LA JOLLA IMMUNOLOGY CONFERENCE

ABSTRACT BOOK

2022 Melvin Cohn Award Winner

Zhi Liu

Glucocorticoid signaling and regulatory T cells cooperate to maintain the hair-follicle stem-cell niche

Maintenance of tissue homeostasis in steady state or under stress is dependent on the proper communication between the stem cells and the supporting cells in the same niche. In addition to promoting immune tolerance, regulatory T cells (Treg) have recently emerged as a critical component of the stem cell niche in the hair follicle (HF), injured muscle, bone marrow, and small intestine to support stem cell differentiation or maintain their quiescence. How Treg cells sense the dynamic signals in the niche microenvironment and communicate with stem cells during tissue regeneration is mostly unknown. Here, by using HF as a model to study Treg cell–stem cell crosstalk, we uncover a hitherto unrecognized function of steroid hormone glucocorticoid in instructing skin-resident Treg cells to facilitate HF stem-cell (HFSC) activation and HF regeneration. Ablation of GR signaling in Treg cells blocked depilation-induced hair regeneration and natural hair growth without affecting Treg's immune suppressive function. Mechanistically, GR and Foxp3 cooperate in Treg cells to induce transforming growth factor β3 (TGF-β3), which activates Smad2/3 in HFSCs and facilitates HFSC proliferation. The present study identifies crosstalk between Treg cells and HFSCs mediated by the GR–TGF-β3 axis, highlighting a possible means of manipulating Treg cells to support tissue regeneration.

Young Investigator Talks

Session I: Eclectic Immunity

Kiyokazu Kakugawa
DYNAMIC TRANSLOCATION OF THEMIS FROM THE CYTOPLASM TO THE NUCLEUS IS ESSENTIAL FOR ITS FUNCTION IN T CELLS

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THEMIS functions as an adaptor during proximal T cell receptor (TCR) signaling. THEMIS has also a nuclear localization signal (NLS) and a significant amount of THEMIS resides in the nucleus. To investigate the importance of nuclear THEMIS, we deleted the NLS sequence to force THEMIS exclusively in the cytoplasm (THc). Similar to Themis null mice, T cell development was impaired in THc mutant mice, suggesting that THEMIS may have nuclear roles. Next, we established Themis mutant mice, with exclusive nuclear localization of THEMIS by inserting an SV40-NLS sequence into Themis (THn). Mature T cell numbers were decreased in these mice similar to THc and Themis null mice. Moreover, THc/n double mutants, in which THEMIS is present in the cytoplasm and nucleus but unable to translocate to either compartment, showed a similar phenotype, indicating that THEMIS translocation to and/or from the nucleus is essential for its function. Finally, we fused Themis with ERT2, which retains THEMIS in the cytoplasm and allows for THEMIS translocation to the nucleus upon Tamoxifen administration. In this fusion protein knock-in mouse (THert2) T cell development was also impaired. However, addition of Tamoxifen resulted in the appearance of normal mature T cells in the periphery, indicating that THEMIS translocation is important for its function. These data show that THEMIS functions, not only as an adaptor, but also as a messenger linking proximal TCR signals with nuclear events important for T cell differentiation and activation.

Wan-Lin Lo

A SINGLE AMINO ACID SUBSTITUTION IN THE ADAPTOR LAT IS A EVOLUTIONARILY CONSERVED BIOCHEMICAL FEATURES, TO ENSURE PROPER T CELL TOLERANCE AND RESPONSES

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The mature T cell repertoire is selected for recognition of self-peptide:MHC during thymic development. T cells must therefore discriminate between brief interactions with self-peptide:MHC and prolonged binding to agonist. The kinetic proofreading model posits that certain TCR signaling nodes serve as molecular timers to facilitate such discrimination. However, the physiological significance and pathological consequences of disrupting this regulatory mechanism are unknown. Here, we reported that accelerating normally slow phosphorylation of LAT Y136 via introduction of an adjacent mutation G135D disrupts ligand discrimination in vivo. The enhanced self-reactivity of G135D LAT T cells triggers excessive thymic negative selection and promotes T cell anergy. During Listeria infection, G135D LAT endows T cells with greater expansion in responses to very weak stimuli, but disrupts the balance between effector and memory responses. Moreover, despite enhanced engagement of central and peripheral tolerance mechanisms, G135D LAT mice are predisposed to autoimmunity. Our data thus reveals the physiological importance of kinetic proofreading to balance tolerance and immunity.

Martina Zoccheddu

EXPLORING THE ROLE OF EXTH17 IN AUTOIMMUNE ARTHRITIS

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Rheumatoid arthritis (RA) is the most common systemic autoimmune disease. T helper 17 (Th17) cells are found in the inflamed synovium in RA and believed to be involved in disease progression. However, IL-17-targeted interventions have been ineffective in trials. One hypothesis is that due to plasticity, Th17 can lose IL-17 expression converting into so-called exTh17. However, the role of exTh17 in the arthritis pathogenesis remains unknown. To explore this, we have leveraged SKG mice, an autoimmune Th17-driven model of arthritis. We generated SKG-based Th17 fate mapping mice carrying two cytokine reporters and an inducible tdTomato cassette in the ROSA26 locus (R26tdTomfl), which is activated by an IL-17A driven Cre. TriTh17 mice (IL-17CreIFNYFPIL-10eGFPR26tdTomflSKG) were utilized to generate initial evidence of Th17 cell plasticity during autoimmune arthritis. We found that exTh17 cells are arthritogenic and are specifically localized in arthritic joints. Bulk RNA sequencing analysis of exTh17 shows a specific gene signature with Cd44 and S1pr4 being genes of interest. A gene signature resembling mouse exTh17 was found in single cell clusters of human CD4+T cells from RA synovium. Spatial transcriptomic data further demonstrated co-localization of such gene signatures with fibroblast, in human arthritic synovium samples, suggesting that a crosstalk between fibroblast and T cells might be involved in the Th17-exTh17 conversion. In vitro co-culture of naïve CD4+T cells from TriTh17 mouse and synoviocytes showed enhanced Th17 conversion into exTh17. We conclude that exTh17 can promote autoimmune arthritis progression and that synoviocytes might play a role in the Th17-exTh17 conversion.

Session II: Protective Immunity and Immune Regulation

Bryan McDonald

CANONICAL BAF COMPLEX ACTIVITY LICENSES CD8+ T CELL EFFECTOR CELL FATES

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CD8+ T cells provide a critical layer of host protection against all manner of pathogens and malignancies. Chromatin remodeling is a critical component of differentiation and acquisition of cytotoxic functions in activated CD8+ T cells, but the mechanisms by which remodeling is achieved in a site-specific way to promote appropriate effector cell fates are poorly understood. Here we identified a role for the ARID1A-containing canonical BAF (cBAF) complex in regulating chromatin accessibility and differentiation of antiviral effector CD8+ T cells. Arid1a was required for CD8+ T cell differentiation into terminal effector and tissue-resident memory cells, but was not required for the formation of long-lived circulating memory cells. Mechanistically, cBAF acted to promote chromatin accessibility at thousands of loci bound by important effector-associated TFs including BHLHE40, T-bet, and BATF. Understanding how chromatin remodeling is regulated in differentiating effector CD8+ T cells will yield insight into optimizing vaccines and adoptive cell therapies for cancer.

Elena Yun Hsuan Lin

SMALL INTESTINE AND COLON TISSUE-RESIDENT MEMORY CD8+ T CELLS EXHIBIT TRANSCRIPTIONAL AND FUNCTIONAL HETEROGENEITY & DIFFERENTIAL DEPENDENCE ON THE TRANSCRIPTION FACTOR EOMESODERMIN

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Tissue-resident memory CD8+ T (TRM) cells are a subset of memory T cells positioned at barrier sites, critical for host defense against infection. Recent studies have begun to reveal substantial, previously underappreciated heterogeneity in CD8+ TRM cell populations across different tissue sites. Nevertheless, CD8+ TRM cells in particular tissue compartments, such as the small intestine lamina propria and the colon, have not been studied in detail. We show that CD8+ TRM cells in the intraepithelial and lamina propria layers of the small intestine and colon exhibit distinct differences in their transcriptional signature and expression of phenotypic markers, granzymes, and cytokines. We also uncover an unexpected, tissue-specific role for the T-box transcription factor Eomesodermin, known to suppress TRM cell formation in the skin, liver, and kidney. Our data demonstrate that Eomesodermin is dispensable for CD8+ TRM formation in both the small intestine and colon. Following CD8+ TRM cell formation, Eomesodermin promotes the maintenance of established CD8+ TRM cells in the small intestine but is dispensable in the colon. Together, our study provides new insights into intestinal CD8+ TRM cell heterogeneity and differential dependence on transcriptional regulators in TRM cell formation vs. maintenance.

Rasika Patkar

NLRP3 CONFERS REGULATORY T CELL-MEDIATED CONTROL OF INTESTINAL INFLAMMATION

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The pro-inflammatory role of inflammasomes has been well established in innate immune cells. Nevertheless, recently, several studies have uncovered that different inflammasome components are also crucial in regulating many different T cell responses. Through RNA-sequencing analysis of regulatory T (Treg) cells as well as CD4+ conventional T cells isolated from different tissues under different physiologic and pathologic conditions, we found selectively elevated expression levels of Nlrp3 and other genes associated with the NLRP3 inflammasome pathway in intestinal Treg cells from mice with autoimmune-mediated inflammation. Next, by taking both mixed bone marrow chimera and Treg cell-specific gene targeting approaches, we have shown that mice with Treg cells devoid of NLRP3 exhibited an

elevated Th17 response, a type of T cell response that is known to be involved in inflammatory bowel diseases (IBD), specifically in the intestine. The loss of the control of intestinal Th17 responses is likely not due to a general defect in Treg cell biology as we found that Treg cell frequencies and function otherwise were not altered by NLRP3 deficiency. Collectively, this study will help gain mechanistic insights into the previously underappreciated anti-inflammatory role of NLRP3 in Treg cells particularly in controlling Th17 responses in the intestine. Ultimately, our work should guide effective therapies for chronic intestinal disorders such as IBD, in which the anti-inflammatory vs. proinflammatory role of NLRP3 is still debated.

Hiutung Chu
COMMENSAL BACTERIA PROMOTE TYPE I INTERFERON SIGNALING TO MAINTAIN IMMUNE TOLERANCE

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Type I interferons (IFN) exert a broad range of biological effects important in coordinating immune responses. Host and microbial factors regulate IFN production, triggering a signaling cascade that has classically been studied in the context of pathogen clearance. In particular, commensal bacteria have been shown to induce IFN to protect against viral infections. Yet, whether immunomodulatory bacteria operate through IFN pathways to support immune tolerance remains elusive. Here, we demonstrate microbiota-dependent IFN signaling is required for priming tolerogenic T regulatory cells (Tregs) by intestinal dendritic cells (DCs). DCs deficient in IFN signaling through deletion of IFNAR-1 display dysregulated cytokine production in response to the commensal bacteria Bacteroides fragilis, resulting in blunted downstream Treg responses. Single cell RNA sequencing of gut tissues demonstrated that colonization with B. fragilis promotes a distinct type I IFN gene signature in Tregs during homeostasis and intestinal inflammation. Moreover, B. fragilis-mediated protection during experimental colitis

was abrogated in IFNAR1-deficient mice. Altogether, our findings demonstrate an important role of microbiota-mediated immune tolerance via tonic type I IFN signaling.

Session III: Innate and Innate-Like Immunity

Thomas Riffelmacher

Metabolic fuel choices control MAIT cell functions at homeostasis and after infection

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Mucosal-associated-invariant-T (MAIT) cells are an innate-like T cell subset that recognizes microbial-derived vitamin B metabolites. In contrast to conventional T cells, MAIT cells have an antigen-experienced phenotype and express memory markers by default. When activated, MAIT cells rapidly produce copious amounts of cytokines, resembling IFN+ Th1- or IL-17+ Th17 effectors cells. While memory-like vs effector-like states in conventional CD4 and CD8 T cells are controlled by mutually exclusive metabolic states, the question remains as to which metabolic programs MAIT cells adopt at steady-state and after infection. Here we integrate scRNA-seq analysis with single-cell metabolic characterization of MAIT cells from humans and mice. We discovered a memory-like metabolic program that is acquired in the thymus and at steady state in the periphery. We identify a novel cluster of circulatory MAIT cells which is metabolically similar to IFN+ MAIT1-like cells and preferentially consumes glucose. MAIT17 clusters, most prevalent in mice, uniquely engage in fatty acid uptake and mitochondrial metabolism. Following exposure to bacteria, mouse MAIT cells expand as CD127-KLRG1+ and CD127+KLRG1-populations that adopt divergent transcriptomic and metabolic profiles with enhanced functionality. They remain altered long-term. CD127+, but not KLRG1+ MAIT cells engage in MAIT17-like metabolic and effector pathways and protect mice from lung infection with Streptococcus pneumoniae. In contrast, KLRG1+ MAIT cells depend on Hif1a-driven glycolysis and remain metabolically dormant but ready to respond, more rapidly engaging multiple metabolic programs to protect from viral infection.

Trever Greene

TYPE I INTERFERON EXHAUSTION IN PLASMACYTOID DENDRITIC CELLS: AN UNDERLYING MECHANISM, COST, AND BENEFIT.

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Balance is an essential virtue of the immune system. Responses which protect against pathogens can themselves quickly become aberrant and even life-threatening. Type I interferons (IFN-I) are essential for protection against viral infections, and pDCs produce a thousand-fold more IFN-I per capita than any other cell-type. As such pDCs are critical for the early systemic control of many viral pathogens. However, following their immediate response to infection these cells completely lose their capacity for IFN-I production. Importantly, this "pDC Exhaustion" is conserved across species and is observed in a diverse cohort of viral infections and cancer. Despite this ubiquity, it was previously unclear how or why pDCs become exhausted. To answer this question, we investigated the exhausted pDC transcriptome and identified alterations in pDC metabolism that underlie pDC exhaustion. Specifically, we identified a single metabolic enzyme (LDHB), which is suppressed in exhausted pDCs. Restoration of LDHB rescued pDC IFN-I production. Furthermore, we showed that LDHB expression was essential for optimal IFN-I production by human and mouse pDCs and promoted viral control and adaptive responses against mouse viral infections. Conversely, we demonstrated that restoring LDHB to pDCs in vivo during infection worsens virus-induced-colitis, suggesting that pDC exhaustion exists as a system to balance the potential of pDCs to control infection with the potential detrimental impacts of excessive and aberrant pDC function. Altogether our results identify a novel regulator of pDC function, restoration of which can reverse pDC exhaustion, and determine an evolutionary purpose for this previously unexplained phenomenon.

Caroline Wathieu
LOSS OF THE TRNA-MODIFYING ENZYME ELP3 IMPAIRS TUFT CELL DIFFERENTIATION AND THE ANTI-HELMINTH IMMUNE RESPONSE THROUGH ATF4 UPREGULATION

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The role of tRNA modifications in immunity only starts to be elucidated. Elp3 is the catalytic subunit of Elongator which promotes the mcm5s2 chemical modification of some tRNAs necessary for efficient protein translation. We previously described that tuft cell numbers dramatically decreases upon Elp3 inactivation in intestinal epithelial cells (IECs). Tuft cells expand in response to parasite infection and initiate anti-helminth type 2 immunity through IL-25 production which triggers ILC2 activation and subsequent IL-13 secretion. To further investigate the role of Elp3 in tuft cell differentiation and in the anti-helminth immune response, the gastrointestinal nematode parasite Nippostrongylus brasiliensis and rIL-13 treatment were used to expand tuft cells in vivo. Upon N.brasiliensis infection, the genetic inactivation of Elp3 in IECs resulted in impaired tuft cell amplification, reduced IL-25 production, ILC2 activation and goblet cell expansion, and consequently delayed expulsion of parasite worms. Upon rIL-13 treatment, only tuft cell differentiation was impaired, suggesting a specific role of Elp3 in tuft cell fate determination. By performing single cell RNA-sequencing on IECs, we demonstrated that expression levels of the transcription factor Atf4 were enhanced upon Elp3 deficiency. Likewise, Atf4 overexpression in IECs in vivo mimicked the phenotype observed in mice lacking Elp3. By using multiple mouse and organoid models, we showed that Atf4 is a negative regulator of tuft cell differentiation. Collectively, our data provide the first insights into molecular mechanisms underlying the key role of tRNA-modifying enzymes and Atf4 in tuft cell differentiation and in the immune response to intestinal parasite infection.

Session IV: Cancer & Anti-Cancer Immunity

Anusha Preethi Ganesan
T CELL STATES AND TCR CLONALITY IN PEDIATRIC BRAIN TUMORS

Anusha-Preethi Ganesan[1,2]
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Brain tumors are the most common solid tumors in children and outcomes remain dismal for a high proportion of patients. Immunotherapies potentiate anti-tumor responses of T cells, however immune response within brain tumors remain largely unknown. We performed paired single-cell transcriptomic and TCR profiling of patient-derived brain tumor-infiltrating T cells to couple T cell molecular program with TCR repertoire and clonality. Contrary to the general notion of a paucity of T cell response in brain tumors, we found striking clonal-expansion of intra-tumoral T cells and shared TCR specificity groups. Clonally-expanded CD8^{+} T cells were enriched for T cell subsets such as tissue-resident memory (T_{RM}) cells, granzyme-K(GZMK)-expressing effector memory and 'cytotoxic effector' T cells, which have been linked to anti-tumor immunity. Clonally-expanded CD4^{+} T cells harbored CD4^{+}CTL-like features. Importantly, clonally-expanded T cells displayed cytotoxicity, cytokine production, *PDCD1* expression and neoantigen-specific gene signatures, suggestive of tumor-reactivity. We observed considerable inter-patient heterogeneity in clonal expansion and PDCD1 expression, therefore we propose that tumor-reactive T cells may be targeted by checkpoint blockers in select patients who demonstrate robust endogenous T cell responses within their brain tumors. Furthermore, we are undertaking studies to define the antigen specificity of the clonally-expanded tumor-infiltrating T cells and assess the *in vivo* function of neoantigen-specific TCR-engineered T cells in mediating tumor control. The ability to perform micro-scaled molecular analyses at high resolution on T cells from patient tumors allows us to identify gene expression programs that may inform patient-specific immunotherapy choices as anti-cancer treatment.

Miguel Reina-Campos

COMMON METABOLIC ADAPTATIONS EMPOWER CD8 T CELL TISSUE RESIDENCY AND ANTITUMOR IMMUNITY

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Lodged in tissues throughout the body, tissue-resident memory CD8 T cells (TRM) contribute a significant portion of the T cell arsenal providing long-term protection from reinfection and tumorigenesis. The regulation of metabolic programming that accompanies TRM adaptation to diverse tissue environments permitting differentiation, survival, and sustained function is not fully established. Understanding how TRM undergo metabolic acclimation to specific tissues may provide key insights into the ability to promote host protection from reinfection and tumor growth. In fact, tumor-infiltrating lymphocytes (TIL) with TRM characteristics possess enhanced antitumor functions and often predict responses to immunotherapy and favorable prognosis. Here, we reveal tissue-specific metabolic adaptations of TRM and highlight the cholesterol biosynthetic pathway, and its upstream regulator Srebp2, to be of particular relevance for small intestine (SI) TRM formation and the antitumor capacity of TIL. Unexpectedly, this tissue-specific adaptation was supported by the production of non-steroidal metabolites, such as ubiquinone, rather than cholesterol. Leveraging this information, we show that genetic and pharmacologic inhibition of squalene synthase (Fdft1), which prevents cholesterol production and promotes accumulation of upstream intermediates, enhances memory formation upon acute viral infection and boosts the antitumor function of CD8 T cells in mice. Together, this study profiles metabolic adaptations of TRM and illustrates how these pathways can be co-opted to potentiate CD8 T cell memory formation in the context of acute infections and revamp CD8 T cell functions in the context of tumors.

Yunqiao Li
INHIBITION OF PLASMA CELL DIFFERENTIATION PROMOTES ANTITUMOR IMMUNITY.

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B cells located in tumor-associated tertiary lymphoid structures (TLSs) participate in anti-tumor immunity. However, the contribution of B cells to anti-tumor immunity needs to be further investigated. The transcription factor Blimp-1 (encoded by Prdm1) is necessary for the generation of plasma cells, the antibody-producing cells. Here we show that Blimp-1 deficiency in B cells promoted antitumor immunity. We found that Blimp-1-deficient B cells increased the expression of costimulatory molecules CD80 and CD86 to enhance effector T cell function. Major histocompatibility complex class II (MHC II) was required for the anti-tumor efficacy of B cell-specific Blimp-1 knockout (BcKO) mice. Experimental maneuvers that impaired antigen recognition or presentation by B cells led to more aggressive tumor growth, whereas growth was inhibited when B cells recognized tumor-specific antigens. Antibodies derived from tumor-bearing mice showed no activity in tumor control. The Blimp-1 inhibitor valproic acid suppressed tumor growth in a B cell-dependent manner. We demonstrate that inhibition of plasma cell differentiation and antibody secretion results in enhanced tumor-associated antigen presentation by B cells and thereby tumor resistance. Our results suggest that B cells could be a potential source of immunotherapy against cancer.

Priyanka Saminathan
BIO-ACTIVE LIPIDS PROTECT AGAINST IMMUNE-RELATED ADVERSE EVENTS DUE TO IMMUNE CHECKPOINT BLOCKADE THERAPY

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Many patients receiving Immune checkpoint blockade (ICB) therapy develop Immune related adverse events (IRAEs), a leading cause for patients to discontinue treatment. Analyzing patients who develop IRAEs can help advance our knowledge of the molecular drivers of these poorly understood off target toxicities. Our recent study of plasma from patients undergoing ipilimumab (anti-CTLA4) or pembrolizumab (anti-PD1) therapy for melanoma, lung cancer or other solid tumors was assessed using high-resolution liquid chromatography-tandem mass spectrometry. We uncovered a novel protective mechanism related to a class of circulating bio-active lipids that suppress ICB-related IRAEs. Significant reduction of bio-active lipids in circulation was associated with increased ICB-mediated IRAEs. Mouse-models (both DSS-colitis and humanized models) were used to show that supplementation with these lipids ameliorated colonic inflammation without impacting ICB-driven tumor regression. We also uncovered a significant correlation between increasing neutrophil counts and decreased bioactive lipids in circulation. These results uncover a previously unidentified regulatory mechanism whereby the identified lipids in circulation specifically suppress deleterious inflammation during ICB therapy, while preserving anti-tumor immunity, suggesting that supplementation of bio-active lipids can be developed as a new therapeutic strategy to improve clinical outcomes in cancer immunotherapy.

Poster Presenters

Poster Session 1:

Sara Quon (I1)

CTCF FACILITATES SUBSET-SPECIFIC CHROMATIN INTERACTIONS TO LIMIT THE FORMATION OF TERMINALLY-DIFFERENTIATED CD8+ T CELLS.

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CD8+ T cells are important for host protection from infections and malignancies, and many current immunotherapies target molecules that alter CD8+ T cell function and differentiation. Although genome organization is known to be important for regulating cell development and function, the changes in spatial chromatin organization accompanying effector and memory CD8+ T cell differentiation remain unknown. Here, we studied how genome organization is integrated with other molecular mechanisms regulating CD8+ T cell differentiation and targeted CTCF, a key factor that regulates genome organization through blocking or facilitating chromatin interactions, to determine how altering interactions affect the CD8+ T cell response. We observed T cell subset-specific changes in intra-TAD interactions at sites related to transcriptional rewiring such as genes encoding for transcription factors that regulate CD8+ T cell differentiation. We next characterized the binding profile of CTCF, a known regulator of chromatin interactions. CTCF binding changed with CD8+ T cell differentiation, and weak-affinity CTCF binding is needed to promote terminal differentiation in both an infection and tumor setting through regulation of transcription programs and transcription factor activity. Strikingly, disruption of a single CTCF binding site upregulated expression of corresponding memory-associated molecules. Thus, our data suggest that chromatin remodelers can fine-tune the CD8+ T cell response through altering interactions that regulate the transcription factor landscape and transcriptome.

Longwei Lu (I2)

DEVELOPMENT OF SENSITIVE BIOSENSORS FOR IMAGING OF T CELL SIGNALING

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Biosensors based on fluorescent proteins (FPs) and fluorescence resonance energy transfer (FRET) allow direct visualization of protein kinases or epigenetic changes and have revolutionized how we study these key regulators in their native biological contexts. However, the limited sensitivity of FRET biosensors hinders their broader applications. We have developed an approach integrating high-throughput FRET sorting and next-generation sequencing (FRET-Seq) to identify sensitive biosensors with varying substrate sequences from large-scale libraries directly in mammalian cells, utilizing the design of self-activating FRET (saFRET) biosensor. The resulting biosensors of Fyn and ZAP70 kinases exhibit enhanced performance and enable the dynamic imaging of T-cell activation mediated by T cell receptor (TCR) or chimeric antigen receptor (CAR), revealing a highly organized ZAP70 subcellular activity pattern upon TCR but not CAR engagement. The ZAP70 biosensor elucidates the role of immunoreceptor tyrosine-based activation motif (ITAM) in affecting ZAP70 activation to regulate CAR functions. A saFRET biosensor-based high-throughput drug screening (saFRET-HTDS) assay further enables the identification of an FDA-approved cancer drug, Sunitinib, that can be repurposed to inhibit ZAP70 activity and autoimmune-disease-related T-cell activation. Furthermore, in the most recent research, we have also developed the first single-FP-based biosensors for tyrosine kinases (e.g., ZAP70, Lck), which should enable the multiplex imaging of T cells and provide multiparameter real-time readouts of different molecules in one single live cell.

Shengyun Ma (I3)

RNA BINDING PROTEIN DDX5 RESTRICTS RORT+ TREG SUPPRESSOR FUNCTION TO PROMOTE SMALL INTESTINE INFLAMMATION

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RORγt+ Regulatory T (RORγt+ Treg) cells play pivotal roles in preventing T cell hyperactivation and maintaining tissue homeostasis, in part, by secreting the anti-inflammation cytokine interleukin 10 (IL-10). Here, we report that Hypoxia Induced Factor 1a (HIF1α) is the master transcription factor for Il10 in ileal RORγt+ Treg. Interestingly, this critical anti-inflammatory pathway is negatively regulated by an RNA binding protein DDX5. As a transcriptional corepressor, DDX5 restricts the expression of HIF1α and its downstream target gene Il10 in ileal RORγt+ Tregs. As a result, T cell-specific Ddx5 knockout (DDX5ΔT) mice with augmented RORγt+ Treg suppressor activities are protected from T cell-mediated ileitis. Genetic ablation or pharmacal inhibition of HIF1 restore ileitis susceptibility in DDX5ΔT mice. The DDX5-HIF1α-IL10 pathway provides novel potential therapeutic targets for ameliorating intestinal inflammatory diseases.

Maximillian Heeg (I4)
TISSUE-SPECIFIC TRANSCRIPTIONAL NETWORKS IDENTIFY HIC1 AS A CRITICAL TRANSCRIPTION FACTOR FOR INTESTINAL TRM DEVELOPMENT.

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CD8+ T cells are a critical component of the immune response to intracellular infections and malignancies. Recently, tissue-resident CD8+ memory cells (TRM) have been shown to provide a first line defense upon reinfection at barrier tissues such as the intestine. However, the transcriptional diversity that allows TRM adaptation to different tissues is not well understood, and it is unclear to which extent TRM can re-adapt to other tissue environments upon reinfection. Here we set out to define TRM in distinct tissues and show that TRM have both shared as well as tissue-specific transcriptional programs. Further, using

transcriptional-regulatory networks, we identified the transcription factor Hic1 as a critical regulator for TRM differentiation. We observed that knockdown of Hic1 hinders TRM formation in the small intestine epithelium in response to both LCMV Armstrong and Listeria monocytogenes infection. In contrast, overexpression of Hic1 favored TRM formation in the small intestine upon infection and provided protection upon reinfection. Mechanistically, Hic1 regulates P2rx7 expression, an eATP receptor previously shown to be important for memory T cell differentiation and TRM homeostasis. Importantly, we found that small intestine TRM are genetically imprinted to reenter the intestine upon secondary infection, whereas this tissue-specific bias was not observed for liver and kidney TRM. In summary, our work highlights the broad transcriptional adaptations of TRM to a range of tissue environments, which may be used as a framework for identifying targets that influence tissue-specific TRM populations in therapeutic contexts.

Thomas Mann (I5)
PROTEIN KINASE C AS A MOLECULAR CLOCK THAT CONTROLS CD8+ T CELL FUNCTION AND EXHAUSTION

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During cancer and chronic viral infections, the persistence of antigen progressively causes CD8+ T cells to differentiate into a dysfunctional PD1+ "exhausted" state, with reduced production of inflammatory cytokines relative to effector cells that form during acute infections. Antigen and costimulation signals activate kinase cascades to induce distinct T cell transcription programs, but how T cells interpret acute and chronic signals to program their transcriptional states remains poorly understood. We found that members of the protein kinase C (PKC) family function together as a "molecular clock," sensing antigen signal duration to drive distinct signaling outputs and divergent transcriptional programs. PKC theta is necessary for maintaining the plasticity and functionality of the least differentiated "progenitor" exhausted cell type. But, during periods of chronic stimulation, T cells degrade PKC theta and selectively maintain PKC eta. PKC eta promotes differentiation of progenitor exhausted cells into a terminally exhausted state, as deletion of PKC eta causes an increase in progenitor cells while agonism of PKC eta is sufficient to promote terminal exhaustion features such as loss of production of the pro-inflammatory cytokines IFN and TNF, and upregulated expression of the exhaustion factor Tox. This "PKC switch" alters downstream signaling in the MAPK pathway to the AP-1

transcription factor family. In summary, continuous signaling through PKCs causes changes in the output from these kinases initially at the protein level, driving transcriptional changes among PKC targets in the AP-1 transcription factor family and thus allowing further widespread transcriptional and functional changes that characterize T cell exhaustion.

Shixin Ma (I6)
ACSS2 and ACLY cooperatively regulate histone acetylation and CD8 T cell exhaustion

Progenitor exhausted (Pro_TEX) T cells in chronic infections and cancer are a unique self-renewing population that mediates long-term immunity and is critical for the success of immunotherapy, but further differentiation of these Pro_TEX cells into terminally exhausted (Ter_TEX) cells with irreversible dysfunction represents an important barrier to current therapies. Ter_TEX cells are accompanied by molecular rewiring at the epigenetic and metabolic levels that have been implicated in mediating their dysfunction. However, the potential crosstalk between metabolic rewiring, particularly within the nucleus, and epigenetic reprogramming in Pro_TEX and Ter_TEX cell fate decision-making is poorly understood. Here, we identify the metabolic enzymes acetyl-CoA synthetase2 (ACSS2) and ATP-citrate lyase (ACLY) as central metabolic rheostats for Pro_TEX and Ter_TEX cell development in infection and cancer. ACSS2 and ACLY preferentially supply nuclear acetyl-CoA in Pro_TEX and Ter_TEX cells, thus regulating state-specific histone acetylation and corresponding gene expression. ACSS2-dependent acetyl-CoA production and histone acetylation are increased in Pro_TEX cells in the absence of ACLY. Therefore, ectopic overexpression of nuclear ACSS2 in CD8 T cells or pharmacological inhibition of ACLY leads to effective tumor control mediated by enhanced effector function and increased Pro_TEX formation. These findings establish a mechanistic link between metabolic rewiring of nuclear acetyl-CoA pools for in situ histone acetylation and CD8+ T cell fate determination.

Angel Ayala (I7)
GLUCOCORTICOID PRETREATMENT OF DONOR CELLS PRIOR TO ALLOGENEIC TRANSPLANTATION IN MICE REDUCES GRAFT-VERSUS-HOST DISEASE

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Bone marrow transplantation (BMT) is a potential curative therapy for many blood disorders, however, it remains underutilized due to major complications, namely graft failure and Graft-versus-Host disease (GvHD). In GvHD, donor immune cells become activated and attack the patient's tissues. The most common initial treatments are immunosuppressives, like glucocorticoids (GCs) which aim to reduce the severity of GvHD and prevent death. Despite the optimized GC-regimen, many patients succumb to GvHD-related death, thus highlighting a need for novel strategies which make BMT a safer treatment option. Previous research demonstrated that GC-pretreatment of human cord blood stem cells enhanced their engraftment upon transplantation into mice, however, they did not assess their impact on GvHD. Since GCs are immunosuppressive, we reasoned that treating BM and spleen cells would reduce the reactivity of T cells being transplanted. In our study, we hypothesized that pretreatment of donor cells prior to transplantation would reduce GvHD severity in mice that receive a BMT. Strikingly, we observed that GC treatment killed T cells in vitro but were able to repopulate following transplantation and appeared less alloreactive. We observed reduced GvHD in mice that received GC treated cells when compared to mice that received vehicle treated cells. Moreover, GC treatment induced apoptosis of conventional T cells while sparing regulatory T cells, suggesting a potential mechanism by which GvHD is reduced in our model. Our results implicate an important role for regulatory T cells in maintaining allogeneic tolerance and highlighting a potential strategy which would make BMT a safer therapeutic.

Kayla Frank (I8)
Novel mechanisms of immune regulation in acute influenza infections

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Influenza viruses cause both seasonal endemic infections—which affect 5-15% of the world's population and lead to about 650,00 annual deaths—and periodic pandemics. Much emphasis is placed on adaptive immune responses to influenza by B and T cells, but several studies have found NK cells are indispensable for host protection. Although we know that NK cells protect against influenza A virus (IAV) infections, the mechanisms by which IAV infection regulates NK cell activation and function are not well understood. Further, we know that the programmed death (PD) pathway inhibits NK and T cell effector functions in cancer and chronic diseases, but its relevance in acute infections is currently unclear. Our studies identify a Mast Cell

(MC)-derived IL-10-dependent mechanism promoting upregulation of PD-1, -L1 and -L2 on NK cells in influenza infections. The presence of Mast Cells, MC-derived IL-10 or NK cell PD-L1 expression is associated with decreased survival in T and B cell deficient Rag1KO mice. However, IL-10 and PD-L1 deficiencies do not improve survival outcomes in Rag1WT mice, and we observe increased immune cell infiltration and lung damage upon infection in these mice. Our future work will investigate the role of the IL-10 and PD pathways in parallel regulation of NK and T cells during IAV infections and will identify therapeutic strategies to harness the immune system to fight influenza virus infections without promoting harmful immunopathology.

Vanessa Delcroix (I9)
SINGLE CELL ATLAS REVEALS CHANGES IN IMMUNE LANDSCAPE OF THE AGING LACRIMAL GLAND

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Dry eye disease is a public health issue affecting millions of Americans and an economic burden for the healthcare system. Aging is the major risk factor for dry eye caused by a chronic inflammation of the lacrimal gland (LG). Current treatments alleviate symptoms but fail to restore LG function.

To understand the changes affecting LG cell populations during aging, we generated the first single cell atlas of the LG comparing "young" (2 months) and "old" (20 months) mice. Unsupervised clustering evidenced that even young LGs contained many tissue-resident immune cells, thus illustrating the role of LG in immunosurveillance upon environmental and microbial insults. Aging induced significant changes in almost all LG cell populations. Our results show new clusters of activated T and B lymphocytes in old mice, with some individual disparities between animals regarding B cell types. To increase the resolution of LG immune landscape, we sorted the CD45+ cells from young and old mice and analyzed them by scRNA-seq. Aging altered the composition of macrophage subtypes and LG sections revealed the presence of giant multinucleated cells in the old LG. Analysis of intercellular communication between clusters showed that macrophages closely interact with many cell types (epithelial cells, fibroblasts, immune cells) and that some signals including MIF (Macrophage migration inhibitory factor) are significantly remodeled by aging.

Altogether, our study offers new insights into the mechanisms of aging and evidence changes in interactions between lacrimal gland epithelial cells and the immune system that may promote the development of dry eye.

Yuhui Sunny Luo (I10)

LONG-DISTANCE COMMUNICATIONS FROM PERIPHERAL CD8 T CELLS TO BRAIN MEDIATES APPETITE LOSS DURING A VIRAL INFECTION

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Bi-directional communications exist between the immune system and the brain in healthy and diseased states. However, little is known about the mechanisms of such interactions. For the first time, we uncover that CD8 T cells are required for appetite loss caused by a sustained viral infection with lymphocytic choriomeningitis virus (LCMV) clone 13 (Cl13) in a mouse model. Furthermore, the activation of two brain regions with established roles in regulating eating behaviors, oval bed nucleus of stria terminalis (ovBNST) and nucleus of solitary tract (NTS), is associated with the appetite loss and is regulated by CD8 T cells. Intriguingly, we find that the induction of appetite loss doesn't require CD8 T cells to exit lymphoid organs, despite the abundance of infiltrated CD8 T cells in the brain during appetite loss. These results suggest that CD8 T cells in the periphery convey appetite-reducing signals to specific neurons in the brain via long-distance communications.

Sudhasini Panda (I11)

Identification and characterization of proteome-wide T cell epitopes from Mtb in active tuberculosis infection

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Tuberculosis caused by Mycobacterium tuberculosis is one of the leading causes of death from a single infectious agent. Identifying dominant epitopes and comparing their reactivity in different states of tuberculosis (TB) infection can help in designing both diagnostics and vaccines. We performed proteome-wide screen of 21,220 Mtb derived peptides in 21 mid-treatment active

TB patients using IFN-γ fluorospot assay. The overall breadth of IFN-γ responses in active TB recognized an average of 10 individual epitopes. In total, we identified 175 individual epitopes, and 37 of those (21.1%) were recognized by 2 or more individuals. These responses were predominantly directed against antigens part of the cell wall and cell processes category. We also found differential reactivity in active TB and LTBI cohorts in terms of clusters of antigens recognized and recognition of TB vaccine candidate antigens. Interestingly, we have identified 9 novel antigens, which have not been described as antigens for Mtb in the past. Additionally, we created a pool of peptides only recognized by individuals with active TB. This active-specific pool has shown higher T cell response in a new active TB cohort compared to individuals with LTBI suggesting a better pool for distinguishing these disease states. Therefore, identifying disease state-specific epitopes and antigens will help in the development of diagnostics, vaccine candidates and correlates of protection.

Tatyana Dobreva (I12)
LEVERAGING LOW VOLUME CAPILLARY BLOOD SELF-COLLECTION TO ENABLE OUT OF CLINIC IMMUNE STUDIES USING SINGLE CELL RNA SEQUENCING

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We describe an experimental and computational platform for performing decentralized immune studies. Small samples of capillary blood (0.1-0.5 mL) can be collected by participants at home with a commercially available push button device. After the participant collects and sends the blood samples to the lab, we perform a multiplexed single-cell RNA sequencing protocol optimized for low blood volume inputs. We have also developed computational infrastructure for automated data processing and streamlined data analysis that enables handling data from large numbers of samples. We are using this platform across multiple studies with academic and commercial partners to create a growing capillary and venous blood single-cell RNA sequencing (scRNA-seq) database, which currently contains over 300 samples and 2.4 million cells with a resolution of 40 cell subtypes.

We provide technical validation of the platform with different data modalities, including single cell and single nuclei RNA-seq, as well as different kinds of input samples, including cryopreserved PBMCs from venous and capillary blood, and nuclei isolated from frozen buffy coat. We also demonstrate that capillary blood gene expression of whole blood samples preserved over 24h on ice is comparable to that of the same samples processed fresh, which allows studies to leverage cold-storage shipping rather than having to process samples

immediately after collection. We also describe initial results of biological studies performed with this platform, including single-point collection and longitudinal sampling.

We are interested in starting new clinical research collaborations leveraging this platform, with a focus on autoimmune diseases and understanding immune health, and a focus on experimental designs that leverage the potential for longitudinal sampling and ad-hoc self-sampling by participants at home during adverse events.

Philip K Farahat (I13)
MODELING DYSTROPHIN IMMUNITY

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The immune system poses a potential barrier to the efficacy of adeno-associated virus (AAV)-based gene therapy for Duchenne muscular dystrophy (DMD). More recently, ongoing clinical trials reveal that some patients develop immunity to the therapeutic transgene, micro-dystrophin (μdys). Although early data has shown that systemic gene therapy leads to widespread expression of udys in muscle, it is still unclear whether this immunity will compromise efficacy and stability of gene therapy for DMD patients. Hindering progress in this area is the lack of reproducible experimental systems to study dystrophin immunity. Here we report the development of a model of inducible dystrophin immunity in dystrophic mice. The immunization of mice with in silico-predicted dystrophin peptides induced dystrophin-specific T cells. The specificity of the response was confirmed by quantifying the induction of IFNg+CD44+ CD8+ or CD4+ T cells in a recall antigen assay. Further, we show through depletion studies that Regulatory T cells (Tregs) constrain the induction of dystrophin-specific T cells. In future studies we will determine whether the induction of dystrophin-specific T cells affects the stability of muscle fibers that re-express dystrophin following AAV-dys gene therapy. Further we will use this model to examine if Treg-inducing biologics suppress dystrophin immunity and improve the efficacy and long-term stability of AVV-udys gene therapy.

Anthony Kusnadi (114)
SARS-COV-2 SPIKE-REACTIVE T CELLS ARE PREVALENT IN HUMAN LUNGS

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The lungs are typically the main organs affected by infection with SARS-CoV-2 and severe COVID-19. Little is known, however, about the SARS-CoV-2-reactive T cells in the lungs because of the difficulty of obtaining this tissue from human subjects. Here, we analyzed lung tissue from a cohort of 29 patients who underwent lung surgery between February and August 2021, after the introduction of vaccination. By using single-cell RNA and TCR sequencing, we detected SARS-CoV-2 spike protein-reactive CD8+ and CD4+ T cells in the lung of 28/29 individuals. Tissue resident memory cells (TRM) comprised a substantial fraction of both the lung CD8+ and CD4+ T cells we analyzed. Also, a subset of highly-expanded SARS-CoV-2-reactive CD4+ T cells with features of cytotoxic CD4+ T cells (CD4-CTLs) was identified in 19 individuals. Our data demonstrate that SARS-CoV-2-reactive lung TRM cells and CD4-CTLs are likely to be prevalent in the population, and these cells may play an important role in protection against severe disease.

Kenna Nagy (I15)
THERAPEUTIC ANTI-GLYCAN ANTIBODIES AGAINST ANTIBIOTIC RESISTANT STAPHYLOCOCCUS AUREUS

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Antibiotic resistance threatens clinical control of bacterial infection as genetic adaptability of microbes outpaces small-molecule development. The combinatorial diversity of antibodies offers a solution to this problem. Under normal circumstances the polymeric glycans of bacterial surfaces avoid adaptive recognition by not binding to MHC molecules, thus being T cell independent and targeted only by low affinity IgM responses. Glycoconjugate vaccines have been developed to overcome this limitation and have shown success for four pathogens. However, this approach has failed to address most bacterial infections including antibiotic resistant Staphylococcus aureus. To overcome limitations in current conjugate vaccine approaches, we optimized anti-glycan B cell help through three convergent prongs: exploiting cognate T cell help, using a B cell-centric adjuvant, and using synthetic minimal glycans. A prototype of a next generation conjugate vaccine was built on a virus-like particle and used to produce nanomolar affinity anti-glycan responses in proof-of-concept studies against eight bacterial glycans. Focusing on Staphylococcus aureus three glycan targets have been selected and used to produce monoclonal antibodies. These antibodies were characterized structurally, biophysically, and by B cell sequencing to confirm high affinity, maturation, and specificity towards the intended targets. The potential therapeutic benefits are currently being tested in three preclinical mouse models: skin, lung, and systemic infection, using passive and active immunization. These preliminary studies have shown efficacy of some of these antibodies.

Lucia Lurman (I16)
EXPOSURE TO FARMYARD MICROBES IN EARLY LIFE OVERCOMES THE IL-2-DEPENDENT GERMINAL CENTER RESTRICTION DURING RESPIRATORY VIRAL INFECTION

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T follicular helper cell (TFH) dependent antibody responses are critical for long term immunity, but can be compromised early in life. Respiratory syncytial virus (RSV), the most common cause

of bronchiolitis in infancy, promotes limited antibody responses in early life when compared to adults leading to increase susceptibility to reinfection. Using a murine model, we recently showed that RSV limits T-cell dependent antibody responses in early life via an IL-2 dependent pathway. Microbiota, and microbiotal products, however have been reported to be capable of promoting antibody-mediated immunity, but whether this is effective in infancy is unknown.

Stimulation of bone marrow derived dendritic cells (BMDC) revealed that the common farmyard microbe Acinetobacter Iwoffii F78, and to a lesser extent LPS, but not RSV nor TLR3, 7 or 9 agonists could promote DC-derived secretion of the pro-TFH factors IL-6 and CD25. Co-culture of A. Iwoffii stimulated BMDCs with naïve CD4+ T cells promoted TFH like differentiation, a process RSV stimulation of BMDCs inhibited. Critically, neonatal exposure to A. Iwoffii enhanced cDC2 frequency, and their expression of CD25, which was accompanied by an increase in TFH, GC B and plasma cell responses to RSV infection in vivo. Together this data highlights the ability of microbiota to boost antibody mediated immunity in early life by overcoming the IL-2-dependent restriction GCs undergo after neonatal viral infection.

Kasia Brzezicka (117)

SUPPRESSION OF AUTOIMMUNE RHEUMATOID ARTHRITIS WITH HYBRID NANOPARTICLES THAT INDUCE B AND T CELL TOLERANCE TO SELF-ANTIGEN

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Autoimmune diseases affect over 4% of the world's population. Treatments are generally palliative or use broad spectrum immunosuppressants to reduce symptoms and disease progression. In some diseases antibodies generated to a single autoantigen are the major cause of pathogenic inflammation, suggesting that treatments to induce tolerance to the autoantigen could be therapeutic. Here we report the development of hybrid nanoparticles (NPs) that induce tolerance in both T cells and B cells. The nanoparticles comprise a lipid monolayer encapsulating a PLGA core loaded with rapamycin (RAPA) that promotes development of regulatory T cells (Tregs). The lipid monolayer displays the protein antigen and a ligand of the B cell inhibitory co-receptor CD22 (CD22L) that act together to suppress activation of B cells recognizing the antigen. We demonstrate that the hybrid NPs decorated with ovalbumin (OVA) elicit tolerance to OVA in naïve mice, as judged by low OVA-specific antibody titers after the challenge. In the K/BxN mouse model of rheumatoid arthritis (RA) caused by B and T cell

dependent responses to the self-antigen glucose-6-phosphate-isomerase (GPI), we show that GPI hybrid nanoparticles delay development of disease, with some treated mice remaining arthritis-free for 300 days. We provide evidence that the mechanism of RA suppression involves induction of B cell tolerance as measured by low anti-GPI antibodies and decreased plasma cell populations, and T cell tolerance as measured by increases in Tregs. The results show the potential of this versatile nanoparticle platform for inducing immune tolerance to a self-antigen and suppressing autoimmune disease.

Yingcong Li (118)
PROFILING PATHOGENIC T CELLS IN COLITIS PATIENTS AND MICE

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Ulcerative colitis (UC) is characterized by an exacerbated immune response in the large intestine, but the pathogenic immune cell remains unknown. To profile the pathogenic T cells in UC, we isolated T cells from colon biopsies of healthy donors and UC patients. Single cell RNA seq analysis indicated that CD4 and CD8 T cells from colitis patients both showed an increased signature consistent with production of IL-17 and related cytokines (TH17/TC17), and T follicular helper (TFH) transcriptomic features. To further address the mechanism of pathogenesis we used the T cell transfer mouse colitis model, we compared the T cells from the colon lamina propria of healthy C57BL/6 mice and colitic Rag1 KO mice transferred with T cells. Consistently, mouse CD4 and CD8 T cells from mice with colitis both showed increased TFH transcriptomic features and clonal expansion. To further validate the pathogenic function of TFH cells, we adaptively transferred Bcl6 KO T cells in to Rag1 KO mice. Compared to WT T cells, Bcl6 KO CD4 T and CD8 T cells induced much less severe colitis with lower expansion and lower pathogenic TH17/TC17 populations. In summary, our study unveiled the pathogenic function of BCL-6 expression in T cells in colitis, a connection between BCL-6 and T cell expansion in the intestine and a selective effect on IL-17 producing T cells, which could lead to new therapies to the disease. The extent to which TFH functions such as IL-21 production contribute to disease or prevention of disease remains to be determined.

Teha Kim(119)

M2E-SPECIFIC MONOCLONAL ANTIBODY COCKTAILS AGAINST INFLUENZA A VIRUS ARE HIGHLY PROTECTIVE, UNIVERSALLY EFFECTIVE, AND VIRAL ESCAPE MUTANT RESISTANT VIA FC-MEDIATED EFFECTOR FUNCTIONS

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Influenza virus has pandemic potential, seasonal epidemics burden the human population, and viral resistance has developed to all available treatment options. A highly protective, universally effective, and escape mutant-resistant therapeutic agent is desperately needed. We developed an effective prophylactic cocktail agent using three Matrix Protein 2 ectodomain-specific monoclonal antibodies (M2e-mAbs) distinct in their M2e epitopes. A low dose of this cocktail protected mice challenged with laboratory and pandemic influenza A virus (IAV) strains significantly better than single M2e-mAb treatments. No viral escape mutants developed in immunocompetent and immunodeficient mice after viral passaging in the presence of single, cocktail, or alternating M2e-mAb treatments. The M2e-mAbs with mouse IgG2a isotype exhibited higher protection than with mouse IgG1 isotype in mice, and the isotype-optimized cocktail therapeutically protected mice challenged with a lethal dose of a laboratory IAV strain. Notably, we discovered that the M2e-mAbs are non-neutralizing but confer full protection via the engagement of activating FcγRs signaling during IAV infection in mice. Our study will critically shape future influenza-therapeutic development.

Greet Verstichel (120)

Pre-TCR signals distinguish between conventional naïve T cells and innate T cells

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In contrast to naïve T cells, thymocytes expressing self-specific TCRs can differentiate in the thymus to innate effector T cells. Although the degree of self-specificity of the full TCR is crucial in determining lineage outcome, increasing evidence suggests that timing of TCR activation and early developmental cues could be involved as well. Here, we aimed to investigate the impact of pre-TCR signaling on T cell fate and functional outcome. The pre-TCR consists of the TCR chain, bound to an invariant pre-TCR chain (pT). pT can be expressed as 2 isoforms, one containing an immunoglobulin-like domain, and a short isoform lacking this domain. We created a mutant line expressing pT solely as a short isoform from the endogenous locus. These mice show impaired proliferation and glycolytic burst as they progress through beta-selection. Surprisingly, not only early stage progression was altered, but the mutation profoundly reduced the number of conventional naïve T cells while leaving innate self-specific T cell numbers intact. T cell lineages like DN T cells and Eomes+ CD8+ T cells were favored in the absence of the long pT isoform. Additionally, to test the role of pT in the selection of pathogenic self-specific T cells, we used the NOD mouse model and introduced a similar mutant line (NOD mice expressing pT solely as a short isoform). The absence of long pT isoform accelerated auto-immune disease onset, suggesting an impact on the selection of pathogenic self-specific T cells as well. These findings reveal the impact of pre-TCR signals on the selection of both homeostatic self-specific T cells as well as pathogenic self-specific T cells in an autoimmune context.

Abraham Phung (I21)
Liposome Encapsulation of Adenoviruses Protects against Pre-Existing Humoral Response

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Oncolytic adenovirus therapy is an emerging treatment for various cancers. The treatment involves infecting and killing cancer cells with an adenovirus, engineered to conditionally replicate in cancer cells, inducing cell death, but remaining harmless to healthy cells. While adenovirus therapies have been approved for intratumor administration, systemic adenovirus treatments have shown limited success due to pre-existing antibodies in the human blood that neutralize the virus. To overcome this shortcoming, a liposome coating procedure has been developed that protects the adenovirus from pre-existing neutralizing antibodies in human

blood. In this study, it is shown that a liposome with optimized formulation protects the encapsulated adenovirus from neutralizing antibodies.

The liposomes are composed of four components: DOTAP, Cholesterol, DSPE-PEG2000, and DSPE-PEG2000-Folate, and are used to encapsulate non-replicating adenovirus vectors expressing the green fluorescent protein (Ad-GFP) transgene and. Protection against neutralizing antibodies was studied by measuring fluorescence from HEK293 cells transduced by liposome-encapsulated adenovirus (DfA) or unencapsulated Ad-GFP in the presence of cell growth media or media spiked with neutralizing serum at 37℃. Experiments showed that DfA demonstrated 8x increased transduction efficiency in human serum compared to unencapsulated Ad-GFP. Optimization experiments demonstrated that cholesterol and DSPE-PEG2000 are critical for serum stability, where removal of cholesterol reduced transduction efficiency in serum by 2x and removal of DSPE-PEG2000 reduced it by 3x. Hypothetically, cholesterol provides protection by improving lipid packing in the liposome while PEG produces a barrier around the liposome. Additional in vivo studies to demonstrate antibody protection in mice are underway.

Katherina Dueker (I22)
TARGETED ISOLATION OF DIVERSE HUMAN PROTECTIVE BROADLY NEUTRALIZING ANTIBODIES AGAINST SARS-LIKE VIRUSES

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After its emergence in 2019, SARS-CoV-2 and its emerging variants of concern (VOCs) have caused millions of infections and deaths worldwide. Like SARS-CoV2 and its predecessor SARS-CoV-1, future spillovers of sarbecoviruses pose a significant threat to global health.

Due to the ongoing emergence of SARS-CoV-2 VOCs such as Omicron and the potential of future coronavirus pandemics, the development of effective vaccines that elicit broad and potent pan-sarbecovirus responses is paramount.

Here we show the targeted isolation and characterization of a large panel of sarbecovirus-reactive broadly neutralizing antibodies (bnAbs) from two SARS-CoV-2 recovered-vaccinated donors. Select bnABs were potent neutralizers of SARS-CoV-2 VOCs, including Omicron. A prophylaxis challenge model using a selection of bnAbs showed robust in vivo protection against a panel of SARS-like coronaviruses. Epitope mapping of the isolated sarbecovirus bnAbs revealed binding to epitopes in a relatively conserved region of the receptor binding domain which conferred neutralization breath.

The panel of bnAbs characterized here provides a wealth of data to inform targeted pan-sarbecovirus vaccine design as well as the design of antibody-based prophylactic or therapeutic agents to combat current and future coronavirus pandemics.

Sara De La Matta (I23)
CYTOTOXIC CD4+ TISSUE-RESIDENT MEMORY T CELLS ARE ASSOCIATED WITH ASTHMA SEVERITY

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Severe asthma represents a distinct endotype that is refractory to corticosteroid treatment. To determine T cell subsets and effector molecules that drive pathogenesis of severe asthma, we performed single-cell transcriptome analysis of airway CD4+ T cells isolated from bronchoalveolar lavage (BAL) samples from 30 patients with mild and severe asthma. We observed striking heterogeneity in the nature of CD4+ T cells present in asthmatics' airways with tissue-resident memory (TRM) cells making a dominant contribution. Notably, in severe asthmatics, a subset of CD4+ TRM cells (CD103-expressing) was significantly increased, comprising nearly 65% of all CD4+ T cells in the airways of male patients with severe asthma when compared to mild asthma (13%). This subset was enriched for transcripts linked to T cell receptor (TCR) activation, cytotoxicity and, following stimulation, pro-inflammatory non-TH2 cytokines (CCL3, CCL4, CCL5, TNF, LIGHT) that could fuel persistent airway inflammation and remodeling. Our findings indicate the need to look beyond the traditional T2 model of severe asthma to better understand the heterogeneity of this disease.

Nicolas Thiault (I24)

Intestinal Intra-Epithelial Natural CD4 CTLs Arise From CD25+ Treg Precursors Upon Thymic Cognate Selection.

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The CD4 T helper cell lineage is initially established and controlled in the thymus by the transcription factor, ThPOK. Sustained expression of ThPOK by mature T cells in the periphery maintains the CD4 T helper commitment and differentiation to different helper fates (Th1, Th17...). However, our group has observed that a significant fraction of CD4 T cells in the intestine are reprogrammed into cytotoxic CD4 T lymphocytes (CD4-CTL). Repeated stimulation of mature CD4 T cells induces the cessation of ThPOK transcription followed by the expression of cytotoxic cell hallmarks (Runx3, granzyme B). How CD4-CTL reprogramming occurs and the environmental cues that are leading this process remain not well understood.

Using a transgenic mouse model, which combines a ThPOK reporter gene with a ThPOK fate mapping construction, we found a population of CD4-CTLs that has never turned on the ThPOK locus. We showed that these CD4-CTLs arise from precursors that develop in the thymus upon a cognate TCR stimulation. Finally, using an in-vitro culture system, we demonstrated that natural CD4-CTLs share a common precursor with Foxp3+ Tregs. Moreover, inhibiting IL-2-dependent de novo differentiation of Tregs greatly favors the development of natural CD4-CTLs.

These findings show that the CD4-CTL population is heterogenous with two distinct origins. During thymic development, natural CD4-CTLs and Treg derive from a common precursor, which commits into either lineage depending on the IL-2 cue.

Phoi Tiet (I25)

EQ101 and EQ102, selective multi-cytokine antagonists, inhibit cytotoxic T cell and NK activity

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Introduction: CTLs and NK cells play important roles in the pathogenesis of various autoimmune and inflammatory diseases, and the activity of these cells are driven by multiple cytokines, including IL-2 and IL-15. EQ101 and EQ102 are selective multi-cytokine inhibitors that target IL-2/IL-9/IL-15 signaling and IL-15/IL-21 signaling, respectively. Here we sought to investigate the impact of these inhibitors on the activity of human cytotoxic CD8+ T cells (CTLs), effector/memory CD4+ T cells, and cytotoxic NK (CD56dimCD16bright) cells.

Methods: Human PBMCs were stimulated with anti-CD3 and CD28 antibodies, rested overnight, labeled with a proliferation dye (Cell Trace Violet), and then stimulated with IL-15, IL-2 or IL-9 for 72hrs. Following stimulation, supernatant was collected for detection of CD8, NK and CD4 relevant cytokines and cells were analyzed for surface activation markers by flow cytometry.

Results: Treatment of PBMCs with EQ101 or EQ102 resulted in a ~50% decrease in proliferation of CD4 T cell subsets, and ~40% decrease of CD8 subsets. In addition, a ~2-fold reduction of the cytotoxic NK cell population was observed with EQ101 and EQ102 treatments. Analysis of the supernatant revealed EQ101 and EQ102 inhibited the production of CD4/CD8 relevant cytokines IL-6, IL-10, IFN-γ, IL-22, TNF-α and Fas/FasL. Furthermore, production of perforin, granzyme A, granzyme B, and granulysin, secreted by CTLs and NK cells, were also significantly reduced. These results demonstrate that the use of selective inhibitors for multiple key cytokines can be an effective strategy in treating T and NK cell-driven autoimmune diseases.

Poster Session II:

Helen McRae (II1)

TARGETING THE BAF-NUCLEOSOME REMODELING COMPLEX IN MACROPHAGES TO BOOST RESPONSE TO IMMUNOTHERAPY

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Tumor-associated macrophages (TAMs) are associated with poor prognosis in multiple solid tumor types and can block the efficacy of existing immunotherapies. Targeting signaling pathways that re-program TAMs from an immunosuppressive "tumor-promoting" to a pro-inflammatory "tumor-fighting" phenotype have shown promise for treating cancer in pre-clinical models. However, in order to find the best method to reprogram macrophages, we need a better understanding of the molecular pathways that control tumor-associated macrophages. The BAF (Brg/Brahma associated factor) complex is a multi-subunit nucleosome-remodeling complex that controls cell type-specific chromatin accessibility and gene expression, including regulating inflammatory gene networks. To study the role of the BAF complex in tumor-associated macrophages, we are using myeloid-specific Cre-drivers to delete BAF complex subunits and studying the effects on macrophage gene expression, tumor growth, and immune function. We are utilizing a combination of flow cytometry, functional genomics and epigenomic techniques in both in vivo and in vitro models. We hypothesize that manipulation of BAF-complex function in myeloid cells could be a potential method to increase response to existing immunotherapies.

Kennidy Takehara (II2)
UNDERSTANDING PROSTATE RESIDENT MEMORY T CELLS

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The prostate is a barrier tissue in contact with a unique microbiome, including bacterial and viral pathogens, which can cause inflammation conducive to development of prostate cancer. However, the resident immune cells within the prostate tissue, in particular CD8+ resident memory T cells (TRM), have not been described or characterized. A detailed understanding of regulatory programs and transcriptional networks that govern T cell adaptation to the prostate will inform the ability to 'program' tissue-tailored immune responses, where immune cells that promote or regulate inflammation can be transcriptionally engineered for trafficking to, retention in, and function within a particular tissue. Therefore, understanding the tissue-specific adaptation of T cells to the prostate microenvironment is essential to informing

prostate-specific disease, including prevention and treatment of prostate inflammation, prostate tumorigenesis, and immunotherapy for prostate tumors. We have validated a model to study prostate TRM and have developed tools to study prostate CD8+ T cells. We find CD8+ T cells within the prostate epithelium up to 500 days following LCMV infection, demonstrating the generation of a stable TRM population. We find prostate TRM have unique features, including expression of functional nuclear AR and unique dynamics of retention molecule CD103. We hypothesize these characteristics are part of prostate-specific TRM adaptation. We expect that identifying the biological adaptations of CD8+ T cells to prostate residency will provide translational information pertaining to prostate inflammation, prostate tumorigenesis, and immunotherapy for prostate tumors.

Amir Ferry (II3)
IDENTIFYING NOVEL REGULATORS OF TISSUE-RESIDENT MEMORY CD8+ T CELLS

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Therapeutic modulation of the immune system via immune checkpoint blockade (ICB) and CAR-T therapy has highlighted the clinical relevance of CD8+ T cell transcriptional states linked to tissue surveillance, memory potential, and cytotoxicity. Tissue-resident memory T cells (TRM) are a recently described subset which exhibit organ-specific and -conserved adaptations that enable rapid protective responses upon encountering pathogens and malignant cells. Numerous studies have shown qualitative differences in patient tumor infiltrating CD8+ T cells, several of which demonstrate the positive prognostic value of CD103+ expression or the enrichment of TRM gene signatures. Therefore, programming TRM-associated adaptations represents an opportunity to develop new, targeted immunotherapy strategies. To identify novel regulators of TRM biology, we performed a bioinformatic screen using several published bulk and scRNA-seq datasets examining CD8+ T cells during acute infection and cancer. High XCL1 expression was conserved across all subpopulations. This chemokine selectively recruits XCR1+ cDC1 cells, which play a critical role in antigen cross presentation and NK cell survival. However, the role of this signaling axis in TRM biology is not well understood. To address this, we have employed genetic models to conditionally deplete XCR1+ cDC1, and other tools to regulate CD8+ T cell XCL1 expression. I will share our insights into the regulation of this axis, its role in TRM biology, and its application in anti-tumor immunity.

Nicole Scharping (II4)

FROM EXHAUSTION TO MEMORY: ALTERING T CELL FATE IMPROVES IMMUNOTHERAPEUTIC RESPONSES TO CANCER

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In cancer, CD8+ T cells have the power to target and kill tumor cells with precision but often fail due to chronic activation from the immunosuppressive tumor microenvironment (TME), differentiating into a dysfunctional cell state known as exhaustion. In healthy tissues, T cells differentiate into tissue-resident memory cells (TRM) in response to infection, which remain lodged in tissues to provide protection from reinfection. When TRM cells are found in patient tumors, they correlate with improved responses to immunotherapy and patient outcomes. Understanding how to manipulate T cell fates to avoid exhaustion and favor TRM characteristics could benefit cancer immunotherapy. To explore differences between these cell states, we screened the core TRM gene expression signatures for genes downregulated as T cells differentiate to terminal exhaustion. Targets were then overexpressed in antigen-specific T cells and adoptively transferred into tumor-bearing mice for analysis. Interestingly, many genes related to protein regulation were identified, including multiple E3 ubiquitin ligases with not well-described functions. When these genes are individually overexpressed in tumor-specific T cells, we found the transduced T cells express less inhibitory receptors and showed enhanced anti-tumor function: increased accumulation in the TME, upregulation of TRM markers, and polyfunctional cytokine production, which resulted in controlled tumor growth and increased mouse survival in multiple cancer models. These results highlight the understudied field of negative regulation of T cell function by protein degradation in T cell differentiation fate and function, and uncovers potential gene targets for immunocellular therapies to favor functional T cell fates in cancer.

Ziyan Xu (II5)

SCAVENGER RECEPTOR CD36 REGULATES INFLAMMATORY AND TYPE-I INTERFERON RESPONSES IN TUMOR-ASSOCIATED MACROPHAGES

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Macrophages are highly plastic cells of innate immune system with diverse functions in tissue homeostasis. Despite their abundance in the tumor microenvironment (TME), the functions of tumor-associated macrophages (TAMs) and their regulation mechanisms remain largely unknown. Macrophages are also key players in metabolic processes including lipid metabolism. Since TME has recently been characterized as a "lipid-rich" environment, we are interested in understanding how lipids in TME regulate TAMs' metabolism and their functions.

In this research, we firstly characterized the lipid metabolic phenotype of TAMs in several mouse tumor models. We found the tolerogenic subset of TAM (with high expression of F4/80 and PD-L1) is lipid-laden and has greater ability to import lipids. We found F4/80hi TAMs highly express scavenger receptor CD36 which binds oxidized low-density lipoprotein (oxLDL) in TME. Using germ-line (Cd36-/-) and myeloid-specific knockout (Cd36flox/flox x Csf1r-Cre) mouse models, we observed that CD36-deficient TAMs decreased their uptake of oxLDL, and decreased expression of immunosuppressive molecules PD-L1 and CD206, and have higher secretion of inflammatory cytokines (TNF, IL-12, IL-1b) and IFNb. Moreover, this reprogrammed functional state of CD36-deficient TAMs leads to better tumor control. We further conducted single-cell RNA-seq and found inflammatory response and type-I interferon response pathways were upregulated in CD36-deficient TAMs. We also showed that knock-out of CD36 spontaneously upregulates IFNb production in macrophages in vitro; and IFNb production is critical for TAM-mediated tumor control in vivo.

In summary, our data suggests that scavenger receptor CD36 plays critical roles in functional reprogramming of tumor-infiltrating macrophages; and blockade of CD36 may have beneficial effects for cancer immunotherapy.

Dan Chen (II6)

A critical partnership between microglia and CD4+ T cells promotes anti-tumor immunity to glioblastoma

The limited utility and efficacy of immunotherapies against glioblastoma is due to a lack of understanding of the unique tissue-specific interactions between the central nervous system (CNS) and the immune system. Here, we identified the types of immune cells providing protection against glioblastoma in the brain by using physiologically relevant preclinical murine models. We demonstrated that stimulating CD4+ 'helper' T cells with the checkpoint inhibitor αCTLA-4 prolonged the survival of glioblastoma tumor-bearing mice, and such improved survival depended on CD4+ rather than CD8+ T cells. Additionally, anti-tumor immunity by CD4+ T cells

required the support from another immune cell type – microglia, which directly interacted with CD4+ tumor-infiltrating lymphocytes (TILs) via MHC-II to sustain their anti-glioblastoma activity. Simultaneously, CD4+ T cells regulated the activation and tumor sensing capacity of microglia in an IFNγ dependent manner, promoting tumoricidal activities of microglia through Axl/Mer mediated phagocytosis of apoptotic glioma cells. Thus, maintaining such mutual partnership between microglia and CD4+ T cells appears to be a key driver for glioblastoma control, providing paramount insights for developing novel therapeutic strategies against such a formidable type of cancer.

Spencer Brightman (II7)

TCR-engineered neoantigen-specific CD4+ T cells mediate immunotherapy of an MHC class II-negative murine squamous cell carcinoma

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The expansion of neoantigen (NeoAg)-specific T cells often accompanies clinical responses to immunotherapies such as immune checkpoint blockade (ICB), adoptive cellular therapy (ACT), and personalized cancer vaccines (PCV), highlighting their importance for antitumor immunity. While NeoAg-specific CD8+ T cells have been studied, the role of NeoAg-specific CD4+ T cells is less well understood. To study the antitumor mechanisms of NeoAg-specific CD4+ T cells, we sorted single CD4+ T cells specific for a mutated clathrin heavy chain epitope (Cltc H129Q) expressed by the murine squamous cell carcinoma VII (SCC VII) tumor. T cell receptor (TCR) sequencing analysis revealed the presence of four distinct TCR clonotypes and expression of these TCRs in primary cells was sufficient to confer preferential recognition of Cltc H129Q as compared to the corresponding wildtype Cltc epitope. Despite differences in TCR avidity, both low and high avidity CD4+ T cells were capable of proliferating to similar degrees in response to tumor antigen in vivo and enhancing primary tumor immunity in a CD8+ T cell- and CD40L-dependent manner. ACT with Cltc H129Q specific CD4+ T cells also synergized with cyclophosphamide treatment to reduce tumor burden in mice with established tumors. Overall, these findings illuminate the mechanistic role of NeoAg-specific CD4+ T cells in tumor immunity, provide insights into the impact of TCR avidity on the helper functions of CD4+ T cells, and highlight the therapeutic potential of ACT with TCR-engineered CD4+ T cells.

Amanda Gavin (II8)

Multiple nucleic acid sensing pathways are regulated by the exonucleases PLD3 and PLD4

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Phospholipase D3 (PLD3) and PLD4 polymorphisms have been associated with several important inflammatory diseases. Mice deficient in both Phospholipase D3 (PLD3) and PLD4 endolysosomal nucleases develop a spontaneous, fatal autoinflammatory syndrome reminiscent of human primary hemophagocytic lymphohistiocytosis (HLH). HLH is characterized by inflammatory liver damage and the production of high levels of cytokines. Survival could be rescued by genetic co-deficiency of TLR9, although abnormalities remained. We show that PLD3 and PLD4 digest ssRNA in addition to ssDNA as reported previously and Pld3–/–Pld4–/– mice accumulate small ssRNAs. Responses to TLR7 and TLR13 ssRNA agonists are exaggerated in Pld3–/–Pld4–/– primary dendritic cell cultures compared to controls, suggesting that both RNA and DNA sensing by TLRs contribute to the excessive inflammation. Unc93b13d/3dPld3–/–Pld4–/– mice, which lack all endosomal TLR signaling, still had elevated type I IFN and additional immunological perturbations triggered by the STING pathway. Our results show that PLD3 and PLD4 regulate both endosomal TLR and cytoplasmic/STING nucleic acid sensing pathways.

JR Shah (II9)

DEVELOPMENT OF ADENOVIRUS CONTAINING LIPOSOMES PRODUCED BY EXTRUSION VS HOMOGENIZATION: A COMPARISON FOR SCALE-UP PURPOSES

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Adenovirus (Ad) based vectors for gene therapy have demonstrated therapeutic efficacy for cancer treatment. Ad requires the coxsackievirus and adenovirus receptor (CAR) to enter cancer cells. Because of this requirement, naked Ad delivery is not promising for treating cancers that do not have CAR receptors. To overcome this challenge, Ad-encapsulated liposomes were developed that enhance the delivery of Ads and increase therapeutic efficacy.

1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) liposomes were synthesized by incorporating DSPE-PEG-2000, DSPE-PEG-2000-folate, and human serum albumin (HSA). Cationic empty liposomes were manufactured to which an anionic recombinant human Ad expressing green fluorescent protein (GFP) were added, which resulted in encapsulated Ad liposomes through charge interaction. Optimization of the liposome formula was carried out with an extrusion process, which is ideal for laboratory-scale small batches. Later, the optimized formulation was manufactured with a homogenization technique – a high shear rotor-stator blending, that is ideal for large-scale manufacturing. Efficient and spontaneous Ad encapsulation is critical for cell membrane fusion and transduction.

Comparative in vitro transduction analysis with a CAR deficient CT26 cell line-based model system used a fluorescence intensity plate reader. It confirmed that liposomes manufactured with both processes are equivalent in in vitro transduction performance despite the different processing techniques. These Ad liposomes increased the transduction efficiency by ~100x compared to the naked Ad (p=0.0089). Comparative physicochemical characterization of Ad liposomes was performed that included particle size, zeta potential, and Cryo-EM. Additional comparative storage stability studies and in vivo studies of Ad liposomes are underway.

Estefania Quesada Masachs (II10)
MACROPHAGE INFILTRATION IS INCREASED IN PANCREATA OF PATIENTS WITH TYPE 1 DIABETES

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Type 1 diabetes (T1D) is characterized by autoimmune destruction of insulin-producing beta cells within the islets of the pancreas, where macrophages are one of the predominant immune cell infiltrates. We used a supervised machine learning approach to characterize and quantify the distribution of macrophage populations throughout whole pancreatic sections from non-diabetic (n=5), autoantibody positive (Aab+, prediabetic) (n=5), and T1D (n=5) organ donors. Across the entire tissue, and across every pancreatic region of interest (islet, peri-islet and exocrine), macrophage infiltration was highest in donors with T1D. Within the pancreas, the peri-islet regions, in close proximity to islets, had the highest relative macrophage infiltration across all the groups independently of the disease status. However, in the extended spatial distribution analysis up to 200 µm from islet perimeter, we did not observe a clear density

gradient of macrophages for any of the groups. Additionally, a greater proportion of macrophages expressed HLA-II in and near islet regions of the T1D group. In the T1D cases, there was a strong and statistically significant correlation between the percentage of HLA-II+ macrophages in and around islets and the percentage of beta cells expressing HLA-II+, while no such correlation was observed when comparing these HLA-II+ beta cells with the percentage of macrophages. The close proximity of macrophages to the islets suggests that they interact and communicate with the beta cells even in normal conditions, and their higher density and level of activation in the T1D pancreas suggests that they may play a role in T1D initiation and progression.

Hannah Rattu Mandias (II11)
THE INVOLVEMENT OF PBRM1 IN ALVEOLAR MACROPHAGE DEVELOPMENT, METABOLISM, AND IMMUNE FUNCTION

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Pulmonary disorders like respiratory infections are one of the leading causes of death worldwide and result in millions of deaths annually. The immune system's initial line of defense against lung disease are alveolar macrophages (AMs), which play an important role in barrier immunity. In response to lung-specific cytokines like GM-CSF and TGF-β, fetal monocytes differentiate into immunosuppressive AMs that phagocytose apoptotic cells and pathogens. This response to the pulmonary microenvironment is regulated by epigenetic modifications, such as DNA methylation and chromatin accessibility. Notably, aberrant AM development and function is associated with lung malignancies such as respiratory infections, chronic obstructive pulmonary disease, and some types of lung cancer. The focus of this study is to investigate the epigenetic role of PBRM1, the defining subunit of the SWI/SNF family PBAF complex, in dictating AM development and function. We have shown that mice harboring a myeloid-specific deletion of PBRM1 exhibit reduced numbers of endogenous AMs. With RNA-sequencing, we have demonstrated that PBRM1-deficient AMs significantly upregulate genes involved in oxidative phosphorylation pathways, including the citric acid cycle and electron transport chain. This suggests that PBRM1 is an important regulator of AM homeostasis and metabolism. To further this work, I plan to elucidate the requirement for PBRM1 in AM development, chromatin remodeling, and host defense against influenza infection. In the future, this research will

enhance the understanding of epigenetic mechanisms governing PBRM1-dependent processes in lung immunity.

Gabriel Ascui-Gac (II12)
RAMP3 UNEXPLORED RELEVANCE FOR INNATE-T CELL IMMUNITY

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RAMP3 is a chaperone protein that aids in the transport, activity modulation and pharmacological switch of many G-protein coupled receptors (GPCRs), such as chemokine receptors. GPCRs are involved in immune cell recruitment and activation, but the role of RAMP3 in the immune system has not been explored. We found that Ramp3 transcripts are highly expressed in innate-like T cells in the lungs of mice, particularly for mucosal-associated invariant T (MAIT) cells. Ramp3 KO mice have an increased number of lung MAIT cells. These differences are even higher after vaccination with a live attenuated Salmonella vaccine strain containing MAIT-cell antigens. Both at steady-state and after vaccination, MAIT cells from Ramp3-/- mice have increased CXCR6, a chemokine receptor that has been associated with lung homing and memory T cell maintenance in the periphery. Consequently, CXCR6 KO mice have drastically lower MAIT cells numbers after Salmonella vaccination, although these cells have comparable levels of the activation marker CD69 compared to wild-type mice. Despite having a higher number of MAIT cells, Ramp3 KO mice have an increased bacterial burden after vaccination, and worse immunity against bacterial pathogens known to stimulate innate-like T cell responses, suggesting alterations in innate immunity. After vaccination, at the effector phase, the MAIT cell transcriptome in Ramp3 KO mice is altered, with a higher gene signature for autophagy and RANK signaling, but this reverted at the memory phase. Altogether, Ramp3 has an unexpected

role in innate immunity and innate-like T cells, in part through down regulating CXCR6 expression.

Jaroslav Zak (II13)
MYELOID REPROGRAMMING BY JAK INHIBITION TO ENHANCE CHECKPOINT BLOCKADE THERAPY

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Unleashing anti-tumor T cell activity by checkpoint inhibition is effective in many cancer patients, but clinical response rates remain limited. By screening small molecule libraries, we identified JAK inhibitors' ability to rescue T cell function. Despite its documented immune suppressive properties, the prototypical JAK inhibitor ruxolitinib enhanced the efficacy of immune checkpoint blockade in cancer. This effect correlated with loss of suppressive gene expression and acquisition of immunostimulatory molecular markers and T cell stimulatory activity in myeloid cells. In preclinical models, ruxolitinib significantly improved the function and increased the total numbers of activated tumor-infiltrating NK and CD4 T cells compared to checkpoint blockade alone, and the efficacy was conditional on granulocytic cells. Ruxolitinib reshaped the systemic distribution of myeloid cells and their transcriptomic signatures, consistent with a reduction in suppressive programming in the bone marrow. In an ongoing Phase I/II clinical trial of ruxolitinib with nivolumab in Hodgkin lymphoma relapsed or refractory to prior checkpoint blockade therapy, patients showed an interim disease control rate of 76% (13/17). Ruxolitinib profoundly affected myeloid cells in these patients, and reductions in the neutrophil-to-lymphocyte ratio and in specific myeloid transcripts including CD163 correlated with response. Our results support the therapeutic potential of JAK inhibition in combination with checkpoint inhibitors in cancer and highlight the potential of reshaped myeloid immunity to improve immunotherapy.

Matt Maxwell (II4)

Loss of the ARID1A Tumor Suppressor Activates a DNA Replication Stress Driven STING-Type I Interferon Signaling Axis that Promotes Anti-Tumor Immunity

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ARID1A is a core protein subunit of the mammalian Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex and among the most frequently mutated tumor suppressors in human cancers. Immune checkpoint blockade (ICB) clinical trials have identified ARID1A mutations as enriched among patients who respond favorably to ICB in a myriad of solid tumor types in a manner that is independent of microsatellite instability. Thus, the molecular mechanisms that dictate ICB response in ARID1A mutant tumors remain incompletely understood. To investigate, we developed ARID1A deficient murine tumor models that recapitulate anti-tumor immune phenotypes such as increased CD8+ T cell infiltration and activation observed in ARID1A mutant human cancers. Leveraging these tumor models and human tumor RNA-seq data, we discovered that ARID1A deficient cancers display upregulation of an immunogenic interferon (IFN) gene expression signature, including genes known to mediate T cell recruitment and activation. Mechanistically, we observe ARID1A loss induces DNA replication stress in the form of transcription-replication conflicts known as R-Loops which result in a concomitant increase of cytosolic single stranded DNA (ssDNA) and RNA:DNA hybrids, thus mimicking a DNA viral infection. Accordingly, overexpression of the R-Loop resolving enzyme, RNaseH2B or the cytosolic DNase, TREX1 restores the ARID1A-IFN gene expression signature to wildtype levels. Furthermore, we have demonstrated that the ARID1A-IFN gene expression signature and anti-tumor immunity is driven by STING dependent cytosolic DNA sensing and Type I IFN. These findings represent a novel molecular mechanism underlying anti-tumor immunity in ARID1A-mutant cancers and may have translational utility in improving patient selection for ICB.

Fabio Cerignoli (II15)
IN VITRO PHENOTYPING AND POTENCY MONITORING OF CD19 CAR T CELLS USING A COMBINED FLOW CYTOMETRY AND IMPEDANCE BASED REAL-TIME CELL ANALYSIS WORKFLOW

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Advancements in immunotherapy have altered the available treatments for cancer, using the specific ability of the immune system to recognize and kill cancer cells. CAR T cells, involves genetically engineering T cells to target a tumor antigen. Unlike endpoint assays, Agilent xCELLigence RTCA continuously monitors CAR T cell cytolytic activity in real time over multiple days. To determine the quality of the CAR T cells under investigation, orthogonal Agilent NovoCyte flow cytometry assays can be performed to evaluate T cell activation, differentiation, and exhaustion. Here, we combined impedance-based real time cell analysis (RTCA) and flow cytometry workflow for ex vivo cytolytic potency monitoring of CD19-specific CAR T cells (CART19). We also examined phenotypic and functional responses to antigen exposure over time. The potency evaluation and characterization of CAR T cells were performed in several ways:

• CAR expression and T cell phenotyping

• Cytolytic potency by an RTCA cytotoxicity assay

• Cytokine production in response to antigen with a flow cytometry multiplex cytokine detection assay

• Characterization of CAR T cell state following antigen-specific activation.

This powerful workflow can be used to easily measure the cytolytic capacity of CAR T cells in conjunction with an in-depth analysis of T cell cytokine production, cell differentiation, and activation state.

Tao Dong (II16)
ONCOLYTIC ADENOVIRUS WITH LIPOSOMES ENCAPSULATION INDUCES ANTICANCER ACTIVITY IN A CAR-DEFICIENT TUMOR MODEL

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Oncolytic viruses can selectively infect tumor cells potentially inducing selective tumor cell lysis and activation of an immune response that has the potential to turn a "cold tumor" into a "hot tumor" by the release of immune molecules followed by induction of a cellular immune response. Turning an immunosuppressed "cold tumor" into a "hot tumor" by increasing immune cells inside an oncolytic virus infected tumor is a key to improving anticancer activity.

Adenoviruses (Ad) as an oncolytic viral therapy has shown therapeutic effects on local treatment of cancers in clinical trials. However, its more widespread use is hindered not only by the requirement of the tumor cells to have a coxsackievirus and adenovirus receptor (CAR) for effective infections but also by the requirement for local administration since for systemic administration Ad will be removed by the immune response before reaching tumor sites. A liposome encapsulated adenovirus platform was developed to efficiently infect CAR-deficient cancer cells.

The encapsulation of Ad by using cationic liposomes is the key to overcoming CAR-dependent infections and generating a strong immune response against infected tumor cells. Adenoviruses were encapsulated in 200 nm extruded DOTAP-folate liposomes (Df) by charge interaction. The encapsulated Ad (DfA) are able to infect CAR deficient tumor cells and suppress CAR-deficient tumors in vivo. The encapsulated Ads (DfA) were also tested in vitro by incubating with neutralizing antibodies (NAb), and results showed protection against the neutralizing serum. The significant release of immunogenic molecules by DfA was assessed by transfecting CAR-deficient cells in vitro.

Adriana Ayla (II17)
COMMENSAL BACTERIA PROMOTE TYPE I INTERFERON SIGNALING TO MAINTAIN IMMUNE TOLERANCE

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Commensal bacteria are critical to the development and education of the intestinal immune system. Although commonly associated with pathogenic infections, commensal microbes also elicit type I interferon (IFN) responses and are important in the maintenance of intestinal homeostasis. However, the relationship between tonic type I IFN driven by commensal bacteria and tolerogenic immune response is not well defined. Here, we show that commensal bacteria maintain basal expression of type I IFN in dendritic cells (DCs) to promote induction of regulatory T-cells (Treg) response. IFNAR1 deficiency in DCs resulted in dysregulated cytokine production, affecting downstream induction of Tregs. Single cell RNA sequencing of gut Tregs also revealed a type I IFN gene signature in mice colonized with B. fragilis compared to germ-free controls. In the context of disease, IFNAR1-deficient mice are unable to benefit from B.fragilis-mediated protection against experimental colitis. Collectively, these findings unveil the diverse role type I IFN play in the maintenance of intestinal homeostasis.

H. Kay Chung (II18)
EPIGENETIC AND TRANSCRIPTIONAL ATLAS REVEALS A TRANSCRIPTION FACTORS DRIVING CD8+ T CELL TERMINAL EXHAUSTION AND PREVENTING IMMUNE CHECKPOINT RESPONSE.

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CD8+ T cells adopt a range of differentiation states in response to viral and bacterial infections or in tumor. By understanding selective molecular signals that specifically drive certain CD8+ T cell states, we can improve the clinical benefit of immunotherapies. Here, we present transcription factors (TFs) that selectively drive differentiation of the terminally exhausted T

cells (TEX), a population of dysfunctional T cells. The abolition of those TFs effectively prevents loss of function without compromising beneficial- memory or effector T cell states. To identify cell-state selective transcription factors (TFs), we generated an epigenetic/ transcription atlas of CD8+ T cell differentiation states by combining RNA- and ATAC- (Assay for Transposase-Accessible Chromatin) sequencing from multiple studies and our own (10 datasets, 9 T cell states, 139 experiments). According to our systemic analysis, most of the reported TFs were active in multiple cell states; however, we found novel TFs including Zscan20 and JDP2 predicted to be highly selective in terminally exhausted T cells (TEX). We show these TFs are key regulators of TEX formation with experimental validation in the murine chronic infection model. Importantly, the depletion of Zscan20 and JDP2 does not perturb memory differentiation including TRM. As these TFs regulate the expression of genes that are critical for T cell exhaustion and loss of function, depletion of TEX specific TFs enhances the potential for effector cell generation and prevents the progression to a dysfunctional state of which cell state does not respond to checkpoint therapy. Therefore the perturbation of Zscan20 and JDP2 improves responsiveness to PD-1 checkpoint blocking therapy in the tumor model. This study describes epigenetic and transcriptional regulation of heterogeneous T cell states and reports new TFs that selectively promote T cell terminal exhaustion and prevent immune checkpoint therapy.

Carolina Chiale (II19)
DDX3X GENERALLY SUPPRESSES TYPE I INTERFERONS AND LIMITS MAMMARENAVIRUS GROWTH DURING IN VIVO INFECTION

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Mammarenaviruses (MA) cause thousands of annual deaths due to hemorrhagic fever and they evade immune responses by targeting dendritic cells (DCs), which are central players for innate and adaptive immunity. Type I interferons (IFN-I) are critical antiviral and immunoregulatory mediators that limit MA growth in vivo. Our laboratory previously identified that the host factor DEAD-box ATP-dependent-RNA-helicase 3 (DDX3X) suppresses IFN-I production and (independently) promotes MA replication. Here we demonstrate that the DDX3X IFN-I suppressive role extends beyond MA to other viral infections and viral replication intermediates (i.e. dsRNA). In addition, we identified a naturally occurring human disease-causing variant of DDX3X which enhances IFN-I signature in MA-infected fibroblasts without affecting MA replication, indicating that different DDX3X' domains control its IFN-I suppression and pro-viral functions. Finally, we generated mice with DDX3X deficiency in DCs to assess the in vivo impact of DDX3X during MA infection. Mice with DDX3X-deficient DCs had reduced MA loads in DCs

one day after infection, consistent with DDX3X' pro-viral role. Notably, this phenotype appeared consequential as it was accompanied with decreased levels of antigen-specific CD8 T cells. Interestingly, IFN-I levels were not elevated in DDX3X-deficient DCs, suggesting IFN-I suppression by DDX3X is cell-type or species specific. Together, our results extend the pro-viral DDX3X' role to an in vivo MA infection and suggest that DDX3X' IFN-I suppression function relies in different protein domains, may be cell-type specific and could play a role in several viral infections.

Julie Burel (II20)

SINGLE-CELL PROFILING REVEALS DISTINCT SUBSETS OF CD14+ MONOCYTES DRIVE THE BLOOD IMMUNE SIGNATURE OF ACTIVE TUBERCULOSIS

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Previous studies suggest that monocytes are an important contributor to tuberculosis (TB)-specific immune signatures in blood. Here we carried out comprehensive single-cell profiling of monocytes in paired blood samples of active TB (ATB) patients at diagnosis and mid-treatment, and healthy controls. At diagnosis, ATB patients displayed increased

monocyte-to-lymphocyte ratio, increased frequency of CD14+CD16- and intermediate CD14+CD16+ monocytes, and upregulation of interferon signaling genes that significantly overlapped with previously reported blood TB signatures in both CD14+ subsets. In this cohort, we identified additional transcriptomic and functional changes in intermediate CD14+CD16+ monocytes, such as the upregulation of inflammatory and MHC-II genes, and increased capacity to activate T cells, reflecting overall increased activation in this population. Single-cell transcriptomics revealed that distinct subsets of intermediate CD14+CD16+ monocytes were responsible for each gene signature, indicating significant functional heterogeneity within this population. Finally, we observed that changes in CD14+ monocytes were transient, as they were no longer observed in the same ATB patients mid-treatment, suggesting they are associated with disease resolution. Together, our study demonstrates for the first time that both intermediate and classical monocytes individually contribute to blood immune signatures of ATB and identifies novel subsets and associated gene signatures that may hold disease relevance.

Nathmara Nadig (II21)

THE ROLE OF GROWTH FACTORS IN REGULATING CELL-INTRINSIC INNATE IMMUNITY IN VASCULAR ENDOTHELIAL CELLS

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Cell-intrinsic immunity and inflammation is an important factor in infection and chronic diseases. Endothelium plays an important role in the process of inflammation as a crucial participant of the inflammatory process in several diseases like cardiovascular diseases, Alzheimer's disease, diabetes mellitus, atherosclerosis, vasculitis and thromboembolic complications. The four different types of endothelial cells - arterial, venous, capillary and lymphatic, perform different functions based on their location specific in organs and tissues. Previous studies have shown that endothelial cells enhance the production of proinflammatory cytokines such as IFNb, IL-1b, TNF-a, IL-12 and IL-6. We hypothesize that tissue-specific growth factors present in growth media can stimulate or inhibit the cell-intrinsic innate immune response of vascular endothelial cells. In this study, we aim to systematically assess different growth factors present in organ-specific culture media preparations for endothelial cells by studying how they affect cell-intrinsic innate immune responses and cytokine production.

Gerald Coulis (II22)

USING TRANSCRIPTOMICS TO IDENTIFY A NOVEL POPULATION OF FIBROGENIC MACROPHAGES IN MUSCULAR DYSTROPHY

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Resident and infiltrating monocyte-derived macrophages are critical regulators of skeletal muscle homeostasis, but this tight dynamic is disrupted in chronic muscle injury which ultimately contributes to the pathogenesis of degenerative disorders such as Duchenne muscular dystrophy (DMD). DMD is a lethal X-linked disorder that results from mutations in the dystrophin gene, causing necrosis, muscle inflammation and fibrosis. However, it still remains unclear how muscle macrophages contribute to fibrosis during muscular dystrophy. Here, we used single-cell transcriptomics to define the phenotypic complexity and transcriptional profile of skeletal muscle macrophages in healthy and dystrophic muscle. Six clusters expressing distinct transcriptomes were identified, unexpectedly, none corresponded to polarized M1 or M2 macrophages. Interestingly, the most prominent macrophage subset in dystrophic muscle corresponded to a putative fibrogenic population based on its high expression of fibrotic factors, galectin-3 and osteopontin (spp1). Galectin-3+ macrophages were induced by muscle injury and remained chronically activated during muscular dystrophy. To investigate this potential fibrotic function, we conducted spatial transcriptomics of dystrophic muscle that showed expression of fibrotic and fibro/adipogenic progenitor (FAP) genes clustered in galectin-3+ areas. Further, computational inferences of cell communication identified spp1 as a major regulator of macrophages and stromal progenitor communication. In light of these results, we are initiating in vivo functional assays using the spp1 macrophage-specific knockout mouse model to assess whether spp1-producing macrophages promote fibrosis in muscular

Katherine Nguyen (II23)
ARID1A LOSS IN MACROPHAGES UPREGULATES EXPRESSION OF PD-L1 AND INCREASES EFFICACY OF PD-L1 CHECKPOINT BLOCKADE

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Tumor associated macrophages (TAMs) are a major component in the tumor microenvironment. TAMs are associated with poor prognosis in solid tumors and promote an immunosuppressive environment, which can result from persistent inflammation. Preclinical studies suggest boosting existing immunotherapies by reprogramming TAMs from a pro-tumor phenotype

towards an anti-tumor one. To better target these TAMs, we must look at the complexes that control them. The BAF complex, an ATP-dependent chromatin remodeler, is an important regulator of inflammatory gene expression. ARID1A, a protein subunit of the cBAF complex plays a crucial role in the epigenetic regulation and differentiation of myeloid cells. Little is known about the effects of Arid1a loss in TAMs and its implications in cancer. We sought to determine the effects of Arid1a loss on macrophages in both an in vivo and in vitro setting. We observed that upon anti-PD-L1 (anti-programmed death ligand-1) treatment, Arid1a myeloid-specific KO mice had improved responses. To investigate the mechanism driving this, we utilized an in vitro system of bone marrow derived macrophages (BMDMs). We observed that Arid1a-deficient (Arid1aKO) macrophages exhibited higher expression of the inhibitory ligand PD-L1, as well as the cell surface marker CD86 and MHC Class II. We found this expression to be interferon independent and operates cell intrinsically in unstimulated macrophages. In conclusion, our results demonstrate that Arid1a loss leads to an improved response to anti-PD-L1 immunotherapy which may be attributed to an upregulation of PD-L1 in macrophages. In the future, our results can help further direct preclinical models/strategies against cancer.

Jyothi Mony (II24)

Imaging- and impedance-based assay for invasion and tumor cell killing by natural killer cells

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Background: Extracellular matrix (ECM) is the acellular structural component in tissues that play an important role in homeostasis. ECM in the tumor microenvironment of solid tumors is altered and can modulate the function of lymphocytes such as the cytotoxic natural killer and CD8+ T cells. We hypothesized that matrigel layer can modulate NK cell invasion and that increasing the distance for invasion would delay the kinetics of tumor cell killing. Also, the function of NK cells would depend on matrix-metalloproteinases for remodeling ECM during invasion.

Methods: Varying volumes of matrigel (50-110 µL/well; 6mg/mL) representing increasing distance for invasion was layered over MCF7-red target tumor cells expressing nuclear-localized red fluorescent protein. NK92 cells were seeded over the solidified matrigel layer at E:T of 3:1 and imaged in green channel using eLive Green (1:1000). NK cell invasion and function was

evaluated using impedance-based measurements of immune cell killing and live cell imaging (4 fields/well) data collected on xCELLigence RTCA eSight.

Results: The impedance readings increase progressively to stabilize as the MCF7 target cells adhere, proliferate, and achieve confluence. The addition of NK92 cells causes impedance to drop back down to levels at the time of NK addiction, due to killing of the target cells. This drop in impedance after NK92 addition, took longer in the presence of matrigel and slowed down progressively with increasing volume from 46h for 50 μL/well to 72 h for 110 μL/well. Consistent with impedance data, the reduction in red fluorescence of target cells and increase in green fluorescence associated with NK92 cells was progressively delayed with increasing volume of matrigel. A similar delay was achieved by using broad spectrum MMP inhibitor, GM6001 (2μM and 10μM), suggesting a role for MMPs. Interestingly, the NK-92 cells induce significant morphological changes in MCF7-red target cells prior to invading all the way through the Matrigel in the presence or absence of GM6001, suggesting an early distal effect.

Conclusion: The results suggest a role for effector functions of NK cells involving cytokines, that is independent from invasion and/or ability to degrade the matrix for the killing of susceptible target cells. The assay also demonstrates the potential for adapting the RTCA eSight platform to systematically study various ECM components and modulation of immune cell function.

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