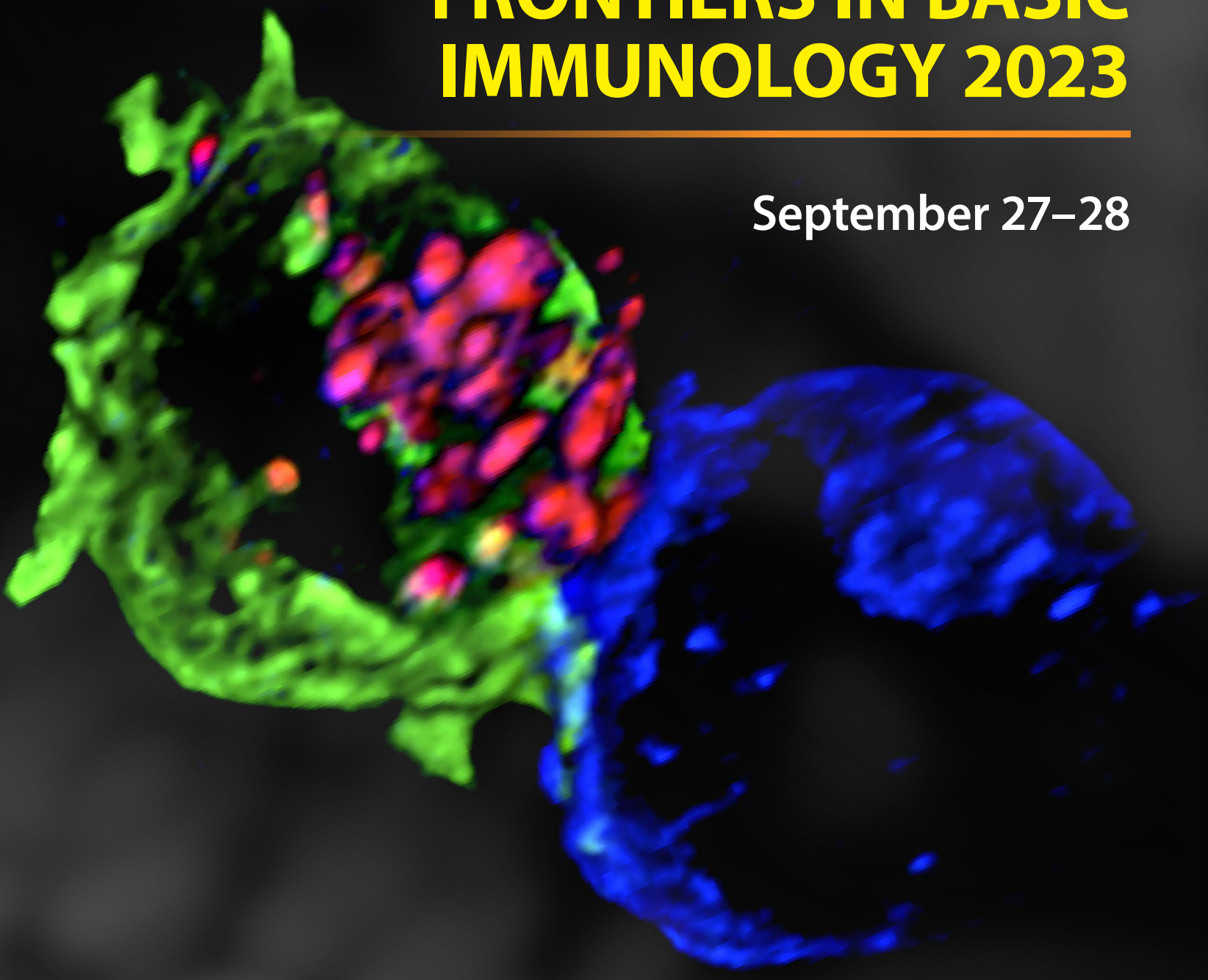
NIH NATIONAL CANCER INSTITUTE

FRONTIERS IN BASIC IMMUNOLOGY 2023



September 27–28

Building 35, Rooms 610/620/630/640
National Institutes of Health, Bethesda, MD

Sponsored by the Center of Excellence in Immunology, Center for Cancer Research, National Cancer Institute

U.S. Department of Health & Human Services | National Institutes of Health

FRONTIERS IN BASIC IMMUNOLOGY 2023

September 27–28

AGENDA

WEDNESDAY, SEPTEMBER 27, 2023

8:30 a.m.	**Welcome Remarks** Glenn Merlino, Ph.D., Scientific Director for Basic Research, Center for Cancer Research, NCI
8:40 a.m.	**Welcome Remarks** Naomi Taylor, M.D., Ph.D., Center for Cancer Research, NCI
SESSION 1	**Immune Cell Development, Thymus-Style** Chairs: Christian Mayer, Ph.D., and Stanley Adoro, Ph.D.
8:45 a.m.	**Transcriptional Determination of T Lineage Fate** Alfred Singer, M.D., Center for Cancer Research, NCI
9:05 a.m.	**CD4 T Cell Differentiation, in the Thymus and Beyond** Rémy Bosselut, M.D., Ph.D., Center for Cancer Research, NCI
9:25 a.m.	**Thymic Formation of CD8 T Cell Repertoire** Yousuke Takahama, Ph.D., Center for Cancer Research, NCI
9:45 a.m.	**The Anatomy, Regulation, and Function of Glucocorticoids Produced in the Thymus** Jonathan Ashwell, M.D., Center for Cancer Research, NCI
10:05 a.m.	**BREAK**
10:35 a.m.	**The Role of the Transcription Factor BRD4 in Thymic Development** Dinah Singer, Ph.D., Center for Cancer Research, NCI
10:55 a.m.	**Notch1 Sets the Molecular Clock for T Cell Development** Roxane Tussiwand, Ph.D., Center for Cancer Research, NCI
11:05 a.m.	**Gene Regulatory Mechanisms Establishing T-Cell Identity** Ellen Rothenberg, Ph.D., California Institute of Technology
11:30 a.m.	**LUNCH BREAK AND POSTER SESSION**
SESSION 2	**Immunity-Pathophysiology Crosstalk** Chairs: Alfred Singer, M.D., and Avinash Bhandoola, Ph.D.
12:45 p.m.	**Not Done Yet: Unexpected Facets of Complement Activities in Cell Physiology** Claudia Kemper, Ph.D., National Heart, Lung, and Blood Institute, NIH
1:05 p.m.	**Immune-Mediated Mechanisms of Adaptive and Maladaptive Tissue Responses** Shruti Naik, Ph.D., New York University

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1:30 p.m. **EGR2: Steering Pathogenic Th17 Cell Function in CNS Autoimmune Disease**
Vanja Lazarevic, Ph.D., Center for Cancer Research, NCI

1:50 p.m. **Immune Calibration of Neuroendocrine Homeostasis**
Chuan Wu, M.D., Ph.D., Center for Cancer Research, NCI

2:10 p.m. **Neuroimmune Interactions in Enteric Nervous System**
Vijay Kuchroo, DVM., Ph.D., Harvard Medical School

2:35 p.m. **BREAK**

SESSION 3 **Cancer Immunology: Part 1**
Chairs: Pamela L Schwartzberg, M.D., Ph.D. and Hyun Park, Ph.D.

3:05 p.m. **The Role of the Intestinal Microbiome in Cancer Immunotherapy**
Marcel van den Brink, M.D., Ph.D., Memorial Sloan Kettering Cancer Center

3:30 p.m. **Understanding Microbiota Regulation of the Tumor Microenvironment and Response to Cancer Therapy**
Romina Goldszmid, Ph.D., Center for Cancer Research, NCI

3:50 p.m. **Turning the Table on Oncogenic Herpesviruses: Vaccination with a Gammaherpesvirus with Targeted Mutations in Genes Required for Replication and Latency Protects Against Wild-Type Virus Pathogenesis in the Host**
Laurie Krug, Ph.D., Center for Cancer Research, NCI

4:10 p.m. **Discovery of Novel Epigenetic Mechanisms that Control T Cell-Mediated Tumor Immunity**
Kai Wucherpfenning, M.D., Ph.D., Dana-Farber Cancer Institute

THURSDAY, SEPTEMBER 28, 2023

SESSION 4 **Innate Immunity**
Chairs: Romina Goldszmid, Ph.D., and Vanya Lazarevic, Ph.D.

8:30 a.m. **Innate Lymphoid Cells: Development, Differentiation, and Dynamics**
James Di Santo, M.D., Ph.D., Pasteur Institute

8:55 a.m. **Different Developmental Pathways Generate Functionally Distinct Populations of Natural Killer Cells**
Avinash Bhandoola, M.B., B.S., Ph.D., Center for Cancer Research, NCI

9:15 a.m. **The Cytokine Receptor DR3 Controls iNKT Cell Immunity**
Hyun Park, Ph.D., Center for Cancer Research, NCI

9:35 a.m. **Memory Responses by Innate T Cells**
Mitchell Kronenberg, Ph.D., La Jolla Institute for Immunology

10:00 a.m. **Regulation of Barrier Immunity**
David Artis, Ph.D., Weill Cornell Medical School

10:25 a.m. **BREAK**

SESSION 5 **Immune Cell Signaling**
Chairs: Laurie Krug, Ph.D., and Jonathan Ashwell, M.D.

10:55 a.m. **Dynamic Membrane Changes Controlling TCR Signaling**
Gillian Griffiths, FMedSci, FRS, Cambridge University

11:20 a.m. **Apoptosis and B Lymphocyte Repertoire Selection**
Christian Mayer, Ph.D., Center for Cancer Research, NCI

AGENDA (CONTINUED)

11:40 a.m.	**Defining the Role of Lck-Coreceptor Association in T Cell Receptor Signaling and Fate Specification** Nicole La Gruta, Ph.D., Monash University
12:05 p.m.	**Formation of the Tetrameric Protein Complex Containing Phospholipase-Cγ1 During T Cell Activation** Lawrence Samelson, M.D., Center for Cancer Research, NCI
12:25 p.m.	**LUNCH BREAK AND POSTER SESSION**
SESSION 6	**Immune Cell Function** Chairs: Daniel McVicar, Ph.D., and Chuan Wu, M.D., Ph.D.
1:40 p.m.	**PI3 Kinase and Balancing T Cell Activation Under Exhaustion** Pamela Schwartzberg, M.D., Ph.D., National Institute of Allergy and Infectious Diseases, NIH
2:00 p.m.	**A Proteostasis Link to Cell Autonomous T Cell Dysfunction** Stanley Adoro, Ph.D., Center for Cancer Research, NCI
2:20 p.m.	**Nutrient-Driven Proliferation in Mucosal Germinal Centers** Jagan Muppidi, M.D., Ph.D., Center for Cancer Research, NCI
2:40 p.m.	**BREAK**
SESSION 7	**Cancer Immunology: Part 2** Chairs: Dinah Singer, Ph.D., and Jagan Muppidi, M.D., Ph.D.
3:10 p.m.	**LAG3: The Third Checkpoint Inhibitor** Dario Vignali, Ph.D., University of Pittsburg
3:35 p.m.	**A Role for Acod1-Mediated Itaconate in Cancer and Metabolic Disease** Daniel McVicar, Ph.D., Center for Cancer Research, NCI
3:55 p.m.	**Steering Cancer Immunotherapies in the High-Dimensional Space of Leukocyte Activation** Grégoire Altan-Bonnet, Ph.D., Center for Cancer Research, NCI
4:15 p.m.	**Metabolic Crosstalk in T Lymphocyte Effector Function** Naomi Taylor, M.D., Ph.D., Center for Cancer Research, NCI
4:35 p.m.	**Closing Remarks**

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Session I

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PP 01

A NON-CANONICAL ROLE FOR CASPASE-1 IN CONTROLLING ANTIMICROBIAL RESISTANCE OF INTRACELLULAR SALMONELLA

AKHADE AS[1], MOSQUERA GV[1], ARRIETA-ORTIZ ML[1], CHEVANCE FFV, KAUR A[1], PETERSON EJR[1], BALIGA NS[1], HUGHES KT[2], and SUBRAMANIAN N[1,3]

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Caspase-1 is a key effector molecule involved in inflammasome activation and has a well-established role in restricting the growth of pathogens by triggering a form of cell death called pyroptosis. Here we reveal a non-canonical, cell death-independent role for caspase-1 in controlling the transcriptional state and drug resistance of an intracellular pathogen. Using Pathogen-sequencing, a method for sensitive transcriptional profiling of minuscule numbers of intracellular bacteria from infected macrophages, we show that host caspase-1 decreases the resistance of intracellular *Salmonella* to endogenous cationic antimicrobial peptides, and to a cationic polypeptide antibiotic used as a last-line drug in Gram-negative bacterial infections. These effects of caspase-1 were independent of its enzymatic activity but dependent on its ability to repress the activation of a two-component signal transduction system in intracellular bacteria. These effects were also independent of caspase-11. Our data suggest an activity and inflammasome-independent role for caspase-1 later in infection in restricting intracellular *Salmonella* which evade initial inflammasome-dependent restriction by caspase-1. Our findings thus take host caspase-1 beyond the well-studied inflammasomes and tie it to signal transduction and drug resistance of an intracellular pathogen with possible implications for host-directed therapy to combat antimicrobial resistance.

PP 02

THE OXIDOREDUCTASE CLIC4 IS REQUIRED TO MAINTAIN MITOCHONDRIAL AND PHAGOCYTIC FUNCTIONS IN BONE MARROW DERIVED MACROPHAGES

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[2]Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Center, National Institutes of Health. Bethesda, MD, USA.

The Chloride Intracellular Channel-4 (CLIC4) is one of six highly conserved proteins in the CLIC family that share high structural homology with glutathione-S-transferase (GST)-omega in the GST superfamily. While CLIC4 is a multifunctional protein that resides in multiple cellular compartments, the discovery of its enzymatic glutaredoxin-like activity in vitro suggested that it could be one of the essential oxidoreductases in cells. Here, we found that the C57BL/6 males that are 3 months of age and older are smaller in size and have a significant splenomegaly characterized by larger white pulp in comparison to spleens from wildtype males.

The bone marrow derived macrophages (BMDMs) from C57BL/6 males were found to contain high reactive oxygen species (ROS), have decreased mitochondrial oxygen consumption rate and a decreased phagocytosis function of cells post-stimulated with LPS and IFNg or both for 6 and 24 hours.

In the absence of CLIC4, however, LPS and IFNg decreased phagocytosis was associated with high expression level of the negative regulator of mitochondrial ROS, UCP2 and lower expression of many highly essential cytokines for macrophages activation or polarization signaling. Furthermore, transcriptomic profiling of BMDMs post-stimulated with LPS and IFNg revealed a time dependent downregulation of genes associated with many pathways including interferons alpha and gamma responses, oxidative phosphorylation, inflammatory responses as well as TLR4 signaling. Also, the secretion of inflammatory cytokines and chemokines were found to decrease in Clic4-KO BMDMs with time.

Our current results highlight a critical role for CLIC4 in maintaining redox-homeostasis and mitochondrial functions in BMDMs as well as macrophages signaling pathways. The exact mechanism of how CLIC4 exerts its function is yet to be fully elucidated. To date, our findings raise the possibility of targeting CLIC4 to induce macrophage hypophagia as a mechanism of innate immune exhaustion to improve some essential therapeutic outcomes for inflammatory diseases and related cancers.

PP 03

MPER DIRECTED BROADLY NEUTRALIZING ANTIBODIES TOWARDS HIV-1

ARORA J[1,2], DONOFRIO G[1,2], SANKHALA R[2,3], DUSSUPT V[1,2], THOMAS P[1,2], DORIA-ROSE N[4], MENDEZ-RIVERA L[1,2], SMITH L[1,2], SLIKE BM[1,2], WIECZOREK, L[1,2], POLONIS VR[1], VASAN S[1,2], AKE J[1], JOYCE MG[2,3] AND KREBS SJ[1]

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Producing broadly neutralizing antibodies (bNAbs) is a major goal for HIV-1 vaccines due to their ability to neutralize multiple strains of HIV by blocking virus engagement with the target cell. bNAbs against HIV-1 target several sites of vulnerability on the envelope, including the conserved membrane proximal external region (MPER). Highly potent bNAbs, such as VRC42, PGZL1 and 4E10, directed against MPER have been isolated from several HIV-1 infected patients. Here, we report the isolation and characterization of MPER-specific bNAbs derived from a chronically infected untreated cohort donor, RV149.1027. Plasma from this donor was able to neutralize 100% of a panel of 34 heterologous viruses. Neutralization fingerprinting analysis revealed that neutralizing antibodies had a predicted specificity targeting MPER. Using probes, MPER-specific single B cells and antigen-negative B cells (CD19+/IgD-/IgM-) were sorted from donor RV149.1027 by flow cytometry. Sorted B cells were cultured in 384-well plates and supernatant was tested for neutralization. B cell receptors (BCRs) were sequenced from cells demonstrating potent neutralization and were subsequently cloned into expression vectors for monoclonal antibody (mAb) production. Antibodies were characterized for binding, epitope mapping, and structural studies. Two bNAbs, MHRP01 and MHRP02, were confirmed to show specificity for MPER as they neutralized HIV-2 chimeric viruses that carry HIV-1 MPER. MHRP01 has striking similarities to 4E10, VRC42, and PGZL1, including identical heavy/light chain gene usage, high potency (IC_{50}<10ug/ml), >96% neutralization breadth, and binding to MPER using similar critical residues. As these MPER-directed mAbs were isolated from several individuals infected with different HIV-1 subtypes, MPER represents a common epitope which has the potential to elicit similar B cell responses in unrelated patients, making it an enticing target for vaccine design.

PP 04

APPLICATION OF METABOLOMIC ANALYSIS TOWARDS THE DISCOVERY OF BIOMARKERS OF IMMUNOGENECITY AND EFFICACY OF PARASITIC VACCINES

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Leishmaniasis is a neglected tropical disease caused by *Leishmania* parasites which is prevalent in approximately 88 countries, yet no licensed human vaccine exists against it and treatment options remain limited. Towards control of leishmaniasis, we have developed *Leishmania major Centrin* gene deletion mutant strains (*LmCen-/-*) as a live attenuated vaccine, which induces a strong Th1 response to provide IFN-γ-mediated protection to the host. However, the immune mechanisms of such protection remain to be understood.

Metabolic reprogramming of the host cells following *Leishmania*-infection has been shown to play a critical role in pathogenicity. Therefore, our goal was to study the metabolic changes associated with the *LmCen*$^{-/-}$ strain to identify the immune mechanism of protection and biomarkers of immunogenicity

C57/BL6 mice were infected with wild type *L. major* (*LmWT)*and *LmCen*$^{-/-}$. The infected ear tissues were collected 7 days post infection and analyzed by untargeted LC/MS mass spectrometry, and the data were analyzed with the Metaboanalyst 5.0 for pathway analysis and Metscape 3.1.1 for integrative network analysis. To verify the results from MS analysis, murine bone marrow-derived dendritic cells, were infected with *LmWT* and *LmCen*$^{-/-}$. BMDCs were cultured with inhibitors or agonists of tryptophan metabolism, and the expression levels of genes of interest were measured via qRT-PCR.

Our results show that distinct metabolic reprogramming occurs in the host cells infected with virulent or live attenuated *Leishmania* parasites. We have identified that Tryptophan metabolism is differentially regulated between the *LmWT* infection and *LmCen*$^{-/-}$ immunization. The *LmWT* infection promotes the anti-inflammatory Kynurenine-AhR and FICZ-AhR signaling, while the *LmCen*$^{-/-}$ immunization uses tryptophan for the increased synthesis of the pro-inflammatory mediator, melatonin.

Application of metabolomic analysis to vaccine studies identified immune mechanisms of protection or pathogenicity and may help identify novel biomarkers of vaccine efficacy of a live-attenuated vaccine candidate for Cutaneous Leishmaniasis.

PP 05

ARGININE SENSOR CASTOR1 MEDIATES COLON EPITHELIAL HOMEOSTASIS AND REPAIR IN COLITIS BY REGULATING INTERLEUKIN IL-6/STAT3-MEDIATED INFLAMMATION

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Cytosolic arginine sensor for mTORC1 subunit 1 (CASTOR1) is a newly discovered arginine sensor, which negatively regulates mTORC1 activity. We have previously shown that CASTOR1 is tumor suppressive in KSHV-induced cellular transformation and breast cancer by regulating the mTORC1 activity and cell proliferation. However, the role of CASTOR1 in immune response and inflammation has not been investigated before. In this study, we generated a CASTOR1 knockout mouse model and used it to investigate the role of CASTOR1 in acute inflammatory bowel disease (IBD). We found that mice with CASTOR1 knockout were resistant to body weight loss, maintained intact intestinal barriers, and showed increased cell proliferation and decreased epithelial apoptosis during dextran sulfate sodium (DSS)-induced intestinal epithelial injury and acute colitis. Mechanistically, CASTOR1 knockout promoted intestinal crypt proliferation and regeneration by inducing interleukin-6-associated reparative inflammation and STAT3 activation. Consequently, treatment with berberine chloride induced mTORC1 activation and relieved IBD impairment while treatment with rapamycin exacerbated acute inflammation. Furthermore, recombinant IL-6 protein suppressed inflammatory immune response while stattic blocked IL-6/STAT3 signaling and exacerbated inflammation in DSS indued colitis mice. Together, these findings provided evidence that CASTOR1 deficiency and activation of mTORC1 could prevent and block intestinal inflammatory disorders. Our results indicate that CASTOR1 likely contribute to DSS-induced colitis, and that activation of the mTORC1 pathway could facilitate colon epithelial regeneration by activating the IL-6-STAT3 pathway, thus providing a new target for the treatment of colitis-related diseases.

PP 06

CLINICAL AND FUNCTIONAL SPECTRUM OF RAC2-RELATED IMMUNODEFICIENCY

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Mutations in the small Rho-family GTPase, RAC2, critical for actin cytoskeleton remodeling and intracellular signal transduction, have been associated with neonatal severe combined immunodeficiency (SCID), infantile neutrophilic disorder resembling leukocyte adhesion deficiency (LAD), and later onset atypical SCID.

We investigated a cohort of 54 RAC2 patients (23 previously reported) from 37 families. Data were collected from referring physicians and literature reports with updated clinical information. Patients were grouped by presentation: neonatal SCID (n=5), infantile LAD-like disease (n=5), or atypical SCID (n=44).

Disease correlated to RAC2 activity: Constitutively active, RAS-like mutations caused neonatal SCID, dominant negative mutations caused LAD-like disease, while activating mutations caused atypical SCID. Significant T- and B-lymphopenia with low immunoglobulins were seen in most patients; myeloid abnormalities included neutropenia, altered oxidative burst, impaired neutrophil migration and visible neutrophil macropinosomes. Among 42 atypical SCID patients with clinical data, upper and lower respiratory infections and viral infections were common.

Twenty-three distinct *RAC2* mutations, including 15 novel variants were identified. Using heterologous expression systems, we assessed downstream effector functions including superoxide production, PAK1 binding, AKT activation, and protein stability. Confocal microscopy showed altered actin assembly evidenced by membrane ruffling and macropinosomes. Altered protein localization and aggregation were observed.

All tested RAC2 mutant proteins exhibited aberrant function. Most mutants produced elevated superoxide; mutations unable to support superoxide formation were associated with bacterial infections. *RAC2* mutations result in immune dysfunction causing a spectrum of disease ranging from early-onset severe combined to later onset combined immunodeficiencies depending on RAC2 activity.

PP 07

MICROBIOTA INDUCED HUMORAL IMMUNITY IS DRIVEN BY AUTONOMOUS SKIN-INTRINSIC GERMINAL CENTERS

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The skin is one of the largest barrier sites which harbors a plethora of microorganisms. Whether skin microbiota promotes humoral responses remains unknown. Here we demonstrate that colonization with common skin commensal *Staphylococcus epidermidis* profoundly modulates cutaneous immunity that results in local and systemic responsiveness with antigen-specific IgG antibodies. Such response is uncoupled from inflammatory signals and is associated with the induction of dermal tertiary lymphoid organs that closely mimic classical germinal centers. Of note, such responses occur in a tissue-autonomous manner as evidenced by the preserved IgG2b/c phenotype in mice deficient in professional lymphoid organs. Our work proposes a model by which commensal specific antigens are collected in the hair follicle leading to the Langerhans cell-dependent generation of cutaneous T follicular helper cells via local Treg plasticity that further expands the skin B cell pool and facilitates IgG production. This mechanism is central to the host's ability to control local cutaneous bacterial burden. It also protects against systemic infection with the same microbe demonstrating the importance of this line of defense in maintaining barrier integrity. Collectively, our observations highlight the autonomous potential of skin organizational flexibility and for the first time demonstrate that B cells represent an indispensable local cutaneous compartment that actively maintains skin immunity.

PP 08

AN ANERGIC-LIKE TRANSCRIPTIONAL PROFILE AND EXPANSION OF CLONOTYPES ASSOCIATED WITH AUTOIMMUNITY OBSERVED IN PERIPHERAL B CELLS FROM HCV-ASSOCIATED B CELL NON-HODGKIN'S LYMPHOMA PATIENTS

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Chronic hepatitis C virus (HCV) infection remains a global health issue, with its role in B cell lymphoproliferative disorders, including B cell non-Hodgkin's lymphoma (BNHL), of increasing concern. Epidemiological and clinical evidence strongly support a causal role of chronic HCV infection in the pathogenesis of BNHL; however, the molecular mechanisms underlying this association are poorly understood. Likely, chronic HCV infection contributes to lymphomagenesis via indirect and/or direct mechanisms. The former is achieved via chronic BCR and/or CD81 stimulation and the latter via viral infection of B cells. To help elucidate this relationship, we performed RNA-sequencing on peripheral B cells collected from chronic HCV-infected patients with or without BNHL, as well as BNHL-only patients and healthy controls. In peripheral B cells from HCV/BNHL patients, we observed enrichment of a transcriptional signature associated with B cell anergy. This anergic-like transcriptional phenotype included overexpression of inhibitory receptors, pro-apoptotic genes, and BCR signaling inhibitors, as well as enrichment of anergic-like gene sets identified in autoimmune- and viral-associated anergic B cells. Furthermore, BCR repertoire analysis of our RNA-seq data identified significant clonal expansion in peripheral B cells from HCV/BNHL patients, with clonotype frequencies ranging from 9.6% to 73%, and we identified 7 expanded clonotypes corresponding to 6 Ig variable gene loci. Specific variable gene usage for expanded clones included Ig genes whose encoded BCRs have been associated with viral- and non-viral-related lymphoma, autoimmunity, and autoreactivity, including *IGKV3-20*, *IGKV3-15*, and *IGHV3-48*, as well as Ig genes with few or no known association with lymphoproliferative or autoimmune disorders. We also observed a strong positive correlation between differentially expressed epigenetic regulatory genes and degree of clonal expansion, suggesting epigenetic regulation may be involved in B cell anergy. Our data support an indirect mechanism of action in HCV-associated BNHL and suggest that dysregulation of B cell anergy, in a manner similar to that observed in autoimmunity, may play a role. Studies are underway to further interrogate peripheral clonal expansion via BCR-sequencing, and to determine if clonal expansion and transcriptional signatures observed in the periphery are recapitulated in matched lymphoma tissue. We believe these results have implications not only for viral-associated lymphomagenesis, but may also shed light on the biological mechanisms regulating B cell anergy.

PP 09

LUNG CANCER IMMUNOREGULATION: UNIQUE ROLE OF CD8+ T CELLS AND IFN-γ

KRATZMEIER CM[1,2], KHALIL MA[1], LIM I[1] , AHUJA HK[1] , BANERJEE A[1] , and KRUPNICK AS[1,2]

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The lungs provide a unique immunoregulatory environment for CD8+ T cell biology when compared to other organs. Therefore, we hypothesize that immunoregulation of lung cancer by CD8+ T cells differs from that of other malignancies. To investigate this hypothesis, we compared two primary carcinogenesis models of 3-MCA-induced fibrosarcoma and urethane-induced lung cancer in B6 wild type or CD8+ T cell deficient mice on a B6 background (B6CD8-/-). The absence of CD8+ T cells increased sarcoma growth (199±65 vs. 511±138 mm3 for B6 vs B6CD8-/- mice respectively p=.03) but decreased the growth of lung cancer (1.7±0.2 vs. 0.6±0.13 cm3 for B6 vs B6CD8-/- mice respectively p=.006). We then evaluated multiple other tumor models including B16 melanoma, EG7 lymphoma, and LLC lung cancer to validate our initial findings. For all non-lung cancer tumors, the presence of CD8+ T cells decreased tumor growth, whereas the presence of CD8+ T cells accelerated lung cancer growth. Cytokine analysis of the tumor beds revealed a significant increase of Th-1 polarizing cytokines, IFN-γ and TNF-α, in lung cancer-bearing but not B16 melanoma-bearing mice in the presence of CD8+ T cells. To support this finding in the context of human malignancies, gene expression analysis mined from The Cancer Genome Atlas Program (TCGA) revealed increased expression of IFN-γ in patients with lung cancer in comparison to patients with other types of cancer including pancreatic and colon cancer. Furthermore, we found that the majority of IFN-γ producers in the tumor bed and surrounding tissue of LLC-bearing mice are the tumor cells themselves, whereas the IFN-γ producing cells in B16-bearing mice consisted of host immune cells. Neutralization of IFN-γ and TNFα resulted in an increase in B16 melanoma tumor growth, which follows the canonical role of type I cytokines in controlling the growth of malignancies. For LLC lung cancer, however, neutralization of IFNγ and TNF-α led to a decrease in tumor growth suggesting a tolerogenic role for these cytokines. In addition, we observed in the tumor infiltrating lymphocytes of LLC-bearing mice an IFN-γ and TNF-αdependent expansion of CD8+Foxp3+ T cells which was not present in melanoma-bearing mice. Therefore, and contrary to the accepted dogma, we believe that an increase in Th-1 polarizing cytokines, mediated by lung cancer associated CD8+ T cells, facilitates the growth of this malignancy. Our data suggests that immunoregulation of lung cancer is unique and may require novel immunomodulating strategies that may not necessarily apply to other malignancies.

PP 10

POSTPRANDIAL CHANGES TO SYSTEMIC METABOLISM IMPRINT DURABLE CHANGES ON T CELL IMMUNE RESPONSES

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While costimulatory and cytokine signals can shape an antigen-stimulated T cell immune response, it is now clear that nutrient availability and intrinsic metabolic pathways play key roles in T cell function and fate. Indeed, the effects of post-prandial metabolism on T cell function and fate are unstudied. Here we show that short periods of fasting and refeeding can have long-lasting effects on T cell immunity. Directly ex vivo, T cells from fed hosts have higher mitochondrial capacity and volume compared to T cells from fasted hosts. Remarkably, these metabolic phenotypes persist after activation and 7 days of expansion in vitro. Further, when OT-I naïve T cells from congenically mismatched fasted and fed mice were co-transferred into Vaccinia^{OVA}-infected hosts, fasted T cells failed to fully engage an effector response and formed dramatically fewer memory T cells after viral clearance. Metabolomic profiling of serum revealed serum triglycerides as a likely culprit for postprandial metabolic reprogramming. Chylomicrons enriched from lymphatics of fed mice were sufficient to impart metabolic reprogramming on fasted T cells in a manner antagonized by the chylomicron protein apoCIII. T cells from LDL receptor (LDLR)-deficient mouse had the metabolic phenotype of fasted T cells and were insensitive to fed serum, confirming the role for triglyceride uptake in post-prandial T cell metabolic programming. Therapeutic T cells expanded in vitro from fasted mice failed to control tumor growth compared to those expanded from fed mice. Our data suggest that T cells are exquisitely sensitive to systemic metabolic changes in the postprandial period and inherit those metabolic programs for several generations. Further, our study highlights the need to consider diet content and timing as key factors in immunology, in immune cell analysis, vaccination strategies, and the generation of cellular therapies for disease including cancer.

PP 11

DEATH RECEPTOR 3 IS A COSTIMULATORY MOLECULE FOR NATURAL KILLER T CELLS

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Invariant NKT (*i*NKT) cells are a small population of thymus-generated T lymphocytes with innate-like phenotype and function that play a disproportionally important role in immune regulation and surveillance. *i*NKT cells are generated upon strong agonistic TCR signaling by glycolipid-loaded CD1d molecules, which differs from conventional T cells that are positively selected by weak TCR engagement with peptide/MHC. Whether *i*NKT cells are also distinct in their costimulatory requirement, however, has remained largely unresolved. Here, we report that the TNF superfamily receptor death receptor 3 (DR3) plays a previously unappreciated costimulatory role in peripheral *i*NKT cell activation. We found that the co-injection of agonistic anti-DR3 antibodies significantly augmented the activation and proliferation of mature *i*NKT cells in α-GalCer injected mice. Moreover, *i*NKT cells of α-GalCer and anti-DR3 co-injected mice dramatically increased their expression of proinflammatory cytokines, resulting in severe thymic atrophy. The DR3 costimulatory effect was strictly dependent on *i*NKT cells, because *i*NKT-deficient *Traj18*$^{-/-}$ mice were refractory to α-GalCer and anti-DR3 costimulation-induced inflammation. Because DR3 ligation alone did not suffice to activate *i*NKT cells, and DR3 exerted its effects only in the context of TCR costimulation, these results strongly support DR3 being a new *bona fide* costimulatory molecule for *i*NKT cells. Considering the interest in utilizing *i*NKT cells in adoptive T cell therapies and as anti-tumor bioreagents, employing DR3 as a tool to boost *i*NKT cell immunity will open new venues in translational and clinical research.

PP 12

HLA CLASS I SIGNAL PEPTIDE POLYMORPHISM INFLUENCES NK CELL FUNCTION

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Natural killer (NK) cells play a crucial role in the innate immune system, and their responses are regulated by various activating and inhibitory receptors. Expression of inhibitory receptors that recognize self-HLA class I molecules prevents auto-reactivity of NK cells and promotes their education, making them functionally competent. CD94/NKG2A is a dimeric inhibitory receptor expressed by subsets of NK cells that binds nonclassical HLA class I molecule HLA-E. HLA-E is expressed by most cells in humans and presents epitopes derived from signal peptides (SPs) of classical HLA class I molecules. Using in vitro assays, we have recently shown that both HLA-E expression levels and binding to CD94/NKG2A are influenced by HLA class I SP polymorphism. Based on these findings, we hypothesized that SP polymorphism may influence NK cell education levels and contribute to inter-individual variation in immune responses. To test this, we performed a "missing-self" assay, in which PBMCs isolated from healthy donors (N=230) were mixed with HLA class I negative K562 cells. NK cell activities were evaluated by flow cytometrical measurements of the CD107a degranulation marker. Analysis of HLA genotypes showed an association between the presence of certain common HLA-A and HLA-C SP variants with the degranulation levels of their $CD56^{bright}$ NK cells. $CD56^{bright}$ cells express high levels of CD94/NKG2A as compared to the rest of blood NK cells and therefore may exhibit higher sensitivity to variation in CD94/NKG2A-HLA-E engagement levels. There was a positive correlation between degranulation in the primary $CD56^{bright}$ NK cells and NKG2A reporter activity against BLCLs when matching for SP genotype. Our data suggest that HLA class I SP polymorphism can differentially contribute to CD94/NKG2A-mediated NK cell education, which ultimately affects their immune response in vivo.

PP 13

NEW PRE-CLINICAL BRAIN METASTASIS MODELS TO INTERROGATE THE ROLE OF THE MICROENVIRONMENT IN IMMUNOTHERAPY EFFICACY.

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Brain metastases (BrM) remain an intractable, deadly complication for advanced melanoma patients. Although immune checkpoint blockades (ICB) have recently shown promising results, only a small subset of melanoma BrM patients benefited from this approach and efficient therapeutic strategies are sTIll desperately needed. Furthermore, detailed studies addressing the contribution of the BrM immune tumor microenvironment (TME) to therapy response are lacking, hindering the identification of resistance mechanisms and potential new targets. Access to BrM paTIent samples is challenging, underscoring the importance of preclinical models to study therapeutic responses. Unfortunately, appropriate immunocompetent preclinical models are scarce, and in most either the immune system is compromised or the brain-blood-barrier (BBB) is artificially disrupted. To address these limitations, here, we developed two novel isogenic immunocompetent melanoma BrM models. These models exhibit a high and stable incidence of BrM without disrupting the BBB through local interaction and display the varied responses to ICB observed in the clinic. Single-cell RNA sequencing and high-parametric spectral flow cytometry of untreated BrM TME revealed a high diversity of T cell subsets and high infiltraTIon of CD8 T cells in the ICBresponsive model (BR1) while neutrophils were enriched in the ICB-resistant model (BR3). Importantly, we uncovered disTInct microglia populations exclusively present in ICB-sensitive BR1 BrM that upregulated genes encoding for T cell-attracting chemokines and antigen presentation. Notably, these microglia positively correlated with T-cell infiltraTIon. Network and cell-cell interaction analysis confirmed the crosstalk between BR1-associated microglia and T-cell subsets. Post-ICB treatment comparative studies highlighted beneficial CD8 T cells and microglia changes induced by ICB uniquely in the responsive BR1. We confirmed the translational relevance of our preclinical models and showed that the specific immune populations present in their TME can also be found in the few recent publicly available datasets from treatment-naïve melanoma BrM patient lesions. To the best of our knowledge, this is the first study characterizing melanoma BrM TME immune composition and their ICB-induced changes in responsive and non-responsive pre-clinical models. Our unique BrM models, mirroring the BrM TME and diversity of ICB response observed in patients, provide a robust plagorm for the much-needed mechanistic studies to optimize BrM therapy.

PP 14

ATTENUATED GERMINAL CENTER (GC) REACTIONS IN SPHINGOSINE-1-PHOSPHATE RECEPTOR 4 (S1PR4) KNOCKOUT MICE STILL PRODUCE HIGH-AFFINITY ANTIBODIES DESPITE SEVERE DEFICIENCIES

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Sphingosine-1-Phosphate (S1P) is a signaling lipid that exists in strict biological gradients to direct vital homeostatic functions including lymphocyte re-circulation. In addition, S1P supports processes during immune activation, such as retention of germinal center (GC) B cells. While these examples are controlled through other well-studied members of the S1P receptor (S1PR1-5) family, little is known about the role of S1PR4 in immunity, despite its high expression on hematopoietic cells.

In a T_H1 footpad immunization model, S1PR4-KO mice mounted attenuated immune responses and developed visibly smaller draining lymph nodes (dLN). Histological and flow cytometric analysis revealed key populations, including T follicular helper (T_{FH}) and GC B cells, were strikingly diminished in S1PR4-KO mice. Since GC reactions are considered the main mechanism underlying antigen (ag)-specific antibody production, we evaluated the humoral response and found similar levels of total and ag-specific IgG isotypes. This was unexpected because the diminished S1PR4-KO GC B cell population had also developed among fewer T_{FH} cells to facilitate affinity maturation and isotype switching. We hypothesize, however, that reducing the ag availability at immunization will challenge the compromised S1PR4-KO GC reactions and reveal differences in the quality of antibodies produced.

In searching for a mechanism, we found that S1PR4-KO mice lacked the early influx of naïve lymphocytes through the dLN, an important step for seeding GCs with ag-specific B and T cells. Because innate immune cells play an acute role in this initial recruitment, we investigated the blood soon after challenge and observed a neutrophil deficiency in S1PR4-KO mice. Through on-going studies, we are working to tie this inflammation-induced neutropenia to the decreased GC response as well as vascularity deficiencies observed in the absence of S1PR4.

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PP 15

3LYSOPHOSPHATIDYLCHOLINE MEDIATES NEUTROPHIL ACTIVITY THROUGH EARLY METABOLIC MODULATION FOLLOWING IMMUNIZATION WITH A LIVE-ATTENUATED *LEISHMANIA* VACCINE

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Leishmaniasis, caused by *Leishmania* parasites, spreads via sandfly vectors and blood transfusions. We are evaluating a *centrin*-deleted *Leishmania major* (*LmCen*$^{-/-}$) parasite as a live attenuated vaccine that has shown safety and efficacy against challenge with wild type *L. major* (*LmWT*) and *L. donovani* in animal models. Differences in immunogenicity between *LmCen*$^{-/-}$ and *LmWT* infections exist; notably, neutrophil-mediated pathogenicity reported in virulent infections is absent in *LmCen*$^{-/-}$ infections. Metabolic regulation that may underlie neutrophil recruitment and functionality remains unexplored. Therefore, we analyzed metabolic reprogramming in neutrophils and their role in *LmCen*$^{-/-}$ immune protection.

C57Bl/6 mice were intradermally infected with *LmWT*^{mCherry} or *LmCen*$^{-/-mCherry}$. 48 hours post-infection, 2.5-3x10^6 parasitized and non-parasitized neutrophils were sort-selected from draining lymph nodes (dLNs). Untargeted metabolomic analyses were performed on neutrophils by mass spectrometry. Neutrophil migration to dLNs was measured via flow cytometry at 24, 48, and 72 hours post-intradermal inoculation with *LmWT* and *LmCen*$^{-/-}$. Transwell assays were performed on parasitized neutrophils to measure migration in the presence and absence of exogenous LysoPC. Simultaneously, in vitro phagocytosis and migration were measured by flow cytometry. Neutrophil activation with and without LysoPC supplementation was measured in the ear and dLNs via flow cytometry.

Mass spectrometry revealed significant enrichment of the bioactive lipid, lysophosphatidylcholine (LysoPC) in neutrophils isolated from *LmCen*$^{-/-}$-infected mice compared to naïve or *LmWT*-infected mice. Peak neutrophil influx to dLNs occured at 48 hours post-infection. Transwell assays revealed enhanced neutrophil migration in presence of exogenous lysoPC in both *LmWT* and *LmCen*$^{-/-}$ infections. In vitro infection of neutrophils revealed increased phagocytic cells and migrated neutrophils measured by LFA-1 expression in the *LmCen*$^{-/-}$ group compared to *LmWT*. LFA-1 expression was enhanced in the *LmCen*$^{-/-}$ group supplemented with LysoPC. In addition, neutrophils in the *LmCen*$^{-/-}$ group expressed greater CXCR2 in the ear, and greater CXCR2 and CXCR4 in the dLNs, compared to *LmWT* when supplemented with LysoPC.

LysoPC enrichment in neutrophils from *LmCen*$^{-/-}$ parasites may promote immune protection following immunization. Further investigating the functional role of neutrophils and the activity of LysoPC in modulating immune responses could aid in the discovery of novel metabolic immune mechanisms and biomarkers of vaccine-induced immunity.

PP 16

CHRONIXC IFN-γ INDUCES PHENOTYPICAL AND FUNCTIONAL CHANGES IN $CD3^+CD4^-CD8^-NK1.1^-$ "DOUBLE NEGATIVE" T-CELLS IN FEMALE MICE WITH REPRODUCTIVE DYSFUCNTION

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Abstract not included by request of author.

PP 17

MURINE CD4+ T CELLS EXHIBIT SEXUALLY DIMORPHIC RESPONSES TO ESTROGEN SIGNALING

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Many autoimmune diseases exhibit sexual dimorphism. 17b-estradiol (estrogen, "E2"), a steroid sex hormone primarily known for its reproductive roles, has also been shown to modulate the phenotype and function of CD4+ T cells. E2 signals through two nuclear receptors, ERa and ERb, which regulate gene transcription through direct DNA binding and other non-genomic mechanisms. We previously showed that in the SAMP/YitFC model of Crohn's-like ileitis, loss of ERb enhanced inflammation selectively in female mice. Additionally, our previous studies showed that loss of ERb resulted in decreased expression of Foxp3 in CD4+ T cells, together suggesting a pro-inflammatory role for ERα and anti-inflammatory role for ERb, potentially in a sex-specific manner.

Using the CD45RB T cell transfer model of colitis, we tested the pathogenicity of CD4+ T cells lacking expression of ERa vs. ERb. Based on prior findings supporting a proinflammatory role for ERa in T cells, we hypothesized that skewing signaling in favor of ERb (through deletion of ERa) would prevent or improve experimental colitis. However, recipients of ERa-KO cells developed more severe disease compared to recipients of WT or ERb-KO cells, indicating that deletion of ERa was not protective. Further, recipients of male ERa-KO and male ERb-KO cells developed comparable disease, suggesting a sex-specific functional redundancy for ERa and ERb in CD4+ T cells. Future studies will identify the contributions of ERa vs. ERb-specific signaling in effector and regulatory T cell subsets and the functional impact of these signaling pathways in males versus females.

PP 18

A CONSERVED ELEMENT IN THE FIRST INTRON OF CD4 HAS ENHANCER FUNCTION *IN VITRO* AT THE INTERMEDIATE BUT NOT DOUBLE POSITVE STAGE OF DEVELOPMENT

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CD4+ helper T cells are responsible for coordinating the adaptive immune response and the regulation of *Cd4* expression is vital during thymocyte development. The majority of developing thymocytes, which express both CD4 and CD8, must make a lineage decision to become either CD4 or CD8 T cells based on the specificity of their TCR for MHC-II or MHC-I plus endogenous peptide and the resulting duration of the positively selecting signal through the TCR. Upon receiving a signal via their TCR, CD4+CD8+ double positive (DP) thymocytes downregulate *Cd8* and become CD4+CD8lo intermediate stage (INT) thymocytes. As a result, thymocytes that use the CD4 coreceptor to stabilize their TCR-MHC-II interaction experience a persistent signal, upregulate *Cd4* expression, and commit to the CD4 lineage, while thymocytes that use the CD8 coreceptor to stabilize their TCR-MHC-I interaction experience a cessation of the signal, terminate *Cd4* expression, re-express *Cd8,* and commit to the CD8 lineage. The regulatory elements involved and the mechanism of *Cd4* upregulation in INT cells as a result of positive selection remains unclear. In this study, we investigated whether a highly conserved cis-regulatory element in the first intron of *Cd4,* which we call NCE, has enhancer function during the INT stage of thymocyte development. Using transient transfections of reporter plasmids, we determined that NCE enhances *Cd4* promoter function in thymoma cell lines arrested at the INT stage of development, but not the DP stage. Consistent with this observation, removing NCE through a CRISPR/Cas9 deletion in INT thymoma cells resulted in lower CD4 expression, while DP thymoma cells are unaffected. In addition, by stripping pre-existing coreceptors and measuring the rate of CD4 cell surface re-expression over time, we demonstrated that the CD4 rate of expression was reduced in NCE-deleted INT cells, but not NCE-deleted DP cells. Taken together, our findings show that NCE has developmental-stage specific enhancer function *in vitro* at the INT, but not DP, stage of development.

PP 19

CRITICAL ROLE OF FIBRINOLYSIS IN HEMATOPOIETIC RECOVERY AFTER MYELOSUPPRESSION

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Bone marrow responds to myelosuppression by expanding the hematopoietic stem cell population. Plasmin, a fibrinolytic protease, has been shown to be required for hematopoietic recovery. Fibrin deficiency alleviates many pathologies seen in plasminogen-deficient ($Plg^{-/-}$) mice such as impaired wound healing and periodontitis. 5-Fluorouracil (5-FU), a highly used chemotherapy drug, induces bone marrow injury, and elevated fibrin deposition in bone marrow has been observed within three days after treatment. Our study aims to evaluate the importance of fibrinolysis in hematopoietic recovery by investigating whether fibrin mediates myelosuppression. Using an *in vivo* model, we determined mouse survival and fibrin deposition after 5-FU treatment to see if hematopoietic recovery is critical for survival. An siRNA targeting fibrinogen (siFibrin) or a scrambled control (siLuciferase) was used to determine whether 5-FU toxicity in $Plg^{-/-}$ mice is fibrin-dependent. We found that plasmin is required for hematopoietic recovery in a 5-FU-induced myelosuppression model, and that reduced fibrin deposition improved survival. Our work suggests that the pharmacological depletion of fibrin via siFibrin partially rescues myelosuppressed $Plg^{-/-}$ mice and improves sustained hematopoietic recovery. Collectively, these data suggest that pharmacological reduction of fibrin may reduce toxicity of myelosuppressive chemotherapy drugs.

PP 20

DUAL SCRNA-SEQ ANALYSIS REVEALS RARE AND UNCOMMON PARASITIZED CELLPOPULATIONS IN CHRONIC *L. DONOVANI* INFECTION

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Leishmaniasis is a parasitic disease affecting more than 90 counties. Visceral leishmaniasis (VL) is the systemic manifestation of this disease caused by the parasite *Leishmania donovani.* VL can be lethal in up to 95% of the cases if left untreated, while distinct rates of relapse are observed in endemic regions. The mechanism of relapse is still unknown, although some studies suggest that low numbers of parasites remain dormant in the host until the immune system becomes permissive for the disease to reemerge. Although phagocytic cells are documented targets of *Leishmania* parasites, it is unclear whether other cell types can be infected. Here, we use unbiased scRNA-seq to simultaneously analyze host cell and *Leishmania donovani* transcriptomes to identify and annotate parasitized cells in spleen and bone marrow in chronically infected mice. Our dual-scRNA-seq methodology allows the detection of heterogenous parasitized populations. In the spleen, monocytes and macrophages are the dominant parasitized cells, while megakaryocytes, basophils and NK cells are found unexpectedly infected. In the bone marrow, the Hematopoietic Stem Cells (HSCs) expressing phagocytic receptors FcγR and CD93 are the main parasitized cells. Additionally, we also detect parasitized cycling basal cells, eosinophils, and macrophages in chronically infected mice. Flow cytometric analysis confirms the presence of parasitized HSCs. Our unbiased dual scRNA-seq method identifies rare, parasitized cells, potentially implicated in pathogenesis, persistence, and protective immunity without prior knowledge of possibly infected cells.

PP 21

ILLUMINATING THE RELATIONSHIP BETWEEN HEPATITIS C VIRUS AND B CELL DISORDERS: DETECTION OF VIRAL GENOME IN PERIPHERAL B CELLS USING RNASCOPE

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Approximately 58 million people are currently infected with Hepatitis C Virus (HCV). HCV is an enveloped, positive-strand RNA virus that primarily infects hepatocytes but has been reported to infect lymphocytes. Epidemiological studies have shown that chronic HCV infection is associated with increased risk of developing lymphoproliferative disorders, including B cell non-Hodgkin's lymphoma. The mechanism underlying this association is unclear. It may consist of direct oncogenic effects or an indirect mechanism. Direct effects refer to potential HCV infection of B cells, while indirect mechanism includes viral interaction with cell surface receptors via chronic antigen stimulation and/or CD81 engagement. The detection of HCV replication in B cells would support the hypothesis of direct virus-mediated lymphomagenesis, where the virus could trigger oncogenic events via intracellular viral proteins. This study aims to further elucidate these mechanisms by visualizing HCV positive and negative strand RNA in B cells using RNAscope fluorescent in situ hybridization. B cells were isolated from whole blood of HCV-infected patients and healthy donors and probed for CD19 mRNA and HCV positive-strand RNA. CD19 serves as a B cell marker and HCV positive-strand RNA indicates the presence of the virus. Preliminary data show successful visualization of CD19 mRNA and HCV positive-strand RNA. Optimization of the negative-strand HCV RNA probe is ongoing. Visualization of this RNA would indicate viral proliferation within B cells. Results from this study will confirm the existence of extrahepatic reservoirs for HCV and provide more direct evidence of viral protein expression in HCV associated lymphoma.

PP 22

ARYL HYDROCARBON RECEPTOR SIGNALING IN STEADY-STATE OR COLITIS INDUCES MUC2 PRODUCTION INDEPENDENTLY OF INTERLEUKIN-22 OR GOBLET CELL PROCESSES.

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Inflammatory bowel disease (IBD) is on the rise globally, posing multifaceted challenges in understanding its intricate causes and improving existing treatments. This necessitates further research into IBD-related mechanisms. Building upon our previous investigation, which showcased the efficacy of indole-3-carbinol (I3C), an aryl hydrocarbon receptor (AhR) ligand, in mitigating colitis severity via interleukin-22 (IL-22) production mediated by immune cells, we delve deeper into the enigmatic role of AhR in intestinal epithelial cells (IECs). Using female Balb/cJ mice, male and female wildtype (WT) C57BL/6 mice, and IEC-specific AhR knockout mice ($AhR^{\Delta IEC}$), we induced colitis chemically and treated the subjects with I3C. We assessed standard clinical parameters, IL-22 levels, colonoscopy findings, and histological changes. Furthermore, we conducted transcriptome microarray analysis, PCR assays, and immunofluorescent staining on colon epithelial cell (CEC)-enriched in vivo or in vitro colon-organoids to scrutinize the gene expression of mucins and goblet cell markers in the presence or absence of AhR.
Our findings illuminate that I3C augments Muc2 expression during colitis. Notably, $AhR^{\Delta IEC}$ mice exhibited an unresponsive phenotype to I3C treatment following colitis induction when compared to their WT counterparts. Remarkably, Muc2 was unveiled as AhR-dependent in IECs, even during steady-state conditions, and $AhR^{\Delta IEC}$ mice lost their capacity to elevate Muc2 levels following I3C treatment during colitis. Goblet cell markers, on the other hand, appeared to rely on IL-22 signaling and remained largely unaffected by AhR modulation.
In conclusion, our results underscore the pivotal role of AhR in IECs in mediating the protective effects of I3C during colitis, regardless of sex. Additionally, we emphasize the substantial AhR-dependency of Muc2 expression, independent of compromised goblet cell development or alterations in IL-22 signaling responses during colitis.

Keywords: colitis, aryl hydrocarbon receptor, mucin 2

PP 23

SUPRA-HOMEOSTATIC FUNCTIONS OF IL-7

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IL-7 is a non-redundant cytokine that promotes T- and B-cell development and is primarily considered as a key regulator of T-cell homeostasis. Here, we report that, at supra-homeostatic concentrations, which are induced in response to lymphopenia, IL-7 exerts additional functions, including the induction of pro-inflammatory chemokines and cytokines. Chemokines and cytokines are produced by peripheral blood mononuclear cells (PBMC) from healthy donors treated with IL-7 at a dose of 1 ng/mL or higher in the absence of concomitant T-cell receptor-mediated stimulation. The most upregulated chemokines are the natural ligands of the major HIV-1 coreceptor, CCR5: CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES. The most upregulated cytokine is TNF-α. $CD4^+$ T-cells and monocytes are the major sources of chemokines and cytokines, but their production requires direct contact between these cells. Studies on the mechanism of production of inflammatory chemokines and cytokines point to a key role of the interaction between membrane TNF-α and receptor TNFR2, and activation of the JAK/STAT and MAPK signaling pathways. These data demonstrate that IL-7, in response to lymphopenia, is a potent inducer of pro-inflammatory chemokines and cytokines in the absence of concomitant T-cell receptor-mediated stimulation.

PP 24

CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES INDUCED BY SPIKE FERRITIN NANOPARTICLE (SPFN) VACCINATION

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Due to the continuous emergence of SARS-CoV-2 variants of concern (VOC), it is important that vaccine strategies elicit broad responses across sarbecoviruses. The Spike protein Ferritin Nanoparticle (SpFN), developed at WRAIR, is a virus-mimicking vaccine displaying 8 stabilized spikes from the original SARS-CoV-2 strain. We know from preclinical studies in rhesus macaques that two SpFN immunizations elicited potent neutralizing antibody titers against multiple SARS-CoV-2 VOC and SARS-CoV-1. SpFN has additional advantages of showing more durable immune responses, and being more thermostable than mRNA vaccines, facilitating its deployment in non-developed countries. The characterization of monoclonal antibodies (mAbs) derived from clinical, post-vaccination samples can inform us of the breadth of an immune response in humans. We isolated over 100 mAbs from participants of a phase 1 clinical trial of the SpFN vaccine, using single B cell cloning strategy. Here we report the characterization of those mAbs using a unique workflow that includes a multiplexed, bead-based technology (Luminex) where we screened mAbs against 35 coronavirus antigens to measure relative binding strength, binding site, and breadth of binding. Of interest are two target sites of neutralization: the N-terminal domain (NTD) and receptor binding domain (RBD), both on the S protein of SARS-CoV-2. Overall, 139 antigen-positive mAbs were isolated, of which 47 mAbs were found to be RBD-directed and 28 NTD-directed. Of the 47 RBD-directed mAbs, 29 (62%) demonstrated cross-binding to the RBD of SARS-CoV-1. Likewise, 10 (62%) of the NTD-directed mAbsbound the S1 of SARS-CoV-1, demonstrating that antibodies generated post SpFN vaccination have breadth of binding to other coronaviruses. Furthermore, epitopes of RBD and NTD-directed mAbs were characterized via competition biolayer interferometry assays, wherein mAbs that compete for the same binding site make up an epitope bin. Four epitope bins were identified for each domain. mAbs were also tested for their neutralization capabilities using pseudotyped virus. Forty-five mAbs were found to neutralize SARS-CoV-2, including some with activity against XBB.1.5 and cross-neutralization to SARS-CoV-1. Finally, we demonstrate that this workflow correlates binding data from the multiplex bead assay to IC50 values from the neutralization assay and shows potential for the multi-plex bead assay to be an accurate and cost-effective selection method. In conclusion, the SpFN vaccine elicits antibodies against multiple epitopes of interest on the spike protein of

SARS-CoV-2, recent variants and SARS-CoV-1, suggesting that SpFN would be a valuable vaccine modality for boosting existing immunity and for the design of novel pan-sarbecovirus vaccine strategies.

PP 25

SPLENIC PD-1+CCR5- NATURAL KILLER (NK) CELLS IN CHRONIC SIV: SHAPING IMMUNE RESPONSES AND PATHOLOGICAL OUTCOMES

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Introduction Secondary lymphoid organs (i.e., spleen and lymph nodes) are vital for a healthy immune response. Unique crosstalk between innate and adaptive immune cells within lymphoid tissue may important for innate responses against pathogens, mediates long lasting and specific humoral and cell-mediated adaptive responses. Crosstalk between Natural Killer (NK) cells and B cells may determine pathogen elimination, vaccine responses and autoimmunity. The interaction between spleen NK cells and adaptive immune cells during SIV remains poorly understood. Thus, this study investigates changes in NK cell populations and their interplay with adaptive immune cells of animals with chronic SIV infection. Methods Using high dimensional flowcytometry, we compared phenotypic and functional changes of cryopreserved innate and adaptive immune cells of the spleen between chronically SIV-infected rhesus macaques (n=10) and uninfected (naïve; n=8) macaques. We also measured and compared cytokines secreted by splenic immune cells following stimulation with Phorbol 12-myristate 13-acetate (PMA) and ionomycin. Results Using FlowSOM-based clustering, we identified diverse NK populations with different phenotypes. We found significant differences in clusters expressing PD-1, CCR5, and CXCR5. In chronic SIV, splenic NK cells ($p < 0.05$) and T cells ($p < 0.001$) showed higher PD-1 expression, indicating increased exhaustion. Among NK cells, CCR5 expression was significantly lower ($p = 0.0062$), suggesting a pro-inflammatory phenotype. A unique PD-1+ CCR5- NK subset was more frequent in chronic SIV, positively correlating with IgG+B cell-secreted MIP-1b and IL-2 (both significantly higher in chronic SIV; $p < 0.05$). Conversely, CXCR5+ CCR5+ PD-1- NK cells were less frequent in chronic SIV, indicating decreased NK B-cell crosstalk. Additionally, in both groups CCR5 expression on NK cells positively correlated with secreted TNF-a (significantly higher in chronic SIV; $p < 0.05$), suggesting a potential role of TNF-a in the increased expression of IgG+B cells in our chronic SIV population. Conclusion In chronic SIV we observe expansion of splenic PD-1+CCR5-NK cells influenced by IL-2 and MIP-1b. These NK cells may trigger pro-inflammatory cytokine release like TNF-a, leading to aberrant increases in B-cell antibody production. Furthermore, given that increased PD-1 expression noted in Kaposi sarcoma has been associated with NK cell functional defects, further investigation of this unique NK cell subset in the spleen in chronic SIV may be of therapeutic value.

PP 26

EXTRACELLULAR VESICLES DERIVED FROM LYMPHATIC FLUID OF MELANOMA PATIENTS IMPAIR DENDRITIC CELL MATURATION

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Spread to regional lymph nodes is the most common site of metastasis from melanoma and almost all solid tumors. Lymphatic metastases begin in the first lymph nodes draining from the primary tumor, i.e., the sentinel lymph nodes (SLN), which is preceded by the development of the premetastatic niche (PMN) initiated by primary tumors. We and others have demonstrated that melanoma-derived lymphatic extracellular vesicles (LEVs) play a critical role in the development of the PMN as they modulate SLN immune subversion. Previously, our group described that EV cargoes (S100A8 and S100A9) interfere with dendritic cell (DC) maturation. In this study, our purpose is to further delineate the mechanism of DC suppression by patient LEVs that may be involved in establishing PMNs in melanoma.

To identify the LEV cargo responsible for SLN immune modulation, we extracted LEVs from the lymphatic fluid of patients with melanoma downstream of primary cutaneous melanomas, compared it with controls (non-malignant post-operative fluid collected from the lymph node dissection field), and examined proteome content using a 10-plex TMT proteomics approach. We identified 255 modulated proteins with ≥1.5-fold change with $p \leq 0.05$ in patient's LEV compared to control, among which several are involved in immunomodulation, including regulation of DC function. To characterize the effect of LEVs on DC suppression, we evaluated the effect of EVs in monocytic ($CD14^+$) and hematopoietic stem cells (CD34+) using DC maturation assays. We found DC maturation (defined by CD83 and CD86) was reduced after in vitro exposure of normal donor DC to patient LEVs compared to control. We also detected the inhibition of Clec9A+ expression on class 1 DC (cDC1), which mediates strong antigen uptake and cross-priming ability. Furthermore, in both sets of DC maturation assays, $CD11c^+HLA\text{-}DR^+$ DCs were diminished, implying that patient LEVs impeded the mechanism of DC maturation. To understand the mechanism of DC suppression in patients' LEVs, we also conducted a total RNA sequencing analysis of subsets of DCs of maturation experiments to evaluate differently regulated transcriptomic profiles. The gene ontology analysis indicates that the hematopoietic cell lineage is the top hit deregulated pathway in patient LEV-challenged DC. Furthermore, preliminary screening of DC markers on SLN from patients who recurred with immunotherapy versus patients who did not recur with immunotherapy revealed substantial variations in maturation and tolerogenic markers on DC when evaluated using a multiplex immunofluorescence imaging (MxIF) platform.

In conclusion, our study revealed that the patient's LEVs differentially altered proteomic cargoes, which may be involved in the suppression of DC functions. Studies are ongoing to validate the mechanistic pathways of DC immunosuppression.

Keywords: Melanoma; sentinel lymph node; dendritic cells; extracellular vesicles.

PP 27

THE ESCRT PROTEIN CHMP5 REGULATES TISSUE-INTRINSIC INFLAMMATION IN SKELETAL MUSCLE

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Macrophages are a key immune cell population required for maintenance of tissue homeostasis through their ability to regulate tissue metabolism, immunity, and inflammation, and to promote repair after injury. In skeletal muscle, the coordinate activity of resident and monocyte-derived macrophages facilitates crucial crosstalk between satellite cells, fibroadipogenic precursors, and myofibers that drives regeneration of damaged muscles. Dysregulation of this crosstalk contributes to conditions such as myopathy, inefficient wound healing, and sarcopenia. Despite their central role in muscle homeostasis and regeneration, the tissue-intrinsic factors and mechanisms that coordinate the recruitment and activity of these macrophages is poorly understood. In this study, we investigated how skeletal muscle homeostasis is regulated by the endosomal-sorting complex required for transport (ESCRT) protein CHMP5, which has recently emerged as a regulator of mammalian tissue development. CHMP5 (charged multivesicular body protein 5) was initially characterized as a member of the ESCRT family of proteins that coordinate membrane scission events in eukaryotic cells. However, recent studies by us and others have revealed non-canonical roles for CHMP5 in development wherein CHMP5 promotes the stability of client proteins required for cellular differentiation and cell fate decisions in both hematopoietic and non-hematopoietic tissues. Using an in vitro model of myogenesis, we found that CHMP5 knockdown impaired the upregulation of key myogenic transcription factors including Myogenin and MyoD and impaired the differentiation of C2C12 myoblasts into myotubes. Furthermore, when wild-type bone marrow-derived macrophages were cocultured with CHMP5-knockdown C2C12 myoblasts, they failed to polarize into pro-regenerative macrophages. Transcriptomic analysis of CHMP5-knockdown myoblasts at various timepoints throughout differentiation revealed upregulation of interferon response-associated transcripts and downregulation of transcripts associated with muscle differentiation. In vivo, mice with muscle specific CHMP5 deletion (CHMP5-KO mice) showed increased pro-inflammatory macrophages in skeletal muscle. H&E staining of tissue sections from CHMP5-KO mice displayed pathologic changes indicative of impaired regenerative capacity. These data together suggest a critical function for CHMP5 in dampening muscle-intrinsic inflammation and promoting muscle homeostasis.

PP 28

SCREENING FOR EPIGENETIC TARGETS TO ENHANCE CAR-T CELL ACTIVITY IN GLIOBLASTOMA

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Glioblastoma is the most common malignant brain tumor. Even with standard of care treatment, the median survival time is only 15 months. Therefore, novel therapies to treat this disease are sorely needed. One promising treatment is chimeric antigen receptor (CAR) T cell therapy. CAR T cells are engineered to kill cancer cells expressing certain antigens; however, this therapy is still being developed and requires more work to be effective in glioblastoma. One strategy for augmenting CAR T cell therapy would be to induce increased expression of the targeted antigens on cancer cells. This could be done using small molecule inhibitors of pathways that regulate expression of these antigens. In these studies, our goal was to screen for epigenetic targets that regulate expression of the CAR T cell target, GD2. We performed both a CRISPR screen of ~500 epigenetic genes and compound library screen of ~320 small molecule inhibitors. For the CRISPR screen, human glioblastoma cells were transduced in vitro with a lentiviral library. Fluorescence-activated cell sorting was used to select cells with the greatest increase in expression of GD2. Amplicons of gRNA sequences from the genomic DNA of high-GD2 cells were sequenced to determine the genes responsible for increased GD2 expression. For the compound library screen, human glioblastoma cells were incubated in vitro with the epigenetic small molecule inhibitors for 72 hours, after which time flow cytometry was used to assess GD2 levels of the glioblastoma cells. The CRISPR screen identified several genes associated with increased GD2 expression. Targeted lentiviral knock-down of the top gene was performed in multiple human glioblastoma cells in vitro. Knocking down the top gene resulted in a specific increase in GD2 expression. The compound library screen identified several small molecule inhibitors associated with increased GD2 expression. One of these compounds was confirmed to increase GD2 in follow-up validation studies. Overall, these results identify multiple mechanisms of increasing GD2 expression in human glioblastoma cells. These findings may inform strategies to improve CAR T cell therapy in glioblastoma treatment.

PP 29

TOWARD MOLECULES THAT CAN BOTH ENHANCE ANTITUMOR IMMUNITY AND INHIBIT AUTOIMMUNITY

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Antibodies that block programmed death-1 (PD-1) have transformed the treatment of many cancers, resulting in enduring therapeutic outcomes for some patients. But these checkpoint inhibitors can also trigger immune-related adverse events (irAE), in which immune cell activity is directed against normal tissues. IrAEs are typically treatment-limiting and management involves administration of systemic immunosuppressants. We are investigating whether bispecific antibodies can be designed to function as tissue-selective PD-1 agonists and whether they may be able to prevent or treat undesirable immune cell activity while preserving anti-tumor immunity.

We term these Location-Specific T-cell Inhibitory Molecules (LoSTIMs). LoSTIMs bind to a tissue-selective surface marker expressed on target cells as well as to PD-1 on T-cells. We hypothesized that LoSTIMs would cluster and activate PD-1 selectively when the bispecific bound both PD-1 and tissue-selective surface marker.

We evaluated whether LoSTIMs that bind the H-2K^b, which is expressed on target cells derived from C57BL/6 mice but not on cells derived from BALB/c or C3H mice, are capable of mediating tissue-selective inhibition of allogeneic T-cell activation. For *in vitro* studies, mouse T-cells were stimulated with allogeneic bone marrow dendritic cells (BMDC) derived from mice of a different background and T-cell activation was measured by carboxyfluorescein succinimidyl ester (CFSE) dilution. For *in vivo* studies, tumor cell lines were implanted subcutaneously into the flank of mice and tumor growth was measured.

LoSTIMs that bind the H-2K^b tissue-selective surface marker inhibited allogeneic activation of BALB/c T-cells by H-2K^b-positive C57BL/6 BMDC. However, they did not inhibit allogeneic activation of BALB/c T-cells by H-2K^b-negative C3H BMDC or allogeneic activation of C3H T-cells by H-2K^b-negative BALB/c BMDC. *In vivo*, H-2K^b-specific LoSTIMs were capable of delaying allograft rejection of MC38 (C57BL/6-derived, H-2K^b-positive) tumor cells implanted in BALB/c mice. A LoSTIM that blocks the binding of PD-1 to PD-L1 was capable of delaying allograft rejection in this manner and in another setting, also functioning as a checkpoint inhibitor, eliciting antitumor immunity against CT26 (BALB/c-derived, H-2K^b-negative) in BALB/c mice.

LoSTIMs that bind the H-2K^b tissue-selective surface marker and PD-1 can mediate tissue-selective immunosuppression, inhibiting allogeneic T-cell activation stimulated by H-2K^b-expressing target cells. LoSTIMs that block PD-1 ligation by PD-L1 can also function as checkpoint inhibitors against cancers that do not express the surface marker. This study supports the development of LoSTIMs that utilize other tissue-selective surface markers and investigations into their ability to ameliorate disease in mouse models of autoimmunity.

PP 30

HIGH RESOLUTION MODELING OF T CELL RECEPTOR RECOGNITION USING DEEP LEARNING

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High resolution structural models of T cell receptors (TCRs) and their engagement with peptide-major histocompatibility complexes (pMHCs) can provide critical insights into immune recognition and facilitate vaccines and therapeutics development. To provide the community with a method to accurately model structures of TCRs and TCR–pMHC complexes from sequence, we have developed the TCRmodel2 algorithm that achieves state-of-the-art TCR-pMHC complex modeling accuracy, with near-native predictions in ~75% of test cases. Our deep learning-based method utilizes several adaptations of AlphaFold to accurately model TCR-pMHC complexes, with focused databases of TCR and MHC sequences to speed up MSA feature building, optimization of the TCR template selection and utilization of pMHC complex structures as templates to improve AlphaFold's pMHC modeling accuracy. The TCRmodel2 algorithm is available via an easy-to-use web server that enables users to submit sequences and obtain models of complexes within 15 minutes, accompanied by an integrated molecular viewer and confidence scores.

PP 31

UNRAVELING MECHANISTIC DETERMINANTS OF CD8 T CELL BIOPHYSICAL HETEROGENEITY

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Cytotoxic T cell responses are crucial in eliminating cancer cells and establishing enduring memory responses against diseases. Recent findings from preclinical and clinical data indicate that, in various types of solid tumors, the quality of the T cell response, rather than the mere presence of T cells, determines the potential success of immune therapy (i.e., ICB). These clinical and preclinical observations underscore the urgent need for developing minimally invasive yet highly predictive biomarkers for assessing immune cell quality. However, the current knowledge gap persists in understanding whether differential T cell function is associated with distinct biophysical properties and how such properties are influenced during T cell stimulation and restimulation. While biochemical endeavors have demonstrated the presence of tumor-reactive T cells with discernible phenotypic differences, identifying predictive patterns has remained elusive. Significantly, to date, no studies have bridged the gap between the biophysical characteristics of T cells and the underlying molecular mechanisms of dysfunction.

We use a microfluidic device called suspended microchannel resonator (SMR) that measures the buoyant mass (BM) of individual cells with a precision near 0.1%. BM is defined as the product between a cell's volume and its density difference relative to the surrounding solution. In our investigations of T cells from mice and healthy donors, we consistently observed a new bimodal distribution, with CD8 T cells clustering around two distinct mass regions. The first is a light population ranging from 5-8 pg, and the second is a heavy population ranging from 10-15 pg. Notably, analyses of volume and density distributions revealed only one population, indicating that neither of these parameters alone accounted for the bimodality of T cell buoyant mass. Thus, while conventional methods like microscopy, light scattering, and density gradient centrifugation failed to distinguish these two T cell populations, they could clearly be resolved by high-precision buoyant mass measurements enabled by the SMR. We verified using scRNA-Seq and flow-based assays that both populations comprised viable cells, and both proliferated when activated by CD3/CD28 beads, although light cells took longer to enter the activated state and displayed slower proliferation rates compared to heavy ones. Light cells also exhibited significantly lower mitochondrial content but were otherwise comparable in terms of conventional surface markers. RNA-Seq also reviewed potential impaired functions of light T cells in migration and differentiation. Ultimately we envision BM to provide informative biomarkers for distinguishing between functional and dysfunctional T cells.

PP 32

THE CD8α–PILRα INTERACTION MAINTAINS CD8+ T CELL QUIESCENCE

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T cell quiescence is essential to preserve a broad repertoire against a large pool of diverse microbial and tumoral antigens, and accumulating evidence suggests that T cell quiescence is actively maintained. Genetic deficiency of intracellular proteins such as FOXP1, TSC1, SLFN2, and BTG1/BTG2, has been reported to abrogate naïve T cell quiescence and inducible deletion of regulatory T cells has been shown to disrupt memory $CD8^+$ T cell quiescence. But the cell surface proteins transmitting such inhibitory signals into T cells to keep T cell quiescence remain largely unknown. CD8α was proposed as a TCR coreceptor and was also demonstrated to be essential for cytotoxic T cell development in the thymus because cytotoxic T lymphocytes were completely eliminated in conventional CD8α knockout mice. However, the function of CD8α on $CD8^+$ T cells in peripheral lymphoid organs remains unknown.

To answer this question, we generated tamoxifen-inducible CD8α conditional knockout mice and found that the Inducible deletion of CD8α on peripheral $CD8^+$ T cells broke the quiescence and reduced the survival of both naïve and memory $CD8^+$ T cells in the absence of antigen exposure. Interestingly, the activation or loss of quiescence caused by CD8α deletion is independent of TCR triggering. Next, we identified PILRα expressed exclusively on the myeloid cells as a new ligand for CD8α in humans and mice. Antibody blockade of PILRα–CD8α interactions by either anti-CD8α mAb (or its Fab fragment) or anti-PILRα mAb also abrogated naïve and memory $CD8^+$ T cell quiescence in the periphery, recapitulating the findings with inducible deletion of CD8α. By contrast, antibody disruption of PILRα–CD8α interactions did not affect the $CD8^+$ T cell positive and negative selection in the thymus.

Our study suggested that the quiescence of T cells, especially CD8 T cells, is actively maintained by myeloid cell–T cell crosstalk through cell surface PILRα–CD8α interactions. Our study provided a further understanding of how T cell quiescence is enforced in the peripheral lymphoid organs.