial proteins with different subcellular distributions, we found that only antigens translocated into intestinal epithelial cell (IEC) cytosol via the bacterial type III secretion system generated robust CD4+ Trm responses. Cytosolic antigens induced >100-fold greater T cell expansion than surface-expressed antigens despite comparable antigen expression levels and programmed Trm rather than default central memory (Tcm) fate. Light-sheet microscopy revealed 250-fold enrichment of intraepithelial Trm cells versus lymphoid tissue-localized Tcm cells for injected versus non-injected antigens, respectively.

MHCII-dependent presentation of cytosolic antigens by IECs was essential for Trm cell development. Conditional IEC MHCII deletion reduced Trm populations and shifted memory composition toward Tcm, while in vivo contact tracing revealed that ~80% of intraepithelial CD103+ Trm cells sustained direct MHCII-dependent interactions with IECs through the memory phase. Single-cell transcriptomics revealed sustained, reciprocal epithelial-T cell signaling. Epithelial-T cell crosstalk activated transcriptional programs that promoted resolution of innate inflammatory signaling, while T cells received signals that drove Trm rather than Tcm programming.

These findings establish a new paradigm: The delivery of antigens into the epithelial cytosol—mimicking type III secretion by extracellular pathogens—may be essential for the development of mucosal vaccines that generate protective Trm cells positioned at barrier surfaces where enteric pathogens are encountered.

Abstract 199

TLR2 Activation Mediates Age- and Genotype-Dependent Microglial Activation and Dopaminergic Neuron Vulnerability via NF-κB and STING Signaling in hLRRK2-R1441G Parkinson's Disease

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Parkinson's disease (PD) is characterized by progressive loss of dopaminergic (DA) neurons, Lewy body pathology, and motor dysfunction. Neuroinflammation driven by activated microglia is a central and self-amplifying feature of PD pathogenesis, correlated with DA neuron loss. Toll-like receptor 2 (TLR2), activated by oligomeric α-synuclein released by dying neurons, triggers MyD88-dependent NF-κB and cGAS-STING signaling cascades, forming a self-reinforcing neuroinflammatory circuit that fuels progressive DA neuron loss. LRRK2, the most common genetic risk factor for familial PD, is highly expressed in microglia and DA neurons, and the hLRRK2-R1441G (TG) mutation amplifies this neuroinflammatory response. Yet whether R1441G amplifies TLR2-driven NF-κB and STING signaling in an age-, genotype-, and route-dependent manner remained unexamined. Using male WT and TG mice at 3 and 6 months, we challenged mice with systemic Pam3CSK4 (LP-IP) or local intrastriatal LP and α-synuclein pre-formed fibrils (PFF-STR). LP-IP induced greater microglial CD68 upregulation in TG vs. WT at both ages, amplified at 6M vs. 3M, confirming age-dependent sensitization of the TG neuroimmune response. Strikingly, nuclear STING expression and p-p65 enrichment were observed within TH+ dopaminergic neurons of the SNpc in TG vs. WT mice, recapitulated by local LP-STR and PFF-STR challenge. Western blot confirmed elevated p-p65 and p-STING in TG mice, accompanied by pro-inflammatory cytokines including IL-1β, TNF-α, and CXCL10, and worsened motor behavior. These findings establish TLR2-driven NF-κB and STING signaling as an age- and genotype-dependent mechanism preferentially targeting TH+ DA neurons in hLRRK2-R1441G PD, identifying the TLR2–NF-κB–STING axis as a promising therapeutic target.

Abstract 200

Attenuated Sindbis Virus Replicons Display Increased Immunogenic Potential In Glioblastoma Models

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Glioblastoma (GBM) is the most common and deadly primary brain tumor in adults. Treatment remains difficult due to its diffuse growth and immunosuppressive tumor microenvironment (TME). Current treatments result in a median survival time of only 12-18 months, underscoring the urgent need for novel therapeutics. Oncolytic viruses offer a promising strategy by selectively infecting tumor cells while stimulating anti-tumor immunity. Alphavirus replicons are attractive therapeutic platforms due to their self-amplifying nature, high therapeutic protein expression, and inability to spread. They can also reprogram the immunosuppressive TME by inducing immunogenic cancer cell death and anti-tumor immune responses, including interferon (IFN) signaling, and can be engineered to deliver therapeutic proteins such as cytokines. However, wild-type (WT) replicons induce host transcriptional and translational shutoff and rapid cytotoxicity, limiting IFN production and therapeutic output. We hypothesize that replicons with reduced cytotoxicity will enhance immune activation and prolong virus-delivered therapeutic protein production. To test this, we constructed an attenuated Sindbis virus (SINV) replicon containing mutations that delay cytotoxicity and enhance IFN signaling. In murine GBM monocultures and macrophage co-cultures, the attenuated SINV replicon reduced cell death and increased IFNβ and pro-inflammatory gene expression. In KR158 monocultures, we observed sustained and higher level GFP expression and increased surface MHC-I levels, indicating enhanced therapeutic protein delivery and antigen presentation. These findings suggest that attenuated SINV replicons increase immunogenic potential and sustain foreign protein expression, supporting their potential as safe and effective immunotherapeutic platforms to improve outcomes in patients with GBM and other cancers.

Abstract 201

Type 1 Interferons Regulate Cellular Redox Balance by Suppressing the Nrf2 Antioxidant Response During Influenza A Virus Infection

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NRF2, a key transcription factor that governs the cellular antioxidant (AOX) response, is critical in maintaining redox balance during viral infections. Disruption of NRF2 activity can lead to accumulation of reactive oxygen species (ROS) and insufficient AOX defenses, a characteristic of influenza pathogenesis. Type I interferons are well-established host factors implicated in antiviral immunity as well as influenza severity. We hypothesize that elevated T1 IFNs may exacerbate disease severity in response to influenza infection by altering cellular redox balance through suppression of the NRF2-driven AOX responses. To test this hypothesis, we infected WT and Ifnar1-/- mice with influenza A virus and sorted alveolar macrophages (AMs), inflammatory monocytes (IM), and neutrophils (PMNs) at D3 post infection for assessment of the NRF2-regulated AOX response. Influenza-infected mice showed increased transcription of NRF2-regulated AOX genes in AMs and PMNs from Ifnar-/- mice compared to WT mice, with the most dramatic effects observed within neutrophils. Direct stimulation of purified neutrophils with T1 IFNs or T3 IFNs (IFN-lambda) increased rather than decreased the expression of NRF2-dependent AOXs. However, neutralization of IFNAR during influenza virus infection of A549 human lung epithelial cells increased the expression of AOXs, NQO1 and HMOX1. These observations suggest that autocrine T1 IFNs produced in response to influenza infection profoundly alter neutrophil redox balance in vivo by increasing their oxidative stress, but this effect is either indirect or dependent on cellular context. Furthermore, manipulating the T1 IFN-NRF2-AOX axis could represent an approach for intervening in influenza-induced acute lung injury (ALI), such as acute respiratory disease syndrome.

Abstract 202

Humoral immunity leads to control of chronic Plasmodium infections

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Chronic, minimally symptomatic Plasmodium falciparum (Pf) infections are common in endemic areas. These infections predispose individuals to secondary bacterial infections and may reduce malaria vaccine efficacy. Thus, determining how chronic infections develop and the immunological changes that occur is essential for ameliorating disease and developing new interventions. Here, we used samples collected from rhesus macaques infected with P. coatneyi, a model of Pf malaria, to determine the host responses leading to control and establishing a chronic infection. Based on whole blood transcriptomics, infections reached chronicity 50-80 days after sporozoite infection. B cell pathways are upregulated during chronic infections and are correlated with parasite control and reduced symptoms. IgG and IgM antibodies against the neutralizing epitope MSP-1-19 peak when parasitemia is controlled and remain elevated. Interestingly, the magnitude of the MSP-1-19 IgM antibody response delineated when each animal would control parasitemia and not MSP-1-19 IgG. Reinforcing this finding, antibodies inhibited parasite growth in vitro and predicted when animals would control parasitemia. Together, this study defines the development of chronic Plasmodium infections and demonstrates that humoral immune response is key to establishing chronicity.

Abstract 203

PFKP in immune evasion and tumor progression

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Phosphofructokinase 1 (PFK1) is one of the key regulatory and rate-limiting enzymes in the glycolysis pathway, converting fructose 6-phosphate and ATP to generate fructose 1,6-biphosphate and ADP. PFKP (PFK, platelet), one of the 3 isoforms of PFK1, is highly expressed in T cell and B cell leukemias, and other solid tumors. Upregulation of PFKP is associated with progression and poor prognosis in leukemia and in various solid tumors. Our previous study shows that PFKP, together with Pyruvate kinase M2, can be phosphorylated, thereby redirecting glycolytic intermediates into the pentose phosphate (PPP) and serine pathways, increasing the levels of the antioxidants NADPH and glutathione, and promoting cancer cell survival. Our recent study demonstrated that PFKP is a nucleocytoplasmic shuttling protein with functional nuclear export and nuclear localization sequences (NLSs). Nuclear PFKP stimulates the expression of C-X-C chemokine receptor type 4 (CXCR4), a chemokine receptor regulating leukemia homing/infiltration, to promote T-ALL cell invasion, which depends on the activity of c-Myc. Our ongoing work identifies that the short C-terminal isoform of PFKP disrupts the PFKP/c-Myc interaction, thereby reshaping pyrimidine metabolism and downregulating PD-L1 expression to inhibit tumor progression.

Abstract 204

Trem2■ macrophages and the epigenetic regulation of macrophage IL-10 in intestinal inflammation

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Regulatory macrophages (Mregs) are a specialized subset of intestinal macrophages that play a role in limiting inflammation and preventing immunopathology through their immunosuppressive function and IL-10 production in the colonic lamina propria. Triggering receptor expressed on myeloid cells 2 (Trem2) has emerged as a marker of this population, though its dynamics during colitis remain largely undefined. Using Citrobacter rodentium as a model of enteric infection, we tracked Trem2+ colonic macrophages by flow cytometry across a 21-day time course every three days. Absolute number of Trem2+ macrophages peaked at day 9, corresponding to the height of inflammation, before declining toward baseline. To investigate the epigenetic state of colonic macrophages during infection, we performed ATAC-seq on sorted colonic macrophages from naïve (day 0) and infected (day 9) mice. Chromatin accessibility at an Il10 enhancer increased at day 9 compared to naïve macrophages, suggesting ongoing epigenetic regulation of Il10 in colonic macrophages during infection. Whether Trem2+ macrophages represent the predominant IL-10-producing myeloid population in the colon during infection remains unknown, as does the mechanism governing Trem2 upregulation and downstream signaling in this context. Given that dysregulated IL-10 production is implicated in both chronic intestinal inflammation and colitis-associated disease, defining the epigenetic mechanisms governing Trem2+ macrophage function may identify novel targets for therapeutic modulation of mucosal immunity.

Abstract 205

Kynurenine-carboxyketoalkene (Kyn-CKA) regulates conventional dendritic cell maturation and function.

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Upregulation of kynurenine synthesis in conventional dendritic cells (cDC) promotes immunosuppressive signaling and is critical for peripheral tolerance. We show that IDO1 upregulation in cDC type 1 and 2 results in the production of the cysteine-reactive metabolite, kynurenine carboxy-ketoalkene (Kyn-CKA). Kyn-CKA attenuates LPS-induced CD40 and CD86 as well as the production of IL-6 and IL-12 by cDCs. Furthermore, we demonstrate that while Kyn-CKA inhibits IDO1-/- cDC activation by LPS, this effect is not recapitulated by kynurenine, indicating that Kyn-CKA is the key bioactive intermediate in the pathway. Notably, while aryl hydrocarbon receptor (AhR) engagement was not required for the effects of Kyn-CKA on CD40 and CD86 expression, AhR-/- cDC2 were partially refractive to the inhibitory effects of Kyn-CKA towards IL-12 secretion. In addition, we observed that cDC primed with OVA/LPS in the presence of Kyn-CKA were less efficient at promoting OT-II T-cell activation and proliferation but generated more TRegs than cDC primed without Kyn-CKA. We then examined the mesenteric lymph nodes (mesLN) of mice treated with Kyn-CKA under steady state and analyzed cDC populations. Within the mesLN, DCs continuously migrate from intestinal tissues to present antigens, controlling the development and functional differentiation of cells in the adaptive immune system. Kyn-CKA administration decreased CD40 and CD86 expression in migratory cDC, and reduced IL-12 and IL-6 expression upon ex vivo activation with LPS. Finally, we demonstrate that Kyn-CKA attenuates cDC activation and migration in an LPS-induced model of pulmonary inflammation and promotes LPS/OVA induced TReg differentiation in an AhR-dependent manner.

Abstract 206

PTEN increases lysosomal acidification and bacterial clearance in macrophages

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Macrophages are essential to controlling Staphylococcus aureus infections. The release of antimicrobial effectors, including reactive oxygen (ROS) and nitrogen species (RNI), along with phagolysosome acidification, is vital for killing S. aureus. In this study, we aim to investigate whether the phosphatase PTEN prevents the killing of gram-positive bacteria and to identify the mechanisms involved. Our data indicate that S. aureus activates PTEN and that PTEN-deficient macrophages enhance the killing of S. aureus. We did not observe any impacts on PTEN regarding ROS and RNI. Interestingly, we found increased lysosomal acidification in both naïve and infected PTEN knockout macrophages. RNAseq analysis showed an enrichment of genes associated with responses to S. aureus, phagosome formation, and phagosomal acidification. Additionally, we noted that PTEN deficiency leads to heightened V-ATPase activity. To determine if PTEN-mediated inhibition of phagolysosome acidification affects S. aureus killing, we treated wild-type and PTEN knockout macrophages with the V-ATPase inhibitor bafilomycin A1. Our data demonstrate that the inhibition of V-ATPase reduced the enhanced bacterial killing resulting from PTEN deletion. These findings suggest that PTEN inhibits S. aureus killing by suppressing V-ATPase-dependent phagolysosome acidification. Consequently, we anticipate that inhibiting PTEN could serve as a promising host-targeted therapy for treating antibiotic-resistant S. aureus.

Abstract 207

Long-term IL12 induces immune dysfunction and T cell death

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Different cytokine strategies have been implemented to improve the metabolic fitness of adoptive T cell therapy (ACT) T cells. One such cytokine, IL12, is a potent inflammatory molecule that enhances central memory T cells (Tcm) and improves tumor control; however, its cytotoxicity has limited its translational potential for clinical administration. To further understand the limitations of utilizing IL12 and identify optimal opportunities to use this cytokine for ACT, we compared activating murine gp100-peptide-specific Pmel T cells and human PBMCs in the presence of either IL2 or IL12, and monitored their ability to develop a Tcm phenotype, produce cytokines, and kill tumor cells in vitro and in vivo. We found that a short duration IL12 (3 days) was sufficient for Tcm development and improved tumor control; however, culturing cells longer reduced proliferation, reduced mitochondrial oxidative phosphorylation capacity, and induced cell death. Interestingly, we observed that cells maintained in IL12 have increased autophagy without increasing mitophagy, suggesting a unique compensatory stress-mediated response. These cells showed increased lipid uptake of fatty acids and altered lipid intermediates, suggesting that continuous IL12 induces changes in lipid metabolism that may suppress T cell health. Together, these findings suggest that T cell fitness can be fine-tuned by altering IL12 timing, which may have significant implications for the future use of this cytokine for ACT expansion and augmentation.

Abstract 208

CD38-NAD+ axis rewrites the fate of CD8+ TRMs with age

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CD8 tissue-resident memory T cells (TRM) protect barrier tissues, and their persistence depends on metabolic fitness. CD38 promotes TRM generation in several tissues, but its physiological role in lung CD8 TRM maintenance and function remains unclear. We hypothesized that the CD38-NAD axis acts as a rheostat of TRM fate: supporting establishment in youth, but under NAD stress and aging, driving excessive accumulation, heightened function, and tissue pathology. In young mice after severe influenza A virus (IAV) infection, CD38 was required cell-intrinsically for efficient lung TRM maintenance and optimal recall protection. Genetic and pharmacologic inhibition showed that this effect depended on CD38 NADase activity. To define the mechanism, purified young lung TRM cells were stimulated ex vivo and exposed to NAD+, which induced dose-dependent cell death that was enhanced by CD38 blockade. Confocal imaging revealed increased PAR/mono-ADP-ribose, γH2AX, and nuclear AIF after NAD+ exposure. With same parameters reduced by PARP inhibition. Functionally, PARP inhibition partially restored viability and reduced early and late apoptosis, whereas ARTC2.2 blockade (s+16a) had no effect, supporting an intracellular, PARP-dependent, mitochondria-linked death pathway rather than an ARTC2/P2RX7 pore mechanism. In an aged IAV model, lung CD8 TRM cells were associated with severe lung pathology and dysplastic repair after acute infection. Combined CD38 blockade and NAD■ supplementation reduced TRM numbers, attenuated tissue pathology, and improved pulmonary function. Together, these findings identify the CD38-NAD-PARP axis as a context-dependent regulator of lung TRM fate and a potential therapeutic target in age-associated chronic lung disease.

Abstract 209

Benchmarking Generative Large Language Models for de novo Antibody Design and Agentic Evaluation in Artificial Intelligence Framework

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Despite major advances in computational antibody engineering, no systematic comparison of modern open-source large language model (LLM) families for artificial and biologically meaningful antibody sequence generation exists, and it remains unclear whether architectural differences matter at compact scales. In this study, five compact transformer variants inspired by prominent open-source LLM families (Llama-4, Gemma-3, DeepSeek-V3, Mistral 7B, and NVIDIA Nemotron-3) were customized and trained from scratch for de novo VH single-domain antibody (sdAb) design. All models were pretrained on 15 million sequences from the Observed Antibody Space (OAS) database, yielding uniformly high generative fidelity: sequence diversity 0.507–0.516 (CV=0.8%), near-complete uniqueness, and novelty 0.925–0.977 (CV=2.2%). The models were then fine-tuned on disease-specific repertoires spanning SARS-CoV-2 (n=4,688), HIV (n=430), HER2 (n=22,778), and Ebola virus (n=2,868). Structural evaluation of top candidates using AlphaFold2, Boltz-2, RoseTTAFold-2, and ESMFold produced mean pLDDT scores of 92.88±1.54 to 93.77±2.16, with no statistically significant differences across architectures (Kruskal–Wallis H=2.06, p>0.05; N=100). These results suggest that, at compact scales, generative performance is driven more by training data and model size than by architectural family. In addition, docking simulations yielded predicted binding free energies of −36.34 to −65.60 kcal/mol. independent validation using IMGT CDR-H3 extraction, BLASTp novelty assessment, and NetMHCIIpan 4.3 profiling confirmed low sequence identity (0–29%), high germline consistency (77–90%), and absence of strong MHC-II binders. Finally, we introduce an agentic evaluation pipeline using the Model Context Protocol (MCP) with Claude Sonnet 4.6 for automated structural analysis and candidate prioritization.

Abstract 210

A Dual-Stream AI Framework for Multi-Perspective Antibody Functional Landscapes

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The arena of computational protein engineering stands at a genuine scientific inflection point. A recognition of AlphaFold with the Nobel Prize signaled a decisive paradigm shift, cementing AI-driven methods as foundational to modern therapeutic discovery. Significantly, this momentum has attracted multi-billion dollar industrial investment in therapeutic protein (Antibody) discovery, due to significant reductions in screening costs, and dramatically accelerated lead optimization timelines and novel development from scratch. Yet the field's ambitions outpace its current capabilities. AI driven models remain constrained by limited generalizability in computational antibody domains, addressing binding affinity prediction, protein–protein interaction (PPI) modeling, and the functional outcomes most consequential to therapeutic translation, including intracellular signaling, immune effector engagement, and broad-spectrum neutralization. This study introduces DualCrossNet, a novel dual stream parallel cross attention framework designed for antibody neutralization prediction, antibody–antigen binding affinity estimation, and protein–protein interaction (PPI) analysis. The architecture integrates generative protein language model embeddings with a custom LLaMA self-attention decoder coupled via classical cross-attention, enabling residue-level information exchange between sequence-aware and interaction-aware representations. Model performance was evaluated through five-fold cross-validation across five benchmark datasets spanning HIV, SARS-CoV-2, SAbDab, Human, and yeast PPI corpora. The system consistently surpassed prior state-of-the-art methods across most of the evaluated tasks. For HIV antibody neutralization prediction, the model achieved an AUC of 0.952 and an AUPR of 93.98%. On the SAbDab binding affinity benchmark, it attained an AUC of 0.942, specificity of 0.895, and AUPR of 0.945, with SARS-CoV-2 affinity estimation yielding an AUC of 0.821 and sensitivity of 0.836. In PPI prediction, DualCrossNet achieved a sensitivity of 0.991, specificity of 0.981, accuracy of 0.997, and AUC of 0.952 on the yeast dataset, while generalization to the human PPI benchmark produced an AUC of 0.997 and accuracy of 0.984. For mutation-driven affinity assessment, the framework outperformed established physics-based and classical attention-based baselines, including EvoEF, FoldX, and AttABseq. Overall, the approach represents a significant advance toward unified, high-accuracy computational protein engineering, accelerating AI-driven antibody discovery, enabling the slashing of current wet lab iteration cycles.

Abstract 211

Aberrant T Cell Glycosylation Drives Thymic Treg Erosion via B Cell-Derived Galectin-3 in Type 1 Diabetes

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Our previous work established that B lymphocytes erode islet-protective regulatory T cells (Tregs) in type 1 diabetes (T1D), revealing an unexpected pathogenic modality for B cells that extends well beyond their canonical roles in autoantibody production and antigen presentation. Here we define the mechanistic basis of this phenomenon and identify a therapeutically actionable axis centered on galectin-3 (Gal3).

Prior literature has documented aberrant Gal3 expression in the thymus of NOD mice, but the cellular source remained unknown. We demonstrate that B lymphocytes are the dominant source of this ectopic thymic Gal3. Concurrently, CD4 single-positive thymocytes in NOD mice display aberrant glycosylation characterized by reduced sialylation and increased terminal galactose exposure relative to healthy controls, as measured by lectin flow cytometry with MAL-II, SNA, and ECL. This glycan remodeling substantially increases ligand availability for Gal3, positioning B cell-derived Gal3 to directly engage developing Treg precursors at a critical checkpoint for lineage commitment.

Pharmacologic inhibition of Gal3 with TD139 restores potent thymic Treg output, selectively expanding Vß3+ Tregs and implicating antigen-specific rather than global Treg recovery. Together, these data reframe B cells as sculptors of the thymic tolerance landscape through a glycosylation-dependent mechanism, and position the B cell-Gal3-glycan axis as a novel therapeutic target in T1D and potentially across autoimmune disease.