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ABSTRACT BOOK

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Oral presentation: Philip I. Marcus Symposium: Interferons and anti-viral immunity

O1

THE GLUTAMATE-CYSTEINE ANTIPORTER XCT IS IMPORTANT FOR THE CGAS DEPENDENT IFN RESPONSE TO HSV AND CYTOSOLIC DNA

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Abstract Content: NRF2 agonists have shown to play an important role in controlling Herpes Simplex virus (HSV) replication. However, the underlying mechanism(s) of the anti-viral effect of NRF2 is still unclear. The NRF2 regulated glutamate-cysteine antiporter SoLute Carrier 7A11 (SLC7A11), also known as xCT, is best known for its importance in managing glutamate levels in the CNS, and has not previously been associated with immunity to virus infection. Here we demonstrate that xCT is important for the interferon response to infection with herpes virus and to stimulation with cytosolic DNA, but not to stimulation with the direct STING agonist cGAMP. Subsequently, inhibition of xCT lead to increased replication of HSV in human keratinocytes while this was decreased by Crispr-activation of xCT. In support of our hypothesis that xCT is important for immunity to HSV, we discovered that the HSV protein 27 (ICP27) targeted the NRF2-xCT axis. Overall, our results demonstrate that xCT is important for resistance to infection with HSV in human keratinocytes and that xCT is important for the induction of IFN by cytosolic DNA and by HSV.

Disclosure of Interest: None Declared
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O2

IDENTIFICATION OF MIDLINE1 AS A CHECKPOINT REGULATOR IN ANTIVIRAL INNATE IMMUNITY

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Abstract Content: Innate immunity to nucleic acids forms the backbone for antiviral immunity and viral-induced diseases. However, the mechanisms that control innate immune activation after sensing nucleic acids are still poorly defined. Here we report that the ubiquitin E3 ligase Midline1 serves as a negative regulator to control antiviral innate immunity against both RNA and DNA viruses. We find knockdown or deletion of Midline1 in human or mouse macrophages enhances production of type I interferon (IFN) in response to double strand (ds) RNA and dsDNA stimulations or RNA and DNA virus infection. Furthermore, mice deficient for Midline1 show more resistant to infections with RNA and DNA viruses because of the enhanced type I IFN production. Mechanistically, we show that Midline1 recruits protein phosphatase 1A (PPM1A) to dephosphorylate TANK binding kinase 1 (TBK1) for its inactivation and block interactions of TBK1 with its upstream adaptors mitochondrial antiviral signaling (MAVS) and stimulator of interferon genes (STING) for dampening antiviral signaling during virus infection. Moreover, Midline1 induces K63-linked ubiquitination of PPM1A to maintain its stability. Collectively, we reveal a critical role for Midline1 as a checkpoint regulator in controlling antiviral innate immunity against both RNA and DNA viruses, thereby providing a potential therapeutic target for controlling viral-induced diseases.

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O3

STAT1 GAIN OF FUNCTION MUTATION IMPAIRS IMMUNE RESPONSE TO VIRAL INFECTIONS

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Abstract Content: Regulation of T cells by interferons and other cytokines, which act via STAT family transcription factors, is critical for host defense. Patients with STAT1 gain-of-function (GOF) mutations develop lifelong ailments, including chronic infections, autoimmunity, and cancer. A characteristic feature of this disorder is chronic

mucocutaneous candidiasis (CMC), related to an exaggerated type 1/interferon (IFN)-gamma bias that antagonizes a type 3/Th17 response required for fungal clearance. However, this type 1/IFN-gamma bias does not explain why almost 40 percent of reported patients exhibit chronic and sometimes lethal viral infections. This observation is unexpected, as cells with STAT1-GOF mutations exhibit increased expression of interferon stimulated genes and patients show more IFN-gamma-producing CD4 T cells. This paradox emphasizes the critical need to understand basic principles of cytokine signaling, specifically how a STAT1-GOF mutation alters cytokine output. To determine the effect of a STAT1-GOF mutation on viral response, we generated a novel conditional knock-in mouse where expression of a common GOF STAT1 mutation, T385M, is Cre-dependent. Using high-dimentional flow cytometry (Cytek Aurora) and RNA-seq, we found that ubiquitous STAT1-T385M expression recapitulates observations found in patients (i.e. elevated STAT1 activation, increased gene expression of STAT1 regulated genes, susceptibility to CMC), as well as a strong type 1/IFN bias at steady state. Despite this type 1 bias, STAT1-GOF mice respond poorly to viral infections that are easily controlled by WT mice, such as MCMV and LCMV-Armstrong. In particular, STAT1-GOF mice exhibit an impaired NK and CD8 T cell effector response not due to exhaustion and develop a cytokine storm seven days after infection, independent of viral load. Unexpectedly, the impaired immune response and cytokine storm is cell extrinsic and not due to one cell type, as when STAT1-T385M expression is restricted to only T cells (CD4-cre), B cells (CD79a-cre), myeloid cells (LysM-cre, CD11c-cre), or ILC1/NK cells (Ncr1-cre), GOF mice respond similarly to WT mice upon viral infection. In fact, the development of a cytokine storm is due to impaired IFN-gamma production by liver NK cells, ILC1s, and iNKT cells early during infection, as administration of IFN-gamma to GOF mice rescues this defect. Furthermore, WT mice treated with IFN-gamma-blocking antibodies also develop a cytokine storm and exhibit impaired lymphocyte response later during infection. In sum, STAT1-GOF mice respond poorly to viral infections due to an unexpected defect in IFN-gamma prodution early during the innate immune response, leading to immunopathology and an impaired adaptive immune response. These results provide insight into why patients with interferonopathies or elevated interferons, such as autoimmune disease, Down Syndrome, and hyperimmunoglobulin E syndrome, respond poorly to viral infections and highlight the importance of properly tuning STAT1 during an immune response.

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Oral presentation: ILC Development & Differentiation

O4

ROLE OF T-BET, RORYT AND RORA IN REGULATING INTESTINAL EPITHELIAL DIFFERENTIATION AND HOMEOSTASIS

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Abstract Content: The generation of lymphoid tissues during embryogenesis relies on group 3 innate lymphoid cells (ILC3) displaying lymphoid tissue inducer (LTi) activity and expressing the master transcription factor RORγt. Accordingly, RORγt-deficient mice lack ILC3 and lymphoid structures, including lymph nodes (LN). Whereas T-bet affects differentiation and functions of ILC3 postnatally, the role of T-bet in regulating fetal ILC3 and LN formation remains completely unknown. Using multiple mouse models together with flow cytometry and single-cell analyses of ILCs and ILC progenitors (ILCP) derived from fetal intestine and LN anlage, we identified a key role for T-bet during embryogenesis and showed that its deficiency rescues LN formation in RORγt-deficient mice. Mechanistically, T-bet deletion skews the differentiation fate of fetal ILCs and promotes the accumulation of tissue PLZF^{hi} ILCP expressing central LTi molecules in a RORα-dependent fashion. Based on our previous findings suggesting *in situ* ILC differentiation in the embryonic intestine, we interrogate ILC1/3 lineage balance in shaping intestinal epithelial differentiation and homeostasis. Altogether, these data unveil an unexpected role for T-bet and RORα during embryonic ILC function and highlight that RORγt is crucial in counteracting the suppressive effects of T-bet.

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O5

TISSUE NICHES AND TRANSCRIPTIONAL PROGRAMS REGULATING ILC1 DIFFERENTIATION DURING ONTOGENY AND INFECTION.

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Abstract Content: ILC1 are found in several tissues with distinct organ-specific phenotypes. We are investigating how this tissue-specific heterogeneity and subset specialization is established and transcriptionally regulated, and how ILC1 acquire effector functions. We could recently show that the transcription factor Hobit is critically involved in these processes. We described different effector stages of committed T-bet^{+} ILC1 throughout various tissues that differentiate along a uniform differentiation pathway across tissues. Specifically, through single cell mRNA sequencing and experimental validation we identified Tcf1^{hi} ILC1 with a “stem-like” expansion potential that mature into more terminally differentiated cytotoxic ILC1. Although Tcf1^{hi} ILC1 preserved the capacity to generate cytotoxic cells across all analyzed organs, the actual differentiation of these effector ILC1 was realized to varying degrees, suggesting that distinct tissue niches may facilitate the generation of cytotoxic ILC1. Employing genetic tracing and multiplex microscopy, our preliminary data suggest that stem-like and cytotoxic ILC1 populate specific tissue niches, and that the self-renewal of stem-like ILC1 and their differentiation to downstream effector stages is spatially regulated within unique anatomical microenvironments. In addition, we found that the composition of local pools of ILC1 is not developmentally fixed. Instead, we identified contexts in which infection leads to a long-lasting reorganization of the tissue niches of ILC1 and thereby improves innate immunity in barrier organs. We will discuss the transcriptional requirements that regulate local ILC1 differentiation during homeostasis and infection. Together, our data provide novel insights into mechanisms of local ILC1 maturation and tissue immunity.

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O6

EMBRYONIC TYPE 3 INNATE LYMPHOID CELLS SENSE MATERNAL DIETARY CHOLESTEROL TO SHAPE MUCOSAL LYMPHOID ORGAN DEVELOPMENT

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Abstract Content: Lymphoid tissue inducer (LTi) cells develop during intrauterine life and rely on developmental programs to initiate the organogenesis of secondary lymphoid organs (SLOs). This evolutionary conserved process endows the fetus with the ability to orchestrate the immune response after birth and to react to the triggers present in the environment. While it is established that LTi function can be shaped by maternal-derived cues and is critical to prepare the neonate with a functional scaffold to mount immune response, the cellular mechanisms that control anatomically distinct SLO organogenesis remain unclear.
We discovered that LTi cells forming Peyer’s patches, gut-specific secondary lymphoid organs, require the coordinated action of two migratory G protein coupled receptor (GPCR) GPR183 (EBI2) and the chemokine receptor CCR6. The unique CCR6 ligand is CCL20, while the ligand for GPR183 is the cholesterol metabolite (i.e. oxysterol) 7α,25-HC, whose production is controlled by the enzyme cholesterol 25-hydroxylase (CH25H). These two GPCRs are uniformly expressed on LTi cells across SLOs, but their deficiency specifically impacts Peyer's patch formation. Our data also identified a fetal stromal cell subset that expresses CH25H and attracts LTi cells in the nascent Peyer’s patches. CH25H activity and oxysterol production can be shaped by the cholesterol content in the maternal diet, suggesting a link between maternal nutrients and intestinal SLO organogenesis.
In the fetal intestine, cholesterol metabolite sensing by GPR183 in LTi for Peyer’s patch formation is dominant in the duodenum, the site of cholesterol absorption in the adult. This anatomic requirement suggests that embryonic, long-lived non-hematopoietic cells might exploit adult metabolic functions to ensure highly specialized SLO development in utero.

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O7

IN VITRO GENERATION OF HUMAN INNATE LYMPHOID CELLS FROM TISSUE-DERIVED CD34+ HEMATOPOIETIC PROGENITORS

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Abstract Content: Innate lymphoid cells (ILCs) are critical effectors of innate immunity and inflammation, whose development and activation pathways make for attractive therapeutic targets. However, human ILC generation has not been systematically explored, and previous *in vitro* investigations relied on inadequate culture conditions or analysis of few markers or cytokines, which are suboptimal to assign lineage identity. We have developed a platform that reliably generates human ILC lineages from $CD34^+$ hematopoietic progenitors derived from cord blood and bone marrow. We showed that one culture condition is insufficient to generate all ILC subsets, and instead, distinct combination of cytokines and Notch signaling are essential for specific ILC subset generation. The identity of natural killer (NK)/ILC1, ILC2, and ILC3 generated *in vitro* was validated by protein expression, functional assays, and both global and single-cell transcriptome analysis, recapitulating the signatures and functions of their *ex vivo* ILC counterparts. Using this platform, we present here an in-depth interrogation of tissue-derived $CD34^+$ progenitors, including gut and tonsil. Altogether, this work presents a resource not only to aid in clarifying human ILC biology and differentiation, but also to serve as an important tool to study dysregulation of ILC functions, which have been implied in various inflammatory diseases in humans.

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Oral presentation: Cytokine induction

O8

MEF2A IS A REGULATOR OF INTERFERON-MEDIATED INFLAMMATION

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Abstract Content: Interferons (IFN) are antiviral cytokines induced by recognition of viral nucleic acids or cellular replicative stress. In particular, the accumulation of cytosolic nucleic acids or the accumulation of DNA lesions has the potential to activate cGAS/STING-mediated IFN induction if not properly constrained. While these responses can be harnessed as tumor therapies, the excessive induction of IFNs and deleterious IFN-mediated inflammation is associated with tissue damage in non-malignant disease. We sought to identify non-canonical IFN-modulatory transcription factors (TF) by examining factors with known genetic alterations that associate with both malignant and inflammatory diseases. Here, we identify MEF2A as a negative regulator of the production of IFNs at homeostasis. The loss of MEF2A function results in the spontaneous induction of type I and III IFNs and downstream ISG expression. Furthermore, DNA lesions that induced DNA damage responses following MEF2A loss resulted in STING-mediated inflammatory activation and genetic deletion of STING maintained homeostatic levels of type I IFN in the absence of this TF factor. We found this response to be independent of the canonical DNA sensors, cGAS and IFI16. Our study is first to connect MEF2A with protection from maladaptive type I IFN responses due to genomic instability triggering non-canonical STING activation across various cell types. We reveal that therapeutic modulation of MEF2A or other members of its family could be beneficial for the management of viral, malignant, and autoinflammatory diseases.

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O9

A BACTERIAL LECITHINASE SHAPES THE INFLAMMATORY LANDSCAPE VIA ACTIVATION OF THE NLRP3 INFLAMMASOME

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Abstract Content: Interleukin (IL)-1 family cytokines including IL-1beta and IL-18 are released in response to host recognition of the pathogen via the inflammasome. Infectious diseases are a major health burden worldwide. The need to find innovative ways to prevent and treat infectious diseases argues that more research is required to understand the molecular basis of host-pathogen interaction. Here, we screened a panel of toxins and identified lecithinase from the human bacterial pathogen *Clostridium perfringens* as an activator of the immune sensor the NLRP3 inflammasome. Lecithinase is an enzymatic toxin which undergoes phagocytosis, resides in LAMP1$^+$ vesicular structures, and induces lysosomal membrane disruption and potassium efflux, triggering NLRP3 inflammasome assembly in macrophages. *C. perfringens* lecithinase induces the release of the inflammasome-dependent cytokines IL-1β and IL-18, and the cytoplasmic protein lactate dehydrogenase, but bypasses the requirement of the pore-forming protein gasdermin D and the plasma membrane rupture mediator protein ninjurin-1 or NINJ1. Further, lecithinase triggers inflammation and lethality via the NLRP3 inflammasome *in vivo*. Indeed, therapeutic modulation of NLRP3 largely protects mice from lecithinase induced inflammation and lethality. Together, these findings identify a pathogen-derived lecithinase as an activator of the NLRP3 inflammasome and delineate the molecular mechanism by which pathogen-associated molecular patterns can activate the mammalian innate immune system.

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O10

THE ROLE OF INTERLEUKINE 17 IN ULTRAVIOLET RADIATION INDUCED SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE IN SKIN AND TUMOR GROWTH

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Abstract Content: Senescent cells are stably viable and active in metabolism and produce inflammatory mediators, a phenomenon called senescence-associated secretory phenotype (SASP). SASP involves the secretion of numerous inflammatory cytokines, growth factors and proteases, which can render the tissue microenvironment immune suppressive, and therefore, has a critical role in immunosuppressive environments for tumor development. Little is known about the role and regulation of SASP in the context of ultraviolet radiation B (UVB) induced immune suppression and skin cancers. Our NanoString analysis showed that IL-17A and IL-17F were upregulated in UVB treated skin. Further studies confirmed that increased IL-17A proteins were detected in skin tissues and tumors of mice which were exposed to UVB. We found that a deficiency in IL-17 receptor (IL-17R-/-) did not have a significant effect on the number of senescent cells in UVB exposed skin. However, SASP biomarkers were significantly inhibited in IL-17R-/- mice compared to wild type controls. Further studies showed that UVB induced SASP and IL-17 production by human keratinocytes. Addition of exogenous IL-17 significantly increased SASP whereas it did not affect cellular senescence. In contrast, addition of anti-IL-17R antibodies inhibited SASP. Notably, blocking IL-17R had similar effects to those observed in UVB treated skin of IL-17R-/- mice. These results reveal a previously unrecognized pathway by which IL-17 regulates UVB induced SASP independently of cellular senescence. Our previous studies demonstrated that UVB induced immune suppression was diminished in IL-17R-/- mice. Immune suppression is considered as a major risk factor for tumor development and UVB induced immune suppression is a long lasting effect. Our results showed that UVB exposure increased the growth of B16F10 mouse melanoma in wild type C57BL/6 mice. Interestingly, the long lasting immune suppressive effects on the enhancement of tumor growth by UVB was diminished in IL-17R-/- mice. Collectively, our studies have demonstrated an important role of IL-17 in UVB induced SASP and immune suppression. Blockade of IL-17 may be exploited to prevention and/or treatment of UVB induced skin cancers.

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Oral presentation: Cytokine signaling

O11

SOCS1 VARIANTS UNDERLY A SPECTRUM OF RARE AND COMMON IMMUNOLOGIC DISEASES

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Abstract Content: Traditionally, human inborn errors of immunity involving JAK-STAT signaling are marked by ultra-rare frequency and high disease severity. One such disorder was recently described in patients with rare autoinflammatory disease harboring loss-of-function mutations of SOCS1, a near-universal inhibitor of JAK function. Shortly after this initial discovery, several studies have uncovered additional *SOCS1* mutations with highly variable phenotypes. The rapid identification and variable penetrance of these mutations suggests that *SOCS1* variation may underly more common immunologic diseases, in addition to the rare forms previously described. To assess this hypothesis, we undertake a forward-genetics approach utilizing a large genetics biobank linked to electronic health records. We demonstrate that individually-rare *SOCS1* variants predicted to be deleterious can readily be identified across genome databases. We associate a diverse array of immunologic disorders with 23 unique *SOCS1* variants spanning all domains of SOCS1. We functionally characterize how each point mutation perturbs the negative regulatory activities of SOCS1, leading to over-active JAK-STAT signaling. Finally, we relate specific cytokine signaling axes to clinical pathogenesis. Together, this study demonstrates that SOCS1 variation underlies an unexpectedly broad spectrum of clinical phenotypes from rare and severe to more mild and common.

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O12

THERAPEUTIC MODULATION OF STING

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Abstract Content: Stimulator of interferon genes (STING) is an essential adaptor protein required for the inflammatory response to cytosolic DNA. Following cGAS activation cGAMP binds and activates STING triggering a conformational change, oligomerization and activation of downstream signalling pathways such as type I IFN, NFkB and autophagy. More recently a growing body of evidence has demonstrated aberrant STING activation as a key driver of inflammatory diseases such as AGS, SAVI, COPA syndrome, ALS, Parkinson's disease and SLE. Here we report the identification and characterization of a potent, selective small molecule inhibitor of STING (STINGi). STINGi inhibited STING dependent signaling but no other innate immune signaling pathways. Mechanistically, STINGi covalently modified Cys^{148} to block STING oligomerization and subsequent activation of type I IFN, NFkB and autophagy. *In vivo* STINGi alleviated the pathological features of experimental AGS in mice. Collectively, our work uncovers a novel mechanism by which STING can be targeted therapeutically and highlights the potential of this strategy for the treatment of STING driven autoinflammatory diseases.

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O13

FAMILY-WIDE IDENTIFICATION OF LATENT STAT DIMERS IN LIVING CELLS

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Abstract Content: Cytokine signalling is dependent on the seven-membered family of transcription factors called Signal Transducer and Activator of Transcription (STAT) proteins. Upon cytokine-induced tyrosine phosphorylation, all STATs assemble so-called “activated” dimers that can bind DNA and regulate transcription. Unphosphorylated STATs, initially thought of as mere precursors waiting to become activated, are now known to have additional vital roles in cytokine signalling. Importantly, these are linked to the assembly of “latent” dimers that adopt an entirely different conformation. Yet, knowledge about STAT protein dimerization in living cells remains incomplete and is particularly fragmentary regarding latent STATs, which hampers understanding of cytokine biology and therapeutic progress. To provide a more complete picture, we developed an autofluorescent biosensor-based translocation assay, which enabled us to determine homo-and heterodimerization of STAT proteins in living cells prior to cytokine stimulation across the entire STAT family. Through comparison with known dissociation constants (K_d) previously obtained from ultracentrifugation experiments in vitro, we inferred that the live-cell assay detected weakly bound protein complexes with K_d values in the millimolar range, and could distinguish binding strengths over at least six orders of magnitude, extending into the low nanomolar range. Using this approach, we tested all 28 possible latent dimer combinations. We found that five of the seven STAT family members assembled homodimers, and we identified an additional two STAT heterodimers. Our results indicated that these dimers generally are of high affinity, with nanomolar dissociation constants, however, there appears to be appreciable variation within this range. Moreover, mutation of a single homologous residue known to dissociate latent dimers of STAT1 and STAT3 had the same disrupting consequences for all latent STAT dimers. We concluded that the assembly of high-affinity homodimers is almost as common before cytokine-induced STAT activation as after. Heterodimerization, in contrast, is much more limited for latent STATs compared to the activated variants. Irrespective of their composition, assemblies of unphosphorylated STATs were stabilized by equivalent side-chain contacts, which suggests that they can all adopt very similar conformations specific for the latent dimers. In summary, these results substantially expand the knowledge of STAT protein interactions and self-association in living cells. This, in turn, improves understanding of how the relationship between latent and activated STATs shapes the outcome of cytokine signals.

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Oral presentation: ILC Homeostasis, Niches and Lifespan

O14

SPATIAL AND FUNCTIONAL COMPARTMENTALIZATION OF SMALL INTESTINE GROUP 2 INNATE LYMPHOID CELLS (ILC2S)

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Abstract Content: Although present in many tissues, ILC2s in the small intestine are prevalent in the lamina propria (LP), where their contributions to type 2 immune responses are well documented. The intestine is an anatomically and functionally complex tissue, however, where luminal signals are transmitted through diverse epithelial and submucosal cell types to the muscularis, the smooth muscle layer encasing enteric neurons that coordinate physiologic responses optimizing peristalsis and absorption. We investigated more carefully how ILC2s distribute across these two domains.

To assess these populations, we physically separated these tissues and performed comprehensive spectral flow cytometric analysis with 38 fluorophore-conjugated antibodies. MS ILC2s comprised 20-30% of total muscularis lymphocytes. In contrast to LP ILC2s, MS ILC2s were IL-25R^{lo} and IL-33R^{+}, and exhibited a distinct phenotype as compared to LP ILC2s and ILC2s from other tissues. Functionally, MS ILC2s, but not LP ILC2s, responded to a single dose of IL-33 by proliferating and producing IL-5, indicating a low threshold for responsiveness to IL-33. Additionally, RNA-Seq analysis showed that MS ILC2s exhibit a distinct transcriptional profile as compared to LP ILC2s, including robust expression of genes that limit inflammation (*Il10ra, Ifngr1, Anxa1, Lgals1 and Socs2*) and sustain tissue health (*Areg, Bmp7, Tgfb1 and Lif*), suggesting a potential role in tissue homeostasis.

We next examined the localization of MS ILC2s, revealing close association with muscularis macrophages (MMs) that play a key role in survival and function of enteric neurons. During *Nippostrongylus brasiliensis* (*N.b.*) infection, Arg1^{+} MMs increase and are greatly diminished in IL-4Rα$^{-/-}$ and γ$_c^{-/-}$Rag2$^{-/-}$ mice, but not Rag2$^{-/-}$ mice, suggesting that ILC2 activation regulates MM alternative activation as assessed by this marker.

MS ILC2s were also closely associated with enteric neurons that coordinate important functions regulating gastrointestinal motility. We found that *N.b.*-induced type 2 inflammation resulted in dysmotility and increased intestinal transit time that was improved in IL-25/ST2/TSLPR triple-deficient mice that disrupt critical activating signals for ILC2s, suggesting that ILC2-induced type 2 immune responses contribute to gastrointestinal dysmotility accompanying acute infection. We also examined *N.b.* infection in mice with neuron-specific IL-4Rα deletion, revealing an unexpected defect in worm expulsion, consistent with a direct role for IL-4/13 signaling in enteric neural activation involved in clearance of this helminth.

Together, our results demonstrate that small intestine ILC2s consist of anatomically and functionally distinct populations made up of LP ILC2s and MS ILC2s. The latter represents a phenotypically and transcriptionally unique population localized in close proximity to MMs and enteric neurons. Our initial experiments suggest direct effects of ILC2s and type 2 immunity on MMs and enteric neurons. We propose that anatomically compartmentalized ILC2s regulate distinct functional domains in the same tissue, creating the potential for precise targeting of outputs that are unique to each population.

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O15

TYPE 1 AND 2 LYMPHOCYTE NICHE BOUNDARIES DURING MIXED INFLAMMATION

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Abstract Content: Allergic inflammation is orchestrated by group 2 innate lymphoid cells (ILC2s) and type 2 helper T (Th2) cells prominently arrayed at epithelial- and microbial-rich barriers. However, ILC2s and Th2 cells are also present in fibroblast-rich niches within the adventitial layer of larger vessels and boundary structures such ducts and airways, and it remains unclear whether they undergo dynamic repositioning during inflammation. Here, using quantitative imaging analysis and lineage tracing mouse models, we show that allergic inflammation drives invasion and accumulation of ILC2 and Th2 cells into lung and liver parenchyma, a domain beyond adventitial sites. However, during concurrent type 1 and type 2 mixed inflammation, IFNγ produced by type 1 lymphocytes directly blocks both ILC2 parenchymal trafficking and cell survival. ILC2 and Th2 cell confinement to adventitia limits mortality during *Listeria monocytogenes* infection. Our results suggest that localization of tissue lymphocytes is tightly regulated during and post-inflammation to promote appropriately timed and balanced immunity.

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O16

INNATE LYMPHOID CELLS ARISE EARLY IN THE FOETAL THYMUS ALONGSIDE DEVELOPING MEDULLARY THYMIC EPITHELIAL CELLS

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Abstract Content: Type 2 innate lymphoid cells (ILC2) contribute to immune homeostasis, protective immunity and tissue repair. Through the production of the cytokines IL-5, IL-4, IL-9 and IL-13, ILC2 promote immunity against helminth infections, but also participate in the inappropriate type-2 inflammation that underlies asthma and allergy. Using a sophisticated reporter mouse model (5xpolychromILC) that enabled us to analyse the expression of 5 key ILC-associated transcription factors (Id2, Bcl11b, Gata3, RORα and RORγt), we recently demonstrated that functional ILC2 can arise in the embryonic thymus from shared T cell precursors, preceding the emergence of $CD4^+CD8^+$ (double-positive) T cells. Confocal microscopy revealed that thymic ILC2 precursors develop in niches with other ILCs, as defined by the expression of ID2. IL-33 producing cells were present in proximity with developing ILC2s, suggesting a potential supportive role in development of ILC2s in the embryonic thymus. Thymic epithelium cells (TEC) also develop early in thymogenesis and require crosstalk with developing thymocytes. Using confocal fluorescence microscopy, we revealed localisation of $ID2^+$ ILCs with cytokeratin 5^+ medullary TECs (mTECs) as early as E13.5, when $BCL11b^+$ pro-T cells have not yet developed. At E17.5, $ID2^+$ cells remain colocalised with mTECs while $BCL11b^+$ pro-T cells are largely absent from these niches. To better understand the co-development of mTECs and thymic ILCs, we are developing an ex-vivo light-sheet microscopy culture system to image embryonic thymus development in compound reporter mice from E12.5, and these results will be discussed. In summary, ILCs have been found to develop early in the foetal thymus before more classical thymocytes, and to associate together with mTECs in medullary niches.

Disclosure of Interest: None Declared
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Oral presentation: Cytokines and metabolism/macrophage

O17

ITACONATE DERIVATIVE SUPPRESSES RIG-I/MAVS AND TYPE I IFN SIGNALING TO POTENTIATE ONCOLYTIC VIROTHERAPY

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Abstract Content: Dysregulated metabolism and defective innate immune responses are common characteristics of transformed cells. This has made it possible to genetically engineer or select oncolytic viruses, which specifically infect and kill cancer cells without harming healthy tissues. However, resistance to oncolytic virotherapy happens and is frequently associated with failure of tumor cells to be infected by the virus. Our recent work demonstrates the potent anti-inflammatory and broad antiviral action of the Kreb's cycle derived metabolite itaconate, namely octyl-itaconate (OI). Unexpectedly, we show that the cell permeable drug metabolite OI enhanced viral infection of different cancer cell lines as well as murine tumor biopsies with several oncolytic viruses including VSV, Measles Virus and Reolysin. Crucially, the sensitizing effect of 4-OI is not observed in non-cancer primary human cells and outperforms the one of the FDA-approved drug dimethyl fumarate. Using patient-derived colon tumor organoids, we demonstrate in an advanced 3D model the capacity of OI to bolster OV infectivity and therapy. Interestingly, the ability of OI to enhance viral spread is ablated upon silencing or CRISPR/Cas9 knock out of the transcription factor Nrf2, due to its capacity to inhibit type I IFN production and response. Mechanistically, we show that OI simultaneously alters the RIG-MAVS and the type I IFN signaling pathways through the impairment of MAVS-TBK1 interaction and the inhibition of the kinase JAK1, respectively. Altogether, our work demonstrates that unconventional application of the drug-derived

metabolite OI in combination with therapeutic living biological agents can result in improved anticancer therapeutic outcomes by targeting the IFN response.

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O18

THE KETONE BODY B-HYDROXYBUTYRATE METABOLICALLY RESTORES T CELL FUNCTION IN SEVERE COVID-19

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Abstract Content: Upon acute infection, the host responds rapidly with a multitude of immunological, metabolic, and behavioral alterations. Among these, anorexia leads to fasting and a metabolic switch to ketogenesis and the production of ketone bodies, mainly β-hydroxybutyrate (BHB). How ketogenesis and in particular ketone bodies metabolically influence the immune response in pulmonary infections, is still not completely understood. By measuring BHB in the serum of patients with acute respiratory distress syndrome (ARDS), we observed decreased levels in patients with COVID-19 compared to influenza patients. Since anti-viral immunity is mainly mediated by $CD4^+$ and $CD8^+$ T cells, we investigated the effect of BHB on T cell immune function. *In vitro* treatment of T cells with BHB, promoted their survival and increased IFN-γ production. By using C13 metabolic tracing we revealed that BHB is used from T cells as an alternative carbon source to fuel oxidative phosphorylation (OXPHOS) and the production of bioenergetic amino acids and glutathione, which is important for maintaining the redox balance. T cells isolated from the blood of COVID-19 patients were exhausted and skewed towards glycolysis but upon treatment with BHB were metabolically reprogrammed towards OXPHOS, displaying increased functionality. Finally, the delivery of BHB as a ketone ester drink was able to metabolically rewire T cells, increasing the production of IFN-γ in SARS-CoV-2 infected mice. In addition, mice that received ketone esters displayed reduced mortality compared to control infected mice. Overall, our data suggest an important role of BHB in the regulation of T cell immune responses in pulmonary infections and that an impairment in the induction of ketogenesis in patients with SARS-CoV-2 infection accounts, at least partially for disease progression. Furthermore, supplementation of ketone ester could prove a cost effective and easy-to-implement alternative therapeutic treatment of SARSCoV-2 infections.

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O19

A NOVEL ROLE FOR ENDOGENOUS PANCREATIC BETA CELL IL-22 SIGNALING IN MAINTAINING SYSTEMIC METABOLIC HOMEOSTASIS

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Abstract Content: We discovered that the cytokine interleukin-22 (IL-22) is an efficient natural inhibitor of cellular stress and improved insulin quality in pancreatic beta-cells in preclinical models of Type 2 diabetes. Importantly, IL-22 completely restored glucose tolerance, suppressed fasting hyperinsulinaemia/hyperproinsulinaemia, and restored insulin sensitivity in obese animals. Treatment of obese animals with IL-22 also showed significant improvements in circulating triglycerides, liver function (AST:ALT ratio) and a reduction in hepatic lipid accumulation. Interestingly, the IL-22 receptor, IL-22Ra1 is highly expressed in the key metabolic organs: the pancreas and liver. Our study aimed to

define the role of endogenous IL-22 in these tissues to provide additional support for IL-22-therapy in metabolic syndrome.

To achieve this, we generated tissue specific IL-22Ra1 knockout mice lacking the receptor in pancreatic alpha-cells ($Ra1^{a\text{-cell }-/-}$), beta-cells ($Ra1^{b\text{-cell }-/-}$) and hepatocytes ($Ra1^{Hep\ -/-}$). We then challenged them with a high fat diet, and measured their glycaemic control, hepatic lipid accumulation, and hepatic markers of cellular stress, lipid, and glucose metabolism. We found that $Ra1^{Hep\ -/-}$ animals had increased hepatic markers of inflammation and cellular stress. Interestingly, we also observed this phenomenon in $Ra1^{b\text{-cell }-/-}$, but not $Ra1^{a\text{-cell }-/-}$ mice. Additionally, $Ra1^{b\text{-cell }-/-}$animals had defective glycaemic control and insulin secretion compared to littermate control animals, which was exacerbated by the high-fat diet. Furthermore, we found that whilst female animals did not gain as much weight as male animals on a high-fat diet, female $Ra1^{b\text{-cell }-/-}$ mice had a worsened phenotype compared to males.
In conclusion, we confirmed the role of endogenous IL-22 signaling in maintaining insulin quality control and healthy hepatic function. We also discovered a novel role for endogenous IL-22 signaling in the pancreatic-beta cell-liver axis and demonstrated the importance of targeting IL-22 to both pancreatic beta-cells and hepatocytes to treat metabolic syndrome.

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Oral presentation: Cytokines and dendritic cells

O20

GM-CSF-MEDIATED LUNG MYELOID CELL–NICHE CROSSTALK IN DEVELOPMENT AND HOMEOSTASIS

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Abstract Content: Fetal myeloid progenitors that seed embryonic tissues during organogenesis differentiate into specialized tissue-resident macrophages upon exposure to critical niche factors. In the lung, alveolar macrophages (AMs) are known to rely heavily on GM-CSF (encoded by *Csf2*) for their development. However, evidence to functionally link components of this intercellular cross talk remains scarce and the relevant sources of GM-CSF, which instruct AM fate, are unknown. To answer this, we have developed novel transgenic *Csf2* reporter mice that were used to not only profile pulmonary GM-CSF production, but also to selectively delete *Csf2* expression from populations of interest. Analysis of *Csf2*-reporter kinetics suggests that pulmonary GM-CSF production is first induced between E16.5 and E18.5. At E17.5, deletion of hematopoietic GM-CSF did not impact AM development. Depletion of epithelial GM-CSF, however, revealed a severe perturbation in the fetal lung monocyte to AM differentiation trajectory. Likewise, we have unequivocally demonstrated that AM survival is critically reliant not on hematopoietic GM-CSF but on epithelial GM-CSF derived from alveolar epithelial type 2 cells (AT2s). Our results demonstrate the nonredundant function of AT2-derived GM-CSF in instructing AM fate, establishing the postnatal AM compartment, and maintaining AMs in adult lungs. This AT2-AM relationship begins during embryogenesis, where nascent AT2s timely induce GM-CSF expression to support the proliferation and differentiation of fetal monocytes contemporaneously seeding the tissue, and persists into adulthood, when epithelial GM-CSF remains restricted to AT2s. Our results also suggest the existence of distinct niches for pulmonary dendritic cell (DC) subsets, where local GM-CSF instructs their final maturation. In our ongoing studies we are exploring this crosstalk between lung DCs and sources of epithelial as well as hematopoietic GM-CSF in more detail.

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O21

CLINICAL DEVELOPMENT OF HETERODIMERIC IL-15 (HETIL-15) FOR CANCER IMMUNOTHERAPY

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Abstract Content: IL-15 is a common γc chain cytokine with important roles in the development, survival, proliferation, and activation of lymphocytes, including natural killer (NK) cells, canonical αβTCR+CD8+, γδTCR+, NKT, ILC1, other ILC subsets and intraepithelial T lymphocytes. IL-15 shares the beta gamma chain receptor with IL-2, the prototypical immunotherapeutic oncology agent and has similar biological effects. However, key differences in production, secretion, stability, and receptor engagement confer unique properties to IL-15, and advantages over IL-2 for the treatment of cancer.
We have identified the mechanism of production of the native form of IL-15 as a heterodimeric membrane-bound and secreted molecule of two polypeptide chains, IL-15 and IL-15Ra. We refer to the native form as heterodimeric IL-15 (hetIL-15). We bio-produced efficiently the circulating endogenous form of IL-15 in the body and initiated its clinical development for cancer immunotherapy. Bio-produced hetIL-15 is in clinical trials for cancer immunotherapy alone and in combination with check point inhibitors [NCI/Novartis Trials (NCT02452268, NCT04261439) hetIL-15, hetIL-15+antiPD-1]. Clinical results indicate that hetIL-15 is safe, and it expands and activates lymphocytes in humans, as expected from the pre-clinical studies.
IL-15 and IL-7 have been recognized as homeostatic cytokines because they are present continuously in the blood and lymph, respectively, at the steady-state and control lymphocyte numbers and activation status. Recent studies reveal a role of hetIL-15 during the initiation of the immune response. We identified IL-15 induction immediately after vaccination as part of a cytokine signature that correlated with efficient antibody development.
hetIL-15 provided as a drug, results in activation of both the innate and adaptive immune system and in the development of long-term memory against pathogens or tumors. The rapid direct action of hetIL-15 on lymphocytes expressing the IL-2/IL-15 beta/gamma receptor is followed by engagement of myeloid and Dendritic Cells. hetIL-15 induces entry of lymphocytes into tumors and promotes the intratumoral accumulation of conventional type 1 dendritic cells (cDC1) and a new population of DCs, $CD103^{int}CD11b^{+}$DCs. An important aspect of hetIL-15 function is the strong action against metastatic disease, as revealed by studies in mice. hetIL-15 reduced circulating cancer cells and prevented both the dissemination and establishment of metastatic disease. These properties make hetIL-15 a unique factor for immunotherapy combinations.

Disclosure of Interest: None Declared
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O22

TYPE I INTERFERON EXHAUSTION IN PLASMACYTOID DENDRITIC CELLS: UNDERLYING MECHANISM AND POTENTIAL BENEFIT.

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Abstract Content: Type I Interferons (IFN-I) are critical cytokines that protect against viral infections. While any cell can make IFN-I, plasmacytoid Dendritic Cells (pDCs) secrete higher quantities and more subtypes of IFN-I than any other cell type. However, following this initial IFN-I response, pDCs become exhausted, losing their capacity to produce IFN-I after both acute and chronic viral infections. Notably, this phenotype is long-term in the chronic setting, favors viral persistence and compromises host defense against secondary infections. It is intriguing that despite this apparent cost of pDC exhaustion such pDC adaptation is preserved across species and occurs in response to multiple types of viral infections. Through investigation of the transcriptional program of non-exhausted and exhausted pDCs we identified losses in pDC metabolic capacity that associated with pDC exhaustion. Mechanistically, we demonstrated that the enzyme lactate dehydrogenase B (LDHB) is repressed in exhausted pDCs while being essential for optimal pDC IFN-I production in mice and humans. Remarkably, the single restoration of LDHB expression was sufficient to recover exhausted pDC function *in vitro* and *in vivo*, and associated with enhanced infection-induced

colitis. Overall, our work identified a novel mechanism for the regulation of IFN-I production in murine and human pDCs via LDHB, and provides a potential explanation for the evolutionary benefit of pDC exhaustion in preventing immunopathology.

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Oral presentation: ILC1 & NKs

O23

GFI1 CO-REGULATES EOMESODERMIN AND FOXO1 TO SCULPT NK CELL MATURATION AND FUNCTION.

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Abstract Content:
Natural killer (NK) cells are innate lymphocytes that protect against virus infections and cancerous cells. To ensure this process occurs effectively, NK cell maturation and function must be tightly regulated by transcription factors. Growth factor independent 1 (GFI1) is a transcription factor that is critical for the regulation of $CD4^+$ T cells and innate lymphoid cell 2 development and function, but has not been implicated in regulating other cell types.
Bioinformatic mining of RNA sequencing (RNAseq) data of NK cells identified that GFI1 is differentially expressed in NK cells. This suggested that, like other immune cell subsets where it plays a key role, it may be important for regulation NK cells. To explore this, we took advantage of $GFI1^{Tomato}$ reporter mice to allow us to generate a blueprint of GFI1 expression *in vivo*. This allowed us to show that GFI1 is differentially regulated during NK cell maturation and thus this transcription factor may be important in their function. Examination of genetic deletion of GFI1 in NK cell subsets revealed that as NK cells matured, in the absence of GFI1 signalling mature NK cells progressively become impaired. This impairment affects multiple key pathways for cell survival and function including proliferation, migration and activation. Indeed, alteration of these pathways almost completely crippled GFI1-deficient NK cells to control B16F10 melanoma tumour development, highlighting the key role GFI1 plays in regulating NK anti-tumour functions.
RNAseq analysis was used to begin to understand the mechanisms by which GFI1 regulates NK cells. This revealed that GFI1 acts as a 'brake', repressing the expression of number of key downstream factors including FOXO1 and Eomesodermin, two transcription factors essential to the programming of NK cells. We explored the co-regulation of GFI1 with these, and how it influenced NK cell positioning in tissues during the maturation process to identify the global importance of GFI1 in defining NK cell developmental and functional states.
Collectively, these results highlight the importance of GFI1 in regulating key processes of NK cell maturation and function and the pivotal nature of this program to mediate effective anti-tumour protection.

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O24

IDENTIFICATION AND CHARACTERISATION OF NOVEL NK CELL-RESTRICTED ACE-NK PROGENITORS

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Abstract Content: Recent reports have identified that innate lymphoid cell (ILC) progenitors (ILCPs) retain their potential to develop into natural killer (NK) cells, contrary to an earlier proposal that indicated that NK cells differentiate from haematopoietic progenitors before their commitment to ILCPs. Indeed, using previously reported criteria for the

purification of NK cell progenitors, dating from before the characterisation of other ILC subsets, we have found that they are heterogeneous and retain the capacity to differentiate into ILC2s and ILC3, when used to reconstitute *Rag2*, *Il2rg* double-deficient hosts. Therefore, to better accurately define NK-restricted progenitors we used polychromic transcription factor reporter mice and single-cell RNA sequencing to identify and characterise a population of bone marrow progenitors exclusively committed to the ILC1/NK lineage. These ILC1/NK lineage-restricted progenitors are defined as Lineage-negative Id2^{+}IL-7Rα^{+}CD25^{-}Bcl11b^{-} NKG2A/C/E^{+} (referred here as aceNKPs). *In vitro*, aceNKPs differentiated into NK1.1^{+}NKp46^{+} innate lymphocytes under neutral conditions with stem cell factor and interleukin (IL)-7, without the requirement for IL-15. Upon maturation, they upregulated EOMES and T-bet expression and produced perforin and interferon (IFN)-γ. Notably, their Bcl11b^{+}NKG2A/C/E^{+} counterparts acquired a similar phenotype but lacked perforin expression, more closely resembling type-1 helper ILCs. Following reconstitution of *Rag2*, *Il2rg* double-deficient recipients, aceNKPs solely gave rise to mature NK cells regardless of their tissue residence, displayed a cytotoxic phenotype, and decreased tumour burden in a model of lung metastasis. In conclusion, our results highlight the multipotency of previously defined NK progenitors and demonstrate that the NKG2A/C/E markers define an aceNKP population in the bone marrow that is restricted to NK lineage production.

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O25

SINGLE-CELL RNA-SEQUENCING ANALYSIS REVEALS T-BET AND EOMES ARE REQUIRED FOR MAINTAINING MATURE NK CELL TRANSCRIPTIONAL PROGRAM

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Abstract Content: T-box transcription factors T-BET and EOMES are best known for directing NK cell development. However, whether they are required for maintenance of the functional program of mature NK cells is still unknown. In this study, we apply single-cell RNA-sequencing (scRNA-seq) on primary human NK cells that are CRISPR-edited to delete both T-BET and EOMES simultaneously. Our data reveal that these two transcription factors are indispensable for maintaining the mature human NK cell transcriptional program.
CRISPR-edited NK cells from 3 donors were either cultured in vitro with low dose rhIL-15 or transferred into NSG mice followed by rhIL15 support. After 1 week, human NK cells were purified by flow sorting (mCD45-, hCD45+, hCD3-, hCD56+) from the spleen of NSG mice. Similarly, after 1 week the in vitro cultured NK cells were also sorted, providing an in vitro comparison to account for in vivo phenotypic changes, and to allow analysis of CD56^{bright} NK cell subset that are abundant in vitro but minimally detected in vivo.
T-BET and EOMES knock-out (KO) NK cells have profoundly dysregulated NK programs in both in vitro and in vivo settings. Cytotoxicity mediators (i.e. *GZMB*, *PRF1*, *GNLY*, *NKG7*), surface receptors (e.g. *KLRF1*, *SLAMF7*), migration molecules (e.g. *S1PR5*, *CX3CR1*, *SELL*) are down regulated. The KO NK cells also have increased expression of some molecules, including *TNFSF18*, which encodes for GITR, a negative regulator of NK cell function, and integrin *ITGB7*.
The transcriptional dysregulation manifested a functional defect where we observed impaired control of K562 tumor in vivo in NSG mice by T-BET and EOMES CRISPR-edited human NK cells ($p<0.001$). In concordance with this impaired tumor control and the downregulation of their transcript expressions, GRANZYME B and PERFORIN protein expressions were significantly decreased in T-BET and EOMES KO NK cells. T-bet and EOMES KO NK cells also had impaired ability to respond to cytokines IL-12 and IL-15 to produce IFN-γ ($p<0.001$).
In addition, unsupervised clustering of the in vitro scRNA-seq data identified a cluster enriched in the T-BET and EOMES CRISPR-edited samples that resembled CD56^{bright} NK cells but also highly expressed ILC-3-associated markers. This cluster has high expression of *RORC*, *AHR* and low expression of *IKZF3*; the surface markers *IL7R* and *KIT* were also highly expressed in this cluster. There are no CD56^{dim} NK cells in the analysis that resembled this ILC-3-like clusters. These data suggest that while CD56^{dim} NK cells require T-BET and EOMES primarily to maintain bona fide NK cell-associated molecules, CD56^{bright} NK cells additionally require T-BET and EOMES to suppress alternative ILC lineage genes.

In summary, this is the first loss-of-function study of primary human NK cells that assesses the transcriptional and functional program regulated by EOMES and T-BET. Our data demonstrate the critical requirement for T-BET and EOMES to maintain identity of mature human NK cells to ensure proper NK cell effector response.

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O26

COMMENSAL MYELOID CROSSTALK IN NEONATAL SKIN REGULATES LONG-TERM CUTANEOUS TYPE 17 INFLAMMATION.

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Abstract Content: Early life immune interactions help shape longer-term skin health and homeostasis. Commensal microbes facilitate neonatal skin accumulation of innate and adaptive T cells, thereby promoting fundamental needs such as immune tolerance to commensals and wound healing. Comparatively little is known about commensal-myeloid cell crosstalk in neonatal skin and the functional consequences of these interactions. Using CyTOF and gnotobiotic mouse models we observed a population of classical monocytes uniquely enriched in the skin of microbially replete neonates. Corroborative studies revealed that skin monocytes rapidly accumulate between D1 and D3 of life, after which their numbers gradually decline. This early monocyte wave was prevented in antibiotic-treated SPF pups as well as in Myd88$^{-/-}$ but not IL1R1$^{-/-}$ mice, suggesting a key role for tonic TLR signaling in their accumulation. To dissect the functional relevance of these cells in cutaneous biology, we developed an antibody-based regimen to temporarily deplete monocytes in the first two weeks of life (NeoDmono). scRNA sequencing revealed a heightened type 17 signature in D15 NeoDmono skin T cells. Flow cytometry assays confirmed sustained elevation of IL-17A production by these cells through adulthood. This reflected a heightened response to commensals as IL-17 production was significantly reduced in antibiotic-treated NeoDmono mice. While there was no visible skin pathology in NeoDmono mice under homeostatic conditions, imiquimod treatment of the ears in adulthood led to significantly increased ear swelling and neutrophils. Taken together, our data demonstrate a previously unappreciated, commensal-dependent regulatory imprinting function of cutaneous classical monocytes in the early life window.

Disclosure of Interest: None Declared
DOI: https://doi.org/10.55567/C22.O26

O27

THE ANALYSIS OF EOSINOPHILIC GASTROINTESTINAL DISORDERS PATHOPHYSIOLOGY BY USING SPATIAL TRANSCRIPTOMIC APPROACHES

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Abstract Content: Eosinophilic gastrointestinal disorders (EGID) are characterized by pathologic eosinophilic infiltration of gastrointestinal tract and lead to organ dysfunction. These disorders include eosinophilic esophagitis (EoE), eosinophilic gastritis (EG), eosinophilic gastroenteritis (EGE), eosinophilic enteritis (EE), and eosinophilic colitis (EC). Conventionally, eosinophils are known as destructive effector cells involved in parasitic infections and allergic reactions. Recently, a growing number of studies have revealed that eosinophils are multifunctional leukocytes involved in various inflammatory and physiologic immune responses. However, high resolution transcriptomic analysis

of these disorders preserving the spatial information are yet to be established. To understand pathophysiology of EGID, we applied a spatial transcriptomics platform, a method that provides visualization and quantitative analysis of the transcriptome in individual tissue sections. Our study, to our knowledge, is the first attempt to characterize EGID through application of spatial transcriptomic approaches.

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O28

ARAP2 IS A POSITIVE REGULATOR OF THE IFN-GAMMA RESPONSE

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Abstract Content: The interferons (IFNs) are secreted by cells during the innate immune response and form an important part of our immune defence against infection. Suppressor of Cytokine Signaling 1 (SOCS1) is the critical negative regulator of IFN and the IL-2 family cytokines. SOCS1 is rapidly induced by these cytokines, acting in a classic negative feedback loop to inhibit cytokine signaling and maintain immune homeostasis. *Socs1*-deficient mice develop severe inflammation resulting in neonatal lethality (Starr et al., 1997), with the impact of SOCS1-haploinsufficiency in humans ranging from autoimmunity to disease susceptibility to severe infection (Korholz et al., 2021). Although the importance of SOCS1 in regulating IFN responses is indisputable, the nuances of how SOCS1 selectively regulates IFN signalling are still being elucidated.
ARAP2, a membrane associated GTPase activator protein, was identified as a SOCS1 interactor and regulator of IFNγ signaling. The interaction occurred through a high-affinity interaction between the SOCS1-SH2 domain and phosphorylated tyrosine residue 415 in ARAP2 (pTyr415; KD = 0.05 μM). CRISPR-Cas9 mediated deletion and siRNA-depletion of ARAP2 in cells diminished STAT1 phosphorylation in response to IFNγ. The reduction in STAT1 phosphorylation correlated with reduced STAT1 nuclear accumulation and transcriptional activity, as measured by immunofluorescence, STAT1-reporter assays, and qPCR analysis of known IFN response genes. Conversely, exogenous expression of ARAP2 enhanced IFN signaling. Moreover, mutation of the key SOCS1-interacting site in ARAP2 (Tyr415 to Phe) phenocopied ARAP2 loss-of-function. Based on these data, we hypothesize that ARAP2 is an adaptor protein with a dual role in regulating the IFN response; one to propagate signaling in the initial stages of the cascade and the other to recruit SOCS1 to complete the negative feedback loop.
Given that immune control of infectious disease relies heavily on an intact IFNγ response, we searched infectious disease patient databanks for nonsynonymous homozygous variants in coding regions of the ARAP2 gene. Four private, four rare and four common, missense ARAP2 variants were identified in 25 patients with viral or bacterial infections, including COVID-19 and Tuberculosis. The impact of these variants on ARAP2 function is currently being explored.
Collectively, this work has identified ARAP2 as a critical component of the IFN response and proposes an alternative hypothesis underpinning the mechanism of SOCS1 action.

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O29

IMMUNITY TO THE MICROBIOTA PROMOTES SENSORY NEURON REGENERATION

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Abstract Content: The microbiota plays a fundamental role in the induction, education, and function of the mammalian immune system. In turn, the immune system operates to sustain and restore tissue function in the context of microbial or environmental exposures. Host-microbiota dialogue is of particular importance at barrier sites that are both home to the microbiota and primary targets of environmental stressors. All barrier tissues, including the skin, are home to a dense network of sensory nerve fibers that are involved in the perception of touch, temperature, pain and itch. Recent work reveals that sensory neurons can also influence other biological processes including host metabolism, inflammation, and protective immunity. These emerging observations underscore our growing understanding of the profound interconnection among biological systems and more particularly between the immune and nervous systems. In the context of infection or injury, host survival requires protection and restoration of all tissue components, each requiring specific repair programs. Based on the profound alliance between the microbiota and its host, we hypothesized that the microbiota could play an important role in bridging biological systems to reinforce tissue protection and restore barrier integrity. In this context, whether immunity to the microbiota can promote neuronal regeneration remains unclear. Here, we show that, upon injury, adaptive responses to the microbiota directly promote sensory neuron regeneration. At homeostasis, commensal-specific Th17 cells colocalize with sensory nerve fibers within the dermis and express a transcriptional program associated with neuronal repair. Following injury, commensal-specific Th17 cells promote axon growth and local nerve regeneration. Mechanistically, our data reveal that the cytokine interleukin 17 A (IL-17A) produced by commensal-specific T cells directly signal to sensory neurons via the IL-17 receptor A, the transcription of which is specifically upregulated in injured neurons. Collectively, our work reveals that microbiota-specific T cells can bridge biological systems by directly promoting neuronal repair, and identifies IL-17A as a major determinant of this fundamental process. Our findings that upregulation of the IL-17A/IL-17RA axis represents a conserved response in injured neurons open the door to novel therapeutic approaches to potentiate sensory recovery after injury, or limit neuropathies in the context of diabetes and chemotherapy.

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O30

GAIN-OF-FUNCTION STAT3 MUTATIONS CONNECT AUTOIMMUNITY AND LEUKEMIA BY PATHOLOGICAL CD8 T CELL DYSREGULATION.

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Abstract Content: STAT3 plays pleiotropic roles in transcription regulation of hematopoietic and non-hematopoietic cells, downstream of surface cytokine and hormone receptors. Overactive STAT3 causes pathology. Germline *STAT3* GOF mutations cause severe early-onset multi-organ autoimmunity: RA, gut enteropathies, autoimmune cytopenias and other pathologies. The mechanisms thereof are largely unknown. In addition, somatic gain-of-function (GOF) *STAT3* mutations are incredibly recurrent in solid-organ and hematological malignancies, including 50% of CD8 T cell large granular lymphocytic leukemias (T-LGL). Notably, T-LGL is often accompanied by autoimmune disease, and somatic GOF *STAT3* mutations have been found in CD8 T cell clones from individuals with rheumatoid arthritis (RA), aplastic anemia (AA), pure red cell aplasia (PRCA), multiple sclerosis (MS) - but also in healthy individuals. This raises the crucial question of whether somatic *STAT3* mutations are a *consequence* of inflammation/proliferation, or a *cause* of autoimmune pathology. Curiously, germline *STAT3* mutations in autoimmunity modify different domains whereas T-LGL somatic mutations cluster overwhelmingly in the SH2 domain. This raises the possibility of differential effects in leukocytes of *STAT3* mutations within versus outside the SH2 domain.

Here, we addressed these questions in 15 humans with STAT3 GOF syndrome and in mice engineered to carry the most common STAT3 GOF syndrome mutation $STAT3^{T716M}$, or the SH2 dimerization interface mutation $STAT3^{K658N}$ found in T-LGL and STAT3 GOF syndrome.
We show that STAT3 GOF in mice and humans causes dramatic accumulation of polyclonal effector CD8 T cells expressing CD57 and the C-type lectin-like receptor NKG2D. Using single-cell mRNA sequencing, CITE-seq and Repertoire and Gene Expression by Sequencing (RAGE-Seq), we identify the landscape of genes and proteins dysregulated by STAT3 GOF in mouse and human CD8 T cell clones. We show that *STAT3* mutations – in different STAT3 domains – drive CD8 clonal expansion and over-expression of a suite of genes involved in CD8 cytotoxic effector functions. Our results implicate STAT3, NKG2D and IL-2/IL-15 signals in coordinately driving CD8 T cell-mediated pathology. Using chimeric mice and CD8 T cell depletion, we demonstrate for the first time that GOF *STAT3*-mutant CD8 T cells actively contribute to autoimmune pathology and lethality. Using genetic deletions and neutralising monoclonal antibody treatments in mice, we identify two possible avenues for targeted depletion, *in vivo*, of these pathological expanded STAT3 GOF effector CD8 T cells.
These results provide a framework for targeting the *STAT3*-mutant "$CD57^{neg}$", "$CD28^{neg}$" or "NK-like" CD8s that circulate as expanded clones and associate with disease in T-LGL, the elderly and in various autoimmune and autoinflammatory disorders.

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O31

CD40-AGONIST TREATMENT CAN REVERSE CHECKPOINT INHIBITOR RESISTANCE IN MELANOMA VIA PRIMING THE INFLAMMATORY RESPONSE OF MACROPHAGES

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Abstract Content: Checkpoint inhibitors (CPIs) have revolutionized cancer treatment, but therapy resistance remains a significant clinical challenge. One strategy to restore anti-tumor activity is to target immunosuppressive tumor-associated macrophages (TAMs) and myeloid cells that suppress adaptive immune responses. Our previous results in the YUMMER mouse melanoma model demonstrated that agonistic CD40 antibody (CD40ag) and CSF1R blockade could overcome PD-1 resistance via functional activation of a specific $CCL22^{+}CCL5^{+}$ IL-12-secreting dendritic cell (DC) subset, but did not indicate a clear role for TAMs, when initiated at day 7 post-tumor injection (early treatment). We hypothesized that the stage of tumor growth might change responsive immune cell populations targeted by CD40ag therapy. To address this, we studied a different treatment scheme in which a single dose of CPIs anti-PD-1 and anti-CTLA4 induced complete tumor regression when applied early, but fail to be effective in delayed administration schemes (after day 10). Specifically, we added CD40ag to a single-dose CPI treatment initiated at different stages of tumor growth, in order to define key myeloid subpopulations and cell-cell interactions leading to successful TME reprogramming versus resistance. In immunocompetent mice, an $iNOS^{low}$ TAM subset exhibited a partial inflammatory response which appeared to be dependent on early tumor T-cell infiltration. Administration of early CD40ag treatment did not generate additional changes in inflammatory markers in the TAM population, despite the significant fraction of $CD40^{+}$ TAMs. However, a delayed CD40ag treatment rapidly upregulated pro-inflammatory markers in a $CD40^{+}Ly6C^{+}$ TAM subset, with $iNOS^{high}$ expression. This approach synergized with CPIs, inducing more than 90% overall survival, in contrast to the monotherapies, which showed initial tumor control but were ineffective on their own long-term. The overall survival of mice treated with a single-dose CPI+CD40ag decreased with initiation delay. Furthermore, in $Rag^{-/-}$ mice, we did not detect $iNOS^{low}$ TAMs and early CD40ag treatment induced an immediate TAM response with multiple proinflammatory markers, but transient in the absence of T cells. By scRNA-seq, early treatment with CD40ag, and in combination with CPIs, increased expression of genes involved in T cell recruitment and activation, such as *Il12b* and *Cxcl9/10*, in responding subsets of DCs and TAMs, respectively. These findings supported the role of CD40ag treatment in activating the innate compartment and establishing an important link with adaptive immunity. Integrating scRNA-seq, flow cytometry and *in vivo* functional data, we determined changes in

myeloid cells associated with successful immune responses as well as TAM subsets limiting immunotherapy. Future studies will focus on immune cell spatial distribution to build and refine relevant interaction networks within the TME to define successful, myeloid-driven therapeutic responses in melanoma.

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O32

ELIMINATING THE IMMUNOTOXICITY OF INTERLEUKIN-12 THROUGH PROTEASE-SENSITIVE MASKING

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Abstract Content: Checkpoint inhibitor (CPI) immunotherapy demonstrates modest efficacy against immunologically 'cold' or immune-excluded tumors. Although interleukin-12 (IL-12) is a powerful antitumor cytokine that enables activation and recruitment of immune cells into tumors, its widespread use in the clinic has been hindered due to severe immune-related adverse events (irAEs). An ideal IL-12 therapy would restrict the proinflammatory effects of IL-12 to the tumor site, while limiting its exposure in the periphery. Here, we solved the IL-12 toxicity challenge by exploiting the preferential overexpression of proteases in the tumor to engineer tumor-selective, masked IL-12. A receptor-based masking domain was fused to IL-12 via a protease-cleavable linker and prevented IL-12 from signaling systemically, whereas proteolytic cleavage of the linker domain by tumor-associated enzymes restored the biological activity of IL-12. We demonstrate that intravenously (i.v.) administered, masked IL-12 produces strong therapeutic effects through remodeling the immune-suppressive microenvironment and renders CPI-resistant tumors responsive, while systemic irAEs are eliminated, boosting the therapeutic index of this promising cytokine. We also show that human patient biopsies (melanoma and breast cancer) can activate the masked IL-12 *ex vivo*, further highlighting the translatability of this approach.

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O33

NOVEL SMALL MOLECULAR INHIBITORS AS POSSIBLE TARGETED THERAPEUTICS FOR BREAST CANCER METASTASIS

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Abstract Content: Breast cancer remains the second leading cause of cancer-related deaths and the most commonly diagnosed cancer among women in the Unites States. For the past several years, research has focused on the role of inflammatory modulators such as interleukin-6 (IL-6) and their role in cancer. Increasing evidence suggests that breast cancer patients with high levels of proinflammatory modulators such as IL-6 and oncostatin-M (OSM) have an overall worse prognosis. Research performed by our lab and others has also shown that OSM promotes tumor progression and metastasis by inducing circulating tumor cell (CTC) numbers, and metastasis to distant sites *in vivo*. Importantly, we have also shown that OSM lingers in the extracellular matrix and is able to "bioaccumulate" providing a long-term active supply of OSM within the tumor environment. While efforts have been made to develop FDA approved therapeutics for OSM, previous studies have all utilized monoclonal antibodies and none have survived clinical trials. For the first time, our research group has developed a stepwise approach towards designing and testing novel small molecule inhibitors (SMIs) against OSM. To determine viable candidates, i*n silico* computational screening of compound libraries towards OSM revealed 16 top potential compounds that interact with the receptor binding region

and inhibit biological function. These top candidate inhibitors were then analyzed for direct interaction with cytokines using fluorescent quenching and chemical shift perturbation NMR (CSP-NMR). *In vitro* efficacy of each SMI was then analyzed by measuring inhibition of all signaling cascades activated by OSM using enzyme-linked immunosorbent assay (ELISA) and immunoblot assays. From these *in silico* and *in vitro* methods, SMI's were then structurally modified with effective functional groups to increase efficacy of binding interaction and reduce toxicity for living systems. Finally, our most promising small molecule inhibitor that has been sufficiently tested for efficacy was examined *in vivo* to evaluate toxicity and determine the maximum tolerated dose. SMI's that prove to be non-toxic were evaluated further *in vivo* utilizing a xenograft mammary breast cancer model, and treatment with our SMI proved to decrease tumor burden when compared to vehicle control. In conclusion, our lab has generated the first ever SMI aimed to inhibit OSM-mediated cancer progression. Our continued efforts aim to develop a novel FDA approved therapeutic to improve outcomes for breast cancer patients.
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O34

IL-27 GENE THERAPY INDUCES EXPANSION OF CD11B+GR1+ MYELOID CELLS WITHOUT COMPROMISING ANTI-TUMOR ACTIVITY

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Abstract Content: Interleukin-27 (IL-27) is a pleiotropic cytokine that exhibits stimulatory/regulatory functions on multiple lineages of immune cells, and has a potential to be used as a therapeutic for cancer. Indeed, we recently found that systemic delivery of IL-27 using adeno-associated virus (AAV-IL-27) exhibited potent inhibition to the growth of tumors in mouse models. In this work, we demonstrate that AAV-IL-27 treatment leads to significant expansion of both mouse and human myeloid cells (MCs). AAV-IL-27-induced expansion of MCs are mainly of the $CD11b^{+}Gr1^{+}Ly6C^{low}$ phenotype, whose expansion is IL-27R-dependent, and requires Stat3 signaling but is inhibited by Stat1 signaling. AAV-IL-27 treatment did not increase the self-renewal capacity of MCs, single cell RNAseq analysis suggests significant expansion of hematopoietic stem cells (HSCs) and granulocyte-monocyte progenitor (GMP) cells. IL-27-induced $Ly6G^{+}$ MCs highly expressed signature genes such as S100A9 and upregulated MHC class I/II molecules. Additionally, IL-27 induced $Ly6G^{+}$ MCs were less immune suppressive and their expansion did not promote tumor growth. In the tumor microenvironment, IL-27 therapy appears to promote $Ly6G^{+}$ MCs to differentiate into MHC class I/II^{high} and $F4/80^{high}$ macrophages. Thus, systemic delivery of IL-27 induces the expansion of MCs via increasing the numbers of HSCs and GMPs in without compromising IL-27 anti-tumor activity.

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O35

DECOY RESISTANT IL-18 EXPANDS HIGHLY FUNCTIONAL C-MAF+ CD8 T CELLS IN THE BONE MARROW TUMOR MICROENVIRONMENT

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Abstract Content: Interleukin-18 (IL-18) is a cytokine that promotes strong type 1 T cell responses, especially IFNγ production, and NK cell activation. However, an endogenous IL-18 binding protein (IL-18BP), induced by IFNγ in a negative feedback loop, limits IL-18 activity. Decoy resistant-18 (DR-18) is a synthetic IL-18 that is impervious to

binding and subsequent biological inactivation by IL-18BP and has previously been shown to generate potent anti-tumor immune responses in preclinical models of melanoma and colon adenocarcinoma. In the solid tumor setting, DR-18 significantly enhances IFNγ production and CD8 T cell activation. Understanding the mechanisms of action of DR-18 in the bone marrow (BM) microenvironment will aid in clinical translation of this drug as a monotherapy and in combination approaches in hematological malignancies. In the lab, we have developed a murine model of autologous stem cell transplantation for myeloma, which generates tumor control that is highly dependent on CD8 T cells and both IFNγ production and signaling within the donor graft. Briefly, mice are injected with Vk*MYC myeloma two weeks prior to lethal irradiation and transplantation with a genetically matched BM and T cell graft. DR-18 is highly efficacious in this setting and promoted tumor-specific immune-mediated control in 62% of treated mice compared to 10% of PBS-treated mice. By multiplex immunohistochemistry, we observed increased CD8 T cell infiltration of regions of healthy BM and within tumor lesions at 6 weeks post-transplant. We next used a triple reporter mouse (IL-10-GFP x IFNγ-YFP x FoxP3-RFP) to show significantly increased IFNγ production, without the use of ex vivo restimulation, in CD8 T cells from BM of DR-18-treated mice. Non-terminal bone marrow aspirates at 3 weeks post-transplant indicated that this phenotype was rapidly induced in response to DR-18. We next performed single cell RNA sequencing on BM CD8 T cells from PBS-treated mice with controlled myeloma or progressive disease and from DR-18 responsive or resistant mice at 6 weeks post-transplant. Additionally, we performed post-hoc TCR sequencing using described TCR pulldown methodology. Response to DR-18 immunotherapy was associated with expansion of a clonal *Maf*-expressing population, that was absent in mice that were relapsing on treatment. This population had high expression of genes encoding perforin, granzyme A, granzyme B and IFNγ. CD8 T cells from mice responding to DR-18 made up the majority of this *Maf*-expressing cluster and expressed *Il18r1* (IL-18 receptor). We confirmed protein-level expression of cytotoxic molecules by flow cytometry from CD8 T cells after DR-18 treatment. Notably, this phenotype was not driven in response to immune checkpoint inhibition with anti-PD-1. Together, these data highlight that DR-18 has potent anti-myeloma effects and generates a highly functional Maf-expressing CD8 T cell subset in the bone marrow of responding mice. Our preclinical data provides compelling rational for the clinical investigation of DR-18 in hematological malignancies.

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O36

INNATE TYPE 2 IMMUNITY CONTROLS HAIR FOLLICLE COMMENSALISM BY DEMODEX MITES

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Abstract Content: *Demodex* mites are obligate commensal parasites of hair follicles (HF) in mammals. Normally asymptomatic, inflammatory outgrowth of mites can accompany malnutrition, immune dysfunction and aging, but mechanisms restricting *Demodex* outgrowth and pathogenesis are not defined. Here, we show that control over mite HF colonization of mice requires ILC2s, IL-13, and its receptor IL-4Ra, but not IL-4 or the adaptive immune system. Epithelial HF-associated ILC2s elaborate IL-13 that attenuates HF and epithelial cell proliferation at anagen onset; in their absence, *Demodex* colonization leads to increased epithelial proliferation and replacement of gene programs for repair by aberrant inflammatory programs leading to loss of barrier function and premature HF exhaustion over time. Humans with rhinophymatous acne rosacea, a nasal inflammatory condition associated with a

high burden of *Demodex*, had increased HF inflammatory cells with decreased type 2 cytokines, consistent with the inverse relationship seen in mice. Our studies uncover a critical role for skin ILC2s and IL-13, which comprise an immune checkpoint necessary to sustain cutaneous integrity and restrict pathologic infestation by colonizing HF mites.

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O37

GROUP-3 INNATE LYMPHOID CELLS PROTECT FROM INTESTINAL INFLAMMATION VIA SYNDECAN-4

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Abstract Content: Group-3 innate lymphoid cells (ILC3s) are a recently described set of tissue resident immune cells which express RORγt and secrete a variety of effector cytokines to orchestrate homeostasis, inflammation, and immunity at mucosal barrier surfaces. Crucially, intestinal ILC3s are dysregulated during inflammatory bowel disease (IBD) with alterations in both frequency and function. It remains unclear how ILC3s sense and respond to the heterogeneity of signals present in the intestinal microenvironment and how they become disrupted during intestinal inflammation. Here, we unexpectedly identify that syndecan-4, a heparan sulfate proteoglycan, is significantly expressed among ILC3s relative to other intestinal immune cells, and that this pathway becomes dysregulated in mouse models of intestinal inflammation and humans with IBD. IL-1 selectively upregulates ILC3-specific syndecan-4, and IL-1R is required for optimal expression *in vivo*. Mice lacking ILC3-specific syndecan-4 exhibit exacerbated susceptibility to intestinal damage and inflammation resulting in greater loss of intestinal epithelial architecture compared to controls. Collectively, our data indicate that syndecan-4 represents a novel pathway by which ILC3s orchestrate intestinal health and limit tissue damage in response to inflammation.

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O38

ILC3S SELECT FOR RORGT+ TREGS AND ESTABLISH TOLERANCE TO INTESTINAL MICROBIOTA

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Abstract Content: Microbial colonization of the mammalian intestine elicits inflammatory or tolerogenic T cell responses, but the mechanisms controlling these distinct outcomes remain poorly understood and accumulating evidence indicates that aberrant immunity to intestinal microbiota is causally associated with infectious, inflammatory, and malignant diseases. Here, we define a critical pathway controlling the fate of inflammatory versus tolerogenic T cells that are specific for the microbiota and express the transcription factor RORγt. We profiled all RORγt$^+$ immune cells at single cell resolution from the intestine-draining lymph nodes of mice and reveal a dominant presence of Tregs and lymphoid tissue-induced (LTi)-like group 3 innate lymphoid cells (ILC3s), which co-localize at interfollicular regions. These ILC3s have interconverting potential with RORγt$^+$ extrathymic Aire-expressing cells, abundantly express major histocompatibility complex class II, and are necessary and sufficient to promote microbiota-specific RORγt$^+$ Tregs and prevent their expansion as inflammatory T helper (Th)17 cells. This occurs through ILC3-mediated antigen-presentation, interleukin-2 gradients, and av integrin. Finally, single-cell analyses demonstrate that ILC3 and RORγt$^+$ Treg interactions are impaired in inflammatory bowel disease. Our results define a novel paradigm whereby ILC3s positively select for antigen specific RORγt$^+$ Tregs, and against Th17 cells, to establish immune tolerance to the microbiota and intestinal health.

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O39

EARLY LIFE INFECTIONS EXTENSIVELY RESHAPE THE TRANSCRIPTIONAL PROFILE AND FUNCTIONALITY OF NK CELLS

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Abstract Content: Infections during early life can have substantially different outcomes and consequences than infections occurring in adulthood. In this study, we have used newborn mice infected with mouse cytomegalovirus (MCMV) to investigate the immunomodulatory effects of a perinatal infection on NK cells. We found that MCMV infection causes a significant shift of NK cell population towards the terminally mature phenotype and severely compromises their functionality, as demonstrated by the reduced ability of NK cells from infected mice to produce cytokines. Such extensive reshaping of NK-cell phenotype occurred only in mice infected during early life and required active virus replication. Remarkably, even infection with heavily attenuated MCMV strains induced NK cell hyporesponsiveness, suggesting that NK cell dysfunction is not due to impaired control of the virus in newborn mice. Mechanistically, the infection caused suppression of principal transcription factors governing NK cell fate and function, such as TCF-1 and Eomes, and resulted in dysregulation of numerous genes and impairment of NK cell function. Finally, we show that RNA virus and bacterial infection similarly impact NK cell function and phenotype. Altogether, our data indicate that early life infections can have profound adverse effects on the functional abilities of NK cells.

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O40

RBP/J REGULATES TISSUE SPECIFIC ADAPTATIONS OF GROUP 2 INNATE LYMPHOID CELLS

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Abstract Content: Recombination signal binding protein for immunoglobulin kappa J region (RBP/J) is a key effector molecule of Notch signaling that facilitates lineage specification of precursors towards the T cell fate and enforces GATA3 expression in Th2 cells. Group 2 innate lymphocytes (ILC2) also require GATA3 for their differentiation while early ILC precursor development has been proposed to rely on Notch signaling. However, the exact role for RBP/J in ILC2 differentiation and function has not been fully elucidated. Here we use an *in vivo* conditional RBP/J expression system to facilitate timed and inducible expression of RBP/J in hematopoietic cells to investigate the role of RBP/J in ILC2 development. Using a combination of bone marrow chimeras and adoptive cell transfers, we report that RBP/J-deficiency yields an anticipated block of T cell development but failed to disrupt ILC2 development across all innate lymphoid development stages. Instead, RBP/J-deficient ILC2s displayed an RBP/J-dependent alteration of ST2 expression in the gut but not the lung or adipose tissue. The modulated cytokine receptor expression led to hyperactivation of gut ILC2s and elevated production of IL-5 and IL-13, which resulted in an expansion of both goblet cells and tuft cells within the intestinal epithelium. Induced expression of RBP/J in RBP/J-deficient ILC2s, or neutralization of IL-33 reversed the IL-5 and IL-13-driven changes in the intestinal epithelium. Taken together, these results indicate that RBP/J dependent activation of Notch signaling in ILC2s constitutes a local gut-specific signal that drives tissue-adaptation of these innate lymphoid cells.

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Oral presentations: Cytokines and T cells

O41

IL-17 PRODUCTION BY SKIN RESIDENT INNATE T CELLS IS HARDWIRED BY EPITHELIAL DERIVED CHOLESTEROL METABOLITES FOR TISSUE IMMUNITY

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Abstract Content: Innate T cells and innate lymphoid cells in barrier tissues are essential for both tissue fitness and immunity against pathogens. γδTCR$^+$ lymphocytes that secrete IL-17 (Tγδ17) localize to dermis and are critical regulators of skin immune responses, but how they mediate their unique function in the skin is largely unknown. Tγδ17 cells express the two prototypic G-protein coupled receptors (GPCRs) CCR6 and GPR183 (EBI2): mice lacking both receptors had significantly diminished dermal Tγδ17 cells and were resistant to psoriasis induction. GPR183 recognizes oxysterols generated by the cholesterol hydroxylase CH25H and functional oxysterols were detected in both the thymus and skin. Analysis of *Ch25h*-reporter mice and unbiased single cell transcriptome analyses showed that interfollicular epithelial cells (IFEs) and a subset of medullary thymic epithelial cells (mTEC) are the depot for oxysterols in skin and thymus respectively. These results demonstrate that developing Tγδ17 cells must be conditioned by sensing cholesterol byproducts synthesized by a discrete subset of mTECs to position in specific oxysterol rich niches in the skin. High cholesterol concentration in the diet increased transcription of oxysterol enzymes and overall GPR183 ligand concentration in the skin, leading to spontaneous IL-17 secretion by Tγδ17 and epidermal hyperplasia. Dietary cholesterol also worsened ear thickening during a psoriasis model in a CH25H and GPR183 dependent fashion. Given that oxysterols can be regulated by infection and inflammation, this sensory circuit directed by cholesterol is predicted to program skin innate T cells' ability to rapidly survey for perturbations in the skin.

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O42

IFN-SIGNATURE TREGS IN VIRAL INFECTIONS

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Abstract Content: The role of regulatory T cells (Tregs) in limiting responses to pathogens, particularly in tissues, remains poorly described. Herein we identify a subset of Tregs with an interferon-stimulated gene-expression signature (ISG-Tregs) that arise in the thymus and represent ~5% of Tregs in secondary lymphoid organs at steady-state. We used scRNA-Seq and a newly generated *Foxp3*-lineage reporter line (*Foxp3-iDTR* mice) to track Tregs in the lungs and peripheral blood following infection with influenza virus. Few Tregs of any type are found in the lung at steady-state; ISG-Tregs appeared by day 3, peaked between days 7-10 (20-30% of all Tregs in the lung), and largely disappeared by day 21 post infection. A second transcriptionally diverse wave of tissue-repair-like Tregs (TR-Tregs) appeared by day 10 and were maintained through day 21 post infection. Using the *Mx1-Cre x Foxp3-iDTR* lineage reporter mouse we determined that the early wave of ISG-Tregs do not give rise to the second wave of TR-Tregs. Consistent with this observation we used TCR-Seq to establish that the TCR repertoire of ISG-Tregs between individual mice overlapped more than the TCR repertoire of ISG-Tregs and TR-Tregs from the same mouse. Thus, ISG-Tregs appear to recognize conserved antigens in response to influenza infection, and these antigens likely differ

from those seen by the second wave of TR-Tregs. Depletion of ISG-Tregs prior to a primary infection did not block the accumulation of ISG-Tregs in the lung during influenza infection. In contrast, depletion of ISG-Tregs prior to a secondary infection with a different strain of influenza did block the accumulation of ISG-Tregs in the lung. Thus, ISG-Tregs in the lung that arise after primary infection may arise from both pre-existing ISG-Tregs or develop in situ following infection. In contrast, prior infection with influenza appears to label all ISG-Tregs capable of responding to influenza with our DTR/GFP reporter, such that subsequent depletion of those cells prevents the emergence of an ISG-Treg population in the lung following a secondary infection. We are currently examining the effect of ISG-Treg depletion throughout a primary influenza infection to determine their functional role. In addition, using a novel human *ACE2* gene replacement mouse (in which the entire ~70,000 bp mouse *Ace2* gene is replaced with the entire ~70,000 bp human *ACE2* gene) we are currently testing whether ISG-Tregs also increase following infection with two distinct SARS COV2 strains. To examine this in human patients we made use of a recent scRNA-Seq meta-analysis of 111 patients with COVID-19 or healthy volunteers. Healthy human volunteers have few ISG-Tregs in peripheral blood. In contrast, ISG-Tregs expand ~16-fold in COVID-19 patients. Interestingly, patients with mild COVID-19 had a significant 50% increase in ISG-Tregs versus patients with severe disease. In contrast, patients with severe COVID-19 showed a substantial increase in an alternative Treg population expressing IL32. Thus, ISG-Tregs represent a new Treg subset that expands rapidly upon infection with influenza or SARS COV2 before being replaced with a distinct wave of tissue-repair-like Tregs.

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O43
NKG2D REGULATES THE EFFECTOR FUNCTIONS OF CD4+ T CELLS AND SHAPES THE INFLAMMATORY PROGRAMS OF MICROGLIA AND MONOCYTE-DERIVED CELLS DURING NEUROINFLAMMATION

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Abstract Content: The role of danger signals in driving aberrant immune responses during multiple sclerosis and experimental autoimmune encephalomyelitis (EAE), a mouse model thereof, remains largely unclear. We performed single cell transcriptome analysis of splenic versus CNS CD4+ T cells at disease peak revealing a transcriptional continuum within CNS CD4+ T cells with distribution skewed by the expression of key effector cytokines and activation markers. One prominent feature associated to CNS as compared to splenic CD4+ T cells was the expression of innate receptors, particularly *Klrk1*, coding for Natural Killer Group 2, Member D (NKG2D), a key innate sensor of cellular danger signals. Moreover, expression of NKG2D ligands was detected in CNS-derived activated microglia and monocyte subsets. CNS derived antigen-specific CD4+ T cells from mice with *Klrk1*-deficiency in the T cell compartment (*Klrk1ΔCD4*) were impaired in the production of inflammatory cytokines, particularly IFN-γ and GM-CSF, as well as in the recruitment of inflammatory myeloid cells, which further displayed transcriptional changes associated with cellular activation and metabolism as compared to littermate controls (*Klrk1Flox*). Importantly, we could demonstrate that *Klrk1ΔCD4* mice show significant resistance to EAE when compared to *Klrk1Flox*. Altogether, our findings suggest the role for the stress-sensing innate receptor NKG2D in the modulation of Th cell-mediated neuroinflammation.

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Oral presentations: Cytokines and B cells

O44
TYPE III INTERFERON DRIVES THYMIC B CELL ACTIVATION AND CENTRAL TOLERANCE

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Abstract Content: The activation of thymic B cells is critical for their licensing as antigen presenting cells and resulting ability to mediate central immune tolerance. The processes leading to thymic B cell licensing are still not fully understood. By comparing thymic B cells to activated Peyer's Patch B cells at steady state, we found that thymic B cell activation starts during the neonatal period and is characterized by TCR/CD40 dependent activation and IgD downregulation, followed by immunoglobulin class switch recombination (CSR) without forming germinal centers. Transcriptional profiling supported the finding of extrafollicular activation of thymic B cells, but also demonstrated a strong interferon signature, which is distinct from peripheral B cell activation. Removal of type I, II or III IFN receptors revealed thymic B cell activation and CSR were primarily dependent on type III IFN signaling. Loss of type III IFN receptor in thymic B cells resulted in reduced activation, and further resulted in reduced thymocyte clonal deletion and thymic regulatory T cell (Treg) development. Together, these findings reveal the importance of type III IFN in generating licensed thymic B cells and suggests innate cytokine signaling in the thymus has a significant impact on the generation of T cell tolerance to B cell presented self-antigens.

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O45

PSYCHOLOGICAL STRESS PROMOTES COLONIC TERTIARY LYMPHOID ORGAN FORMATION THROUGH IL-23 SIGNALING AND CONFERS PROTECTION AGAINST SECONDARY INJURY

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Abstract Content: Psychological stress has been previously reported to worsen symptoms of inflammatory bowel disease. Similarly, intestinal tertiary lymphoid organs (TLOs) have historically been associated with areas of more severe inflammation. There are no studies investigating TLO formation in the context of psychological stress. In addition, the majority of studies examining stress and IBD are performed in mouse models of acute colitis. Here, we use a mouse model of Crohn's disease-like ileitis, the SAMP1/YitFc (SAMP), which develops spontaneous ileitis, but not colitis, without chemical or genetic manipulation. Stress was induced using restraint stress (RS); mice were restrained 3 hours per day for 56 consecutive days.
While RS-SAMP did develop depressive-like behavior, they did not exhibit more severe ileitis, nor did they develop spontaneous colitis. However, stressed mice did have significantly more colonic growths. Upon investigation, it was determined that these growths resembled TLOs due to the high number of B cells, CD4+ T cells, and dendritic cells. The cellular composition of colonic TLOs was determined through deconvolution of whole transcriptome atlas data collected from the GeoMx digital spatial profiler platform and validated with flow cytometry. Interestingly, while there was increased formation of TLOs in RS-SAMP mice, the composition of those TLOs did not differ from unstressed (US) mice.
16s analysis of the stressed SAMP microbiome revealed no persistent genus-level changes. We performed fecal microbiome transplantation (FMT) to account for potential species-level changes or functional alteration. FMT into germ-free SAMP mice using stool from unstressed and stressed mice replicated the behavioral phenotype seen in donor mice. However, there was no difference in TLO formation between recipient mice.
Whole transcriptome atlas analysis of RS-SAMP mice TLOs demonstrated increased expression of the key TLO formation pathway constituents *ltbr*, *il23*, *il23r*, *il22*, and *tnfsf13* (APRIL). Increased production of IL-23 and IL-22 was confirmed with ELISA, and *ltbr* was confirmed with RT-PCR. Previous work has demonstrated several redundancies in the TLO formation pathway upstream of IL-23 signaling. Therefore, we generated SAMP x IL-23r$^{-/-}$ (KO) mice and subjected them to chronic RS. RS-KO mice were unable to increase TLO formation. Furthermore, IL-23, but not IL-22, production was increased in KO. Administration of recombinant IL-22 rescued TLO formation in both US and RS-KO mice.

We then induced a secondary colonic insult in US and RS-SAMP mice with a 7-day 3% dextran sodium sulfate (DSS) regiment. Interestingly, RS-SAMP mice administered DSS had reduced colitis on both histology and endoscopic examination. Mesenteric lymph node cell profiles were not significantly different between US and RS-SAMP mice administered DSS.
Our findings demonstrate that psychological stress induces colonic TLOs through intrinsic alterations in IL-23 signaling, not through extrinsic influence from the microbiome. Furthermore, chronic stress is protective against secondary injury from colitis, suggesting That TLOs may function to improve the intestinal mucosal barrier.

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O46

MEMORY B CELLS IN SARS-COV-2 CONVALESCENT PATIENTS RE-ENGAGE IN THE IMMUNE RESPONSE AFTER VACCINATION

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Abstract Content: Introduction: Upon SARS-CoV-2 infection, serum antibody responses with neutralizing activity are mounted. While a single infection does not provide strong protection against re-infection by virus variants, multiple antigen contacts lead to the emergence of broadly neutralizing antibodies.
Methods: We studied the B cell response of a convalescent individual, who received an additional COVID-19 vaccination one year after recovery. Spike-specific B cells were sorted before and at different time points after the vaccination and they were subjected to high-throughput single-cell RNA sequencing. V(D)J sequences of single B cells were deciphered and used to produce recombinant monoclonal antibodies.
Results: Long-lived memory B cells that originated from the initial SARS-CoV-2 infection re-entered the vaccine response. This process was accompanied by transition of the B cell compartments into an activated state by upregulation of CD11c, T-bet and characteristic integrins. This subset largely overlaps with a B cell subset that was previously described as age/autoimmune-associated B cells and which increases in frequency with repeated antigen contacts. Some B cells expressed IL-10 upon vaccination, which suggests a regulatory function. One week after vaccination, antibody-producing plasmablasts emerged. Expanded clones were detected and the process of antibody maturation was highlighted by the identification of phylogenetic trees. Analysis of *in vitro* expressed monoclonal antibodies indicated that upon hypermutation the neutralizing potency of the derived mAbs increased strongly. Some hypermutations led to differential neutralization of variants of concern (VOCs).
Conclusion: We argue for CD11c+ and T-bet+ B cells being a common subset of activated memory B cells, which can be detected after repeated encounter with the antigen. Even though this subset is often discussed in the context of autoimmunity, it takes part in anti-viral immune reactions and gives rise to hypermutated B cell clones. In addition to classical affinity maturation, a second important function of antibody maturation is the re-diversification of dominant B cell receptor clones via insertion of hypermutations, which alters the binding mode of an antibody to its target. This effect explains the phenomenon that booster vaccinations can lead to increased neutralization of VOCs, which evolved by escape from serum antibody responses.

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Oral presentations: ILC2s

O47

CRISPR-MEDIATED GENOME-WIDE TRANSCRIPTION FACTOR SCREENING IDENTIFIES MEF2D AS A NOVEL REGULATOR OF ILC2 FUNCTION

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) potently drive type 2 immunity by secreting cytokines such as interleukin (IL)-5 and IL-13. ILC2s participate in protective immunity against helminths infections, however overactive ILC2 activity can contribute to the development of atopic diseases such as allergy and asthma. Targeting regulators of ILC2 development and function represents a rational avenue to manipulate type 2 inflammation. However, the rarity of ILCs and their upstream progenitors hampers efforts to discover novel regulators in a high throughput manner. In the present study we optimised an ILC culture and CRISPR-screening protocol to uncover novel regulators of ILC development and function. By manipulating the duration of Notch signalling and provision of cytokines, we engineered an ILC culture assay in which ILC2s and ILC1/NK cells represent the major progeny derived from in vitro expanded common lymphoid progenitors (CLPs). The CLPs were derived from either IL-13-tdTomato reporter mice or Gata3-human-CD2 reporter mice crossed with Cas9-EGFP expressing mice, cultured and transduced with a retroviral library containing sgRNAs targeting 1131 mouse transcription factors. sgRNAs that disrupted the pathway for IL-13 or Gata3 expression were detected using flow cytometry and computational analysis. Several novel transcription factors were identified and validated as regulators of ILC2 development and function using Gata3 and IL-13 as readouts, including Mef2d.
Mef2d is a member of the MEF2 family transcription factors with characterised roles in regulating muscle, heart and neuronal development. In the haematopoietic compartment, MEF2 transcription factors are involved in B cell development, B cell receptor signalling and Treg cell differentiation. The role of Mef2d in ILC2 function and type 2 immunity is so far uncharacterised. Conditional deletion of Mef2d in mouse lymphocytes (Mef2d cKO) resulted in blunted lung type 2 responses in multiple models of allergen-induced intranasal challenge. In an antigen-specific response model, mice were intranasally sensitised and re-challenged with the cysteine protease papain in combination with 2W1S peptide. Mef2d cKO mice harboured lower numbers of ILC2s, and those present expressed lower levels of Gata3, IL-5 and IL-13 in vivo. Consequently, the hallmarks of allergic asthma, including eosinophil infiltration and macrophage Arg1 expression, were reduced in Mef2d cKO mice. A deficiency in the adaptive response was also observed as evidenced by a reduction in both 2W1S-specific and bystander Th2 cells. In a second model of acute type 2 response induced by intranasal ragweed challenge, Mef2d cKO mice developed a suppressed type 2 lung response similar to that observed in the antigen specific model.
Ongoing studies suggest that Mef2d regulates Gata3-dependent and independent pathways in ILC2s. In summary, through our novel genetic screens we have identified Mef2d as a critical regulator of ILC2 biology and type 2 immunity.

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O48

C-MAF ENFORCES CYTOKINE PRODUCTION AND PROMOTES MEMORY-LIKE RESPONSES IN MOUSE AND HUMAN TYPE 2 INNATE LYMPHOID CELLS

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Abstract Content: Group-2 innate lymphoid cells (ILC2s), which are involved in type 2 inflammatory diseases such as allergy, can exhibit immunological memory, but the basis of this ILC2 "trained immunity" has remained unclear. Here, we found that stimulation with IL-33/IL-25 or exposure to the allergen papain induces the expression of the transcription factor c-Maf in mouse ILC2s. Chronic papain exposure results in high production of IL-5 and IL-13 cytokines and lung eosinophil recruitment, effects that are blocked by c-Maf deletion in ILCs. Transcriptomic analysis

revealed that knockdown of c-Maf in ILC2s suppresses expression of type 2 cytokine genes, as well as of genes linked to a memory-like phenotype. Consistently, c-Maf was found highly expressed in human adult ILC2s but absent in cord blood and required for cytokine production in isolated human ILC2s. Furthermore, c-Maf-deficient mouse or human ILC2s failed to exhibit strengthened ("trained") responses upon repeated challenge. Thus, the expression of c-Maf is indispensable for optimal type 2 cytokine production and proper memory-like responses in group-2 innate lymphoid cells.

Disclosure of Interest: None Declared
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O49
INHIBITION OF DIACYLGLYCEROL ACYLTRANSFERASE 2 RESTRAINS GROUP 2 INNATE LYMPHOID CELLS AND ALLERGIC LUNG INFLAMMATION

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) comprise a remarkably potent source of cytokines and have been shown to be central in instructing and sustaining human type 2 immunopathologies including allergic lung inflammation and asthma. ILC2s directly sense alarmins such as interleukin (IL)-33 following allergen or microbial challenge, driving ILC2 proliferation and type 2 cytokine production. However, the precise molecular signatures of IL-33-mediated ILC2 activation remain unknown. Using an RNA-sequencing approach we demonstrate here that in ILC2s IL-33 rapidly induces the expression of diacylglycerol acyltransferase 2 (DGAT2), an enzyme known catalyze triacylglycerol synthesis and lipid storage in lipid droplets. In addition, we observed that IL-33-mediated ILC2 activation leads to elevated fatty acid uptake and storage that requires activity of fatty acid binding protein 5 (FABP5) and fatty acid transporter protein 2 (FATP2). Importantly, lipidomic analysis revealed a selective role of DGAT2 in fatty acid metabolism in ILC2. Moreover, pharmacological inhibition of DGAT2 impaired proliferation and type 2 cytokine production of murine and human ILC2s in a dose-dependent manner. Moreover, in a preclinical mouse model of allergic airway inflammation, we demonstrate that DGAT2 inhibition decreases ILC2 proliferation, lung inflammation and airway hyperreactivity. These observations highlight the crucial role of DGAT2 in ILC2 biology and ILC2-mediated lung inflammation and suggest that DGAT2 inhibitors should be considered as a promising approach for the treatment of asthma.

Disclosure of Interest: None Declared
DOI: https://doi.org/10.55567/C22.O49

Oral presentations: Joint Plenary Session: Cytokines and ILCs - host-microbe context

O50
IL-12 CONTROLS HOST PROTECTION AND PATHOLOGY DURING STERILE INFLAMMATION VIA A TISSUE-RESIDENT CDC1-ILC1 CIRCUIT

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Infection is restrained by the concerted activation of structural cells, tissue-resident and circulating immune cells. While much work has focused on how tissue-resident innate immune cells sense pathogens to elicit a swift effector response and subsequent recruitment of leukocytes to infected tissues, the signals that initiate the activation of tissue-

resident innate immunity during sterile inflammation are not well understood. Our lab has shown previously that that tissue-resident type 1 innate lymphoid cells (ILC1) serve an essential early role in host protection through rapid production of interferon (IFN)-γ following viral infection and acute liver injury, suggesting that ILC1 responses may be primed following structural cell apoptosis. In support of this hypothesis, we found that conventional type 1 dendritic cells (cDC1) endocytose cell-free DNA from dying structural cells to activate the cGAS-STING pathway and IL-12 production. IL-12 signaling derived from cDC1 is sufficient and necessary to induce ILC1 IFN-γ production during viral infection and acute liver injury to promote the survival of host structural cells through limiting viral spread in infected tissues or by increasing Bcl-xL expression in hepatocytes following liver injury. However, this mechanism contributes to host pathology during diet-induced obesity following continuous IL-12 production from adipose-resident cDC1 to potentiate IFN-γ-dependent polarization of recruited monocytes to inflammatory macrophages that contribute to adipose tissue inflammation and systemic insulin resistance. Together these results identify a fundamental tissue-resident circuit composed of cDC1 and ILC1 responses that control the host immune response to dying cells during inflammation.

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O51

RECRUITMENT AND ACTIVATION OF TYPE 3 INNATE LYMPHOID CELLS PROMOTES ANTI-TUMOR IMMUNE RESPONSES AFTER A CISPLATIN TREATMENT.

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Abstract Content: T lymphocyte infiltration to the tumor bed is a major prognostic factor in many cancer types. Immune checkpoint blockade (ICB) is able to trigger long-lasting antitumor immune responses resulting in clinical benefit in various cancer types, but only in a limited number of patients. Tumors that lack lymphocyte infiltrate (also called ‘cold’ tumors) are resistant to ICB monotherapy and some cytotoxic chemotherapies used in a neoadjuvant setting. This led to the hypothesis that increasing T cell infiltration of cold tumors may enhance their sensitivity to checkpoint inhibitors and chemotherapies, as well as the number of responding patients.

ILC3s express the transcription factor RORγt and produce the cytokines interleukin-17 (IL-17) and IL-22. ILC3s include natural cytotoxicity receptor (NCR)+ ILC3s, chemokine receptor type 6 (CCR6)+ ILC3s and fetal lymphoid tissue inducer (LTi) cells involved in the development of secondary lymphoid organs. Conflicting roles for ILC3s have been described in the development of cancer. Accumulation of ILC3s is associated with tumor growth and poor outcome in breast and colorectal cancer. In contrast, such accumulation is associated with a better outcome in non-small cell lung carcinoma (NSCLC) and melanoma. Notably, in NSCLC, ILC3s have also been positively associated with the presence of tertiary lymphoid structures, a known predictor of a favorable clinical outcome, suggesting that ILC3s in tumors might possess lymphocyte recruitment capacity, a typical feature of LTi cells.

Using murine models, we found that CCR6+ type 3 innate lymphoid cells (ILC3s) can trigger an increase in the number of T cells infiltrating a tumor. Shortly after administration of cisplatin chemotherapy, production of the chemokine CCL20 and proinflammatory cytokine IL-1β at the tumor site led to the recruitment and activation of ILC3s. Within the tumor, ILC3 production of the chemokine CXCL10 was responsible for the recruitment of CD4+ and CD8+ T lymphocytes to the tumor. ILC3-dependent infiltration of T cells was essential for antitumor immune responses and increased the efficacy of checkpoint inhibition. Thus, we reveal an essential role of CCL20 and IL-1β, which promote ILC3-dependent antitumor immunity and enhance tumor sensitivity to immunotherapy.

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O52
DISTINCT 3D REMODELING OF IL4-IL13-IL5 LOCI MEDIATES DIFFERENTIAL TYPE 2 RESPONSES IN INNATE VERSUS ADAPTIVE LYMPHOCYTES

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Abstract Content: Type 2 cytokines, IL-4, IL-5 and IL-13 reside on the same chromosome as a cluster and are a hallmark of type 2 immune responses controlling pathogen clearance and tissue repair, and also causing allergies and asthma when dysregulated. Both innate and adaptive lymphocytes are specified selectively produce cytokines; however, their production of pattern of effector cytokines is distinct. Specifically, the ILC2 response is rapid with prominent production of IL-5 and IL-13, but minimal IL-4. In contrast, Th2 responses emerge over time following activation with prominent IL-4 production along with IL-13 but little IL-5. How those two related but distinct type 2 immune cells configure the extended type 2 cytokine loci and elicit selective output of this cassette genes is not well understood. While multiple layers of regulatory mechanisms are expected in play including transcription factor (TF) loading, chromatin accessibility or chromatin conformation, a detailed understanding of contribution from those regulatory mechanisms remains elusive. Here, we aimed to take a holistic view of type 2 cytokine loci before and after activation between ILC2 and Th2 to decipher the molecular underpinning of differential cytokine output.
Transcriptome profiling of activated ILC2s and Th2 cells highlighted a unique transcriptional response in which only few genes, including *Il4, Il5, Il13* and *Csf2*, were massively transcribed in a cell-type specific manner. Notably *Il4* was only induced in Th2 cells with IL-5 being highly produced in ILC2 cells. Global chromatin architecture was mostly stable regardless of cell activation status, in line with previous reports. Nonetheless, the extended type 2 cytokine loci underwent dramatic remodeling during 1 hr stimulation, resulted in differential 3D configurations between ILC2 and Th2. In ILC2, *Il13* and *Il5* loci were aligned in proximity to support parallel transcription of 2 cytokines in the shared topologically associated region (TAD) while *Il4* locus was insulated and separated into a different TAD. In Th2, the insulation of IL-4 was not evident and *Il4* and *Il13* were aligned in proximity while *Il5* locus was located in distance in a separate TAD. By integrating multi-dimensional data on epigenome and TF loading, more than ten putative REs were further characterized and classified into 2 groups: structural REs bound by CTCF and Cohesin, and functional enhancers bound by various TFs and histone acetyltransferase. Mice with individually targeted deletion of 5 defined REs confirmed cell-type specific and activation-dependent roles of these REs in regulating ILC2s and Th2 cells *in vivo*. Cross-referencing these murine REs to human genome revealed conservation in human as well as close proximity to disease associated SNPs linked to allergy, asthma and eosinophil activation. *Therefore, signal-dependent dynamic 3D configuration instructed by the interaction between TFs and REs is likely to underlie the discordant regulation of type 2 cytokines between ILC2s and Th2 cells in mice and human.* These results will significantly advance our understanding of epigenomic control of type 2 cytokines including 3D conformation and may further help understand context dependent pathogenic nature of GWAS mutations linked to allergic diseases.

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Oral presentations: Cytokines and microbiome

O53
REGULATION OF BILIARY IMMUNITY BY TUFT CELLS AND THE MICROBIOME

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Abstract Content: Inflammation and injury in the extrahepatic biliary tree can lead to severe liver and pancreatic injury, and compromises host metabolic function. Despite this, biliary inflammation is poorly understood. We have found that the biliary tree is home to a diversity of immune cells, and exhibits robust transcriptional heterogeneity among epithelial cells. We explored the role of tuft cells—chemosensory epithelial cells with immunomodulatory functions—in the biliary tree using knockout mice and single cell sequencing. We found that abundance of biliary tuft

cells is regulated by bile acids and decreases postnatally. In the absence of tuft cells, we observed spontaneous accumulation of highly activated neutrophils; this was accompanied by an increase in inflammatory gene expression from non-tuft epithelial cells, including elevated expression of TNFa which may be required for neutrophil recruitment. Finally, we found that this altered inflammatory environment was driven by the host microbiome. Our current work seeks to identify the microbiome-dependent factors that can impact biliary immune cell milieu, and the relevant tuft cell effector molecules that prevent aberrant inflammatory responses to the host microbiome at homeostasis.

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O54

THE METABOLITE DERIVED FROM HIGH-SALT DIET-INDUCED GUT MICROBIOTA REGULATES BRAIN TUMOR ENVIRONMENT.

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Abstract Content: The Westernized diet refers to consuming high amounts of saturated fat, sugar, and salt, and low amounts of dietary fiber. Such a diet is currently thought to contribute to non-communicable diseases such as autoimmune diseases, heart disease, and cancer. A high-salt diet (HSD) is known to affect various organs, including the cardiovascular, renal, and central nervous systems. Recently, several studies reported the effect of HSD on cancer due to the enhanced osmolarity of tumor microenvironment. However, it has been known that the concentration of sodium in brain is tightly regulated. That is, there may be another effect of HSD to tumor environment in brain. In addition, the effects of HSD on brain tumor have not been elucidated. In this study, we observed an increased tumor burden in mice who received the high-salt diet. Mice on a high-salt diet also showed a change in their gut microbiota. The specific bacteria significantly increased in HSD group compared to normal-salt diet (NSD) group. This bacteria is known to produce several metabolites, and we measured these metabolites in cecum of NSD and HSD group. Among them, the one metabolite increased in HSD group and we supplemented this metabolite to mice with brain tumor. Then, we observed the exacerbation of brain tumor post the supplement compared to control group. Therefore, we speculated that the HSD exacerbated brain tumor via the increased production of metabolite by altered gut microbiota.

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O55

IGA CONTROLS THE MICROBIOTA FROM DRIVING DYSREGULATED HOST ADAPTIVE IMMUNE RESPONSES

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Abstract Content: IgA is the most abundant immunoglobulin isotype produced in the human body. It coats a substantial proportion of the commensal microbiota, but its function has largely remained elusive. Reports of elevated IgM in previous models of IgA deficiency has suggested that IgM may compensate to control the microbiota. To identify the function the IgA, we generated a novel IgA deficient mouse model termed IgA secretory deficient, IgAsec. In this model, B cells retain the ability to class switch to IgA in the Peyer's patches (PP), but are unable to secrete the antibody, eliminating both luminal IgA and the elevated IgM plasma cells seen in the original model. We observed that the IgAsec deficient mice had dysregulated adaptive immune responses marked by expansions of activated T cells in the intestine tissue and in the PPs. Intriguingly, IgA played different roles in the small and large intestine limiting distinct helper and cytotoxic T cell functions. Using germ-free IgAsec deficient mice and transfers of microbial consortiums, we identified that specific commensal microbes are required to drive the dysregulated adaptive immune responses seen in SPF IgAsec mice. In addition, we found that IgA protected the host from severe inflammatory disease. In IL-10 deficient hosts, IgA deficiency led to increased colitis and mortality. These results reveal that IgA

plays a critical role at mucosal surfaces controlling specific commensal microbes from driving inflammatory T cell responses in intestine.

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Oral presentations: Cytokines and general host defense

O56

A SUBSET OF CD4+ EFFECTOR MEMORY T CELLS LIMIT IMMUNITY TO PULMONARY VIRAL INFECTION AND PREVENT TISSUE PATHOLOGY VIA ACTIVATION OF LATENT TGF-BETA

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Abstract Content: A rapid immune response upon pathogen re-exposure underpins immunological memory, with cross protection against divergent pathogens such as heterologous or novel viral strains requiring cross-reactive memory T cells. Understanding the pathways which control memory T cell formation and function is therefore crucial for the rational design of viral vaccines, and will aid the discovery of therapies to boost anti-viral immunity.

The cytokine TGFβ is a key regulator of mucosal homeostasis by restraining effector T cell function. TGFβ is always secreted as a latent complex which must be activated extracellularly. However, the role of TGFβ activation in regulating memory T cell function remains poorly understood.

Here, we show that a population of CD4+ effector memory T (T_{EM}) cells activate latent TGFβ via expression of an integrin, αvβ8. Integrin αvβ8 expression marks a transcriptionally distinct sub-population of CD4+ T_{EM}, enriched for anti-inflammatory pathways. Loss of αvβ8 on murine CD4+ T_{EM}, but not Foxp3+ Tregs, led to exacerbated virus-specific CD8+ T cell responses following secondary influenza infection, which was associated with enhanced viral clearance. However, although accelerating viral clearance, loss of αvβ8 expression on CD4+ T_{EM} resulted in enhanced lung pathology following secondary influenza infection, which was completely reversed by adoptive transfer of αvβ8+ CD4+ T_{EM}.

These data highlight a novel pathway by which a distinct CD4+ memory T cell subset restrains anti-viral immunity to prevent host tissue damage during secondary viral infection. Such pathways could be targeted therapeutically to either boost memory T-cell-mediated immunity, or restrain host tissue damage during viral infection.

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O57

HISTONE DEACETYLASE 1 RESTRICTS PRO-INFLAMMATORY CYTOKINE RELEASE IN T CELL-MEDIATED ANTIFUNGAL IMMUNITY

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Abstract Content: Life-threatening invasive fungal infections by commensal Candida spp. in immunocompromised individuals represent high healthcare burdens, especially owing to the worrisome rise of antifungal resistance.

Although innate immune responses are pivotal to control fungal infections, antifungal immunity also relies on protective T and B cell responses. Of note, the molecular knowledge about how epigenetic mechanisms mediate or contribute to T cell-mediated immunity, polarization and plasticity upon fungal challenges remain scarce. Here, we use a conditional $CD4^+$ T cell-specific knock-out mouse model lacking histone deacetylase 1 ($HDAC1^{-/-}$). HDAC1 is responsible for reversible chromatin modifications and involved in many biological functions, including inflammatory responses and T helper (T_h) subset activation. We show here that $HDAC1^{-/-}$ $CD4^+$ T cells exhibit cell-intrinsic dysregulated cytokine expression profiles, leading to increased release of pro-inflammatory mediators like IFNγ, IL-17A and IL-22. Hence, HDAC1-deficient mice display increased susceptibility following systemic fungal infections accompanied by severe immunopathology of infected organs. Further, analysis of isolated $CD4^+$ T cells from infected organs and secondary lymphatic tissue revealed HDAC1-dependent alterations in T_h17- regulatory T cell (T_{reg}) plasticity. Our data highlights the crucial role HDAC1 exerts on the control of cytokine release and T cell polarization during the onset of the adaptive response to pathogenic challenge.

Disclosure of Interest: None Declared
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O58

INTERLEUKIN-17 RECEPTOR SIGNALING PROMOTES PANETH CELL STEMNESS AND THEIR ABILITY TO REGENERATE GUT EPITHELIUM AFTER IRRADIATION INDUCED INJURY

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Abstract Content: IL-17A/F signaling through its receptor complex (IL-17RA/RC) plays a vital role in immunity and inflammation. IL-17RA/RC is expressed by Lgr5+ intestinal stem cells (ISCs), progenitor cells and diverse intestinal epithelial cell types including Paneth cells. Furthermore, it has been observed that therapies neutralizing IL-17A or IL-17RA in patients with Crohn's disease (CD) induce adverse side effects. This suggests that IL-17A may play an essential role in intestinal mucosal host defense. Paneth cells and their antimicrobial products play a critical role in mediating small intestinal host defense under homeostatic conditions as well as after injury or infection. Paneth cells have also been shown to gain stemness and repropagate intestinal crypts during inflammation in the absences of Lgr5+ ISCs. The specific role of intestinal IL-17A signaling in Paneth cells to regulate host defense at steady state or after irradiation induced intestinal inflammation remains poorly understood. To address this, we generated Paneth cell-specific *(Il17ra$^{fl/fl}$;Defa6-cre*) *Il17ra* knockout mice as well as lineage tracer (*Defa6-cre*;*mT/mG*) mice. We exposed these mice to gamma irradiation (1200 cGy) to study intestinal injury and regenerative responses. Mice were euthanized 3 and 5 days post irradiation. Under homeostatic conditions no difference in Paneth cell number, morphology and expression of key antimicrobial genes (*Lyz1 and defensins*) were observed in *Il17ra$^{fl/fl}$;Defa6-cre+/-* mice. However, our preliminary data from irradiated *Il17ra$^{fl/fl}$;Defa6-cre+* mice suggest that IL-17RA signaling is important in maintaining intestinal barrier integrity since we observed increased microbial dissemination to the liver and spleen compared to their respective littermate control mice. We also observed increased levels of fecal Lcn2 (a marker for intestinal inflammation) in irradiated *Il17ra$^{fl/fl}$;Defa6-cre+* mice. Lastly, our preliminary data from irradiated *Defa6-cre*;*mT/mG*mice that received anti-IL-17A treatment revealed a reduced ability of Paneth cells to repopulate the intestinal epithelium. Collectively, our data reveals an important role of the IL-17A-IL-17RA axis in promoting Paneth cell-dependent tissue regeneration.

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Oral presentations: ILC3 and LTICs

O59

TOX2 IS REQUIRED FOR METABOLIC ADAPTATION OF ILC3 IN GUT BUT NOT CENTRAL LYMPHOID TISSUES

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Abstract Content: Innate lymphoid cells (ILCs) are immune cells that lack specific antigen receptors but possess similar effector functions as T cells. Interestingly, ILCs and T cells express many of the same transcription factors. One such factor is Tox2, a member of TOX family, that has been shown to play an important role in imposing T cell exhaustion in CD8+ T cells and in the differentiation of T follicular helper cell. However, its role in ILC lineages is unknown.

Tox2 is highly expressed in gut ILC3 as compared to ILC3 from mesenteric lymph node. We generated germline *Tox2*$^{-/-}$ mice and found that they have reduced ILC3 numbers in gut; however, ILC3 in spleen, lymph node or thymus were intact. We generated a conditional knock out allele of Tox2 (*Tox2*$^{fl/fl}$) and crossed it with ER$^{T2\ Cre}$ to ablate Tox2 from mature cells upon tamoxifen administration. Conditional ablation of Tox2 in ILC subsets of adult mice resulted in a significant reduction in gut ILC3, but mesenteric ILC3 numbers remained intact. This result suggested that Tox2 is important for persistence of mature gut ILC3.

Next, we performed transcriptional profiling of adult ILC3 from gut and mesenteric lymph nodes in *Tox2*$^{-/-}$ mice. ILC3 from mesenteric lymph nodes of *Tox2*$^{-/-}$ mice were transcriptionally similar to their wild-type counterparts. However, gut ILC3 from *Tox2*$^{-/-}$ mice appeared transcriptionally distinct. *Tox2*$^{-/-}$ gut ILC3 showed reduced expression of Hexokinase 2 (Hk2), the rate limiting enzyme in glycolysis. Consistent with reduced glycolysis of *Tox2*$^{-/-}$ gut ILC3 as compared to their WT counterpart, supplementing *Tox2*$^{-/-}$ gut ILC3 culture with glucose did not rescue survival whereas the metabolite pyruvate, that is downstream of Hk2, partially rescued the survival of gut ILC3 from *Tox2*$^{-/-}$ mice.

Similar to Tox2, Hk2 was upregulated in gut ILC3 as compared to mesenteric lymph node ILC3. HIF-1α is stabilized at protein level under hypoxia and we observed higher HIF-1α expression in gut as compared to mesenteric lymph node. This suggested gut is more hypoxic than mesenteric lymph node. Further, we found that hypoxia is sufficient to induce expression of Tox2 and Hk2 in ILC3. Because, Tox2 deficiency did not affect HIF-1α expression at the protein level, Tox2 is likely regulated downstream of HIF-1α.

We propose that ILC3 residing in gut adapt to hypoxia via a pathway involving Tox2 and Hk2 upregulation, which in turns supports glycolysis in gut ILC3. In summary, our results establish that ILC3 residing in different tissues are metabolically distinct, and further establish that some of these metabolic differences are transcriptionally controlled by Tox2.

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O60

POSTNATAL ILC HOMEOSTASIS AND FUNCTION IS CONTROLLED BY DISTINCT SUBSET-SPECIFIC MICRORNA REGULOMES

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Abstract Content: Innate lymphoid cells (ILC) are tissue-resident lymphocytes involved in the organogenesis of lymphoid structures and the organism's adaptation to environmental cues during post-natal life. Deregulation of ILC effector responses is involved in multiple inflammatory disorders. However, the underlying regulomes coordinating the functional programs in ILCs are poorly dissected. miRNAs are small non-coding RNA molecules which finetune cell function by regulating gene expression on the post-transcriptional level. Despite their fundamental role in regulating immune cells little is known about the role of miRNAs in controlling ILC homeostasis and function. This is partly due to the lack of genome-wide miRNA profiling studies for ILC resulting from their low abundance and paucity of input material.

Conditional deletion of the miRNA processor gene *DiGeorge syndrome critical region 8* (*Dgcr8*) resulted in virtual absence of peripheral ILC subsets and a lack of the postnatally forming solitary intestinal lymphoid tissues (cryptopatches and isolated lymphoid follicles). Surprisingly, upon abrogation of the miRNA machinery, the fetal ILC compartment and all prenatally forming lymphoid structures (lymph nodes, Peyer's patches) were intact identifying miRNAs as critical regulators for postnatal ILC homeostasis.
To dissect the role of miRNAs in regulating the postnatal ILC compartment we established a small RNA sequencing platform for ultra-low input allowing for robust profiling of the small RNA transcriptome in ILC. Using this approach, we obtained a comprehensive atlas of the miRNA transcriptional landscape of the four major ILC subsets in the small intestine. Remarkably, all ILC subsets clustered specifically based on their miRNome and harbored a unique miRNA transcriptional signature indicating distinct miRNA regulomes in each ILC subset.
To test whether specific miRNomes establish early during ILC development we generated miRNA transcriptomes of the bone marrow common lymphoid progenitor, common helper ILC progenitor and ILC2 precursors. We found that ILC subset-specific miRNA programs observed in fully matured cells were already turned on in bone marrow ILC precursors during ILC and ILC2 lineage commitment indicating developmentally conserved miRNA programs in ILC.
Inducible deletion of *Dgcr8* in ILC revealed subset-specific kinetics of miRNA-deficient ILC and enabled us to study their mRNA transcriptome. By analyzing genes that were upregulated after abrogation of the miRNA machinery and that harbored binding sites for the miRNAs expressed in each subset, we identified immune processes regulated by miRNA target regulomes in ILC. In NKp46^{+} ILC3 genes specifically related to type 1 immune responses were regulated by miRNA while in CCR6^{+} ILC3 miRNA target regulomes predominantly regulated antigen processing and presentation. In contrast, in ILC2 miRNAs regulated genes linked to immune cell activation. By deleting a highly expressed and conserved single miRNA in ILC we observed the regulation of the type 1 transcription factor T-bet and IFNg secretion in CCR6^{-} ILC3 while ILC2 showed an increased activation state. Our results uncover ILC-specific miRNA regulomes which establish during lineage commitment and control the postnatal ILC homeostasis and the individual functional output of ILC subpopulations.

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O61

MICROBIOTA-INSTRUCTED REGULATORY T CELL DIFFERENTIATION IS MEDIATED BY A DISTINCT ANTIGEN PRESENTING CELL SUBSET

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Abstract Content: The mutualistic relationship of gut-resident microbiota and cells of the host immune system promotes homeostasis that ensures maintenance of the microbial community and of a poised, but largely non-aggressive, immune cell compartment. Consequences of disturbing this balance, by environmental or genetic factors, include proximal inflammatory conditions, like Crohn's disease, and systemic illnesses, both metabolic and autoimmune. One of the means by which this equilibrium is achieved is through induction of both effector and suppressor or regulatory arms of the adaptive immune system. In mice, *Helicobacter* species induce regulatory (iTreg) and follicular helper (Tfh) T cells in the colon-draining mesenteric lymph nodes under homeostatic conditions, but can instead induce inflammatory Th17 cells when iTreg cells are compromised. How *Helicobacter hepaticus* and other gut bacteria direct T cells to adopt distinct functions remains poorly understood. Here, we investigated which cells and molecular components are required to convey the microbial instruction for the iTreg differentiation program. We found that antigen presentation by cells expressing RORgt, rather than by classical dendritic cells, was both required and sufficient for iTreg induction. These RORgt^{+} cells, likely to be type 3 innate lymphoid cells (ILC3) and/or a recently-described population of Aire^{+} cells termed Janus cells, require the MHC class II antigen presentation machinery, the

chemokine receptor CCR7, and a_v integrin, which activates TGF-β, for iTreg cell differentiation. In the absence of any of these, instead of iTreg cells there was expansion of microbiota-specific pathogenic Th17 cells, which were induced by other antigen presenting cells (APCs) that did not require CCR7. Thus, intestinal commensal microbes and their products target multiple APCs with pre-determined features suited to directing appropriate T cell differentiation programs, rather than a common APC that they endow with appropriate functions. Our results illustrate the ability of microbiota to exploit specialized functions of distinct innate immune system cells, targeting them to achieve the desired composition of equipoised T cells, thus maintaining tolerance.

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Oral presentations: Cytokines and anti-bacterial defense

O62

A FAMILY OF CONSERVED SHIGELLA EFFECTORS DAMPENS IFN RESPONSES BY BLOCKING CALCIUM SIGNALLING

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Abstract Content: Interferons (IFNs) have long been recognized for the ability to antagonize viral infections of human and murine cells, yet their functions during bacterial infections remain poorly understood. In particular, no bacterial virulence factors have been identified that evade IFN responses. Here we demonstrate that the enteric bacterium *Shigella sonnei* blocks signaling downstream of all families of IFN receptors. We identify the *Shigella* OspC family of type III-secreted effectors as potent inhibitors of JAK/STAT activation and expression of key IFN-stimulated genes (ISGs). Deletion of OspC1 and OspC3 rendered *Shigella* hyper-sensitive to IFN restriction in colonic epithelial cells, a phenotype absent in cells lacking IFN receptors. Similarly, *ospC1/C3*-deficient *Shigella* were attenuated during infection of the murine gut but rescued in mice lacking functional IFN signaling. The underpinning mechanism of IFN inhibition was mediated by blockade of the Ca^{2+} sensor calmodulin (CaM) upon OspC binding. Expression of OspC effectors or infection with OspC-expressing *Shigella* prevented phosphorylation and activation of CaM-associated kinases (CaMKs), inhibiting downstream JAK/STAT signaling. Finally, we observed conservation of OspC effector proteins in diverse human and animal-associated bacteria. All OspC-like proteins were able to bind CaM, but only those from pathogenic species could block IFN, suggesting that OspCs may represent a virulence strategy conserved in diverse pathogens. These data reveal a novel molecular mechanism of IFN inhibition, and exemplify the critical role of Ca^{2+} and IFN targeting in bacterial pathogenesis.

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O63

A HUMAN IFN-INDUCED APOLIPOPROTEIN L WITH DETERGENT-LIKE ACTIVITY KILLS INTRACELLULAR PATHOGENS.

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Abstract Content: Activation of cell-autonomous defense by the immune cytokine, interferon-gamma (IFN-g), is critical to control life-threatening infections in humans. IFN-g induces the expression of hundreds of host proteins in all nucleated cells and tissues, yet many of these proteins remain uncharacterized. We screened 19,050 human genes by CRISPR-Cas9 mutagenesis and identified IFN-g-induced apolipoprotein L3 (APOL3) as a potent bactericidal agent protecting multiple non-immune barrier cell types against infection. Canonical apolipoproteins typically solubilize mammalian lipids for extracellular transport; APOL3 instead targeted cytosol-invasive bacteria to dissolve their anionic membranes into human-bacterial lipoprotein nanodiscs detected by native-MS and visualized by single-particle cryo-EM. Thus, humans have harnessed the detergent-like properties of extracellular apolipoproteins to fashion an

intracellular lysin under IFN-inducible control, thereby endowing resident non-immune cells with a mechanism to achieve sterilizing immunity.

Disclosure of Interest: None Declared
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O64
A BACTERIAL AUTOTRANSPORTER IMPAIRS INNATE IMMUNE RESPONSES BY TARGETING THE TRANSCRIPTION FACTOR TFE3

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Abstract Content: Pathogen evasion of immune defense mechanisms is critical for the colonization of the host. Enterohemorrhagic *Escherichia coli* (EHEC) is a clinically significant human pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome. EHEC genome contains unique clusters of sequences called O islands (OI), which are absent in non-pathogenic *E. coli*. The function of most OI-encoded genes, particularly in the context of innate immunity, remains unclear. By screening a library of 56 EHEC O-island mutants, we have uncovered EhaF, an uncharacterized protein encoded by the OI-14, as an innate immune inhibitor in vitro and in vivo. Bioinformatic and biochemical analyses identified EhaF as an autotransporter—a type of bacterial secretion system with no known innate immune roles. We found that EhaF secreted by EHEC translocates into the cytosol of macrophages and inhibits TLR4 signaling and the ensuing inflammatory responses such as IL-6 and IFNb expression. An unbiased approach involving immunoprecipitation and mass spectrometry identified TFE3—a transcription factor emerging to be to be a key regulator of immune gene expression—as the target of EhaF; EhaF was found to interact with and inhibit TFE3 leading to the impairment of TLR4-mediated inflammatory responses. EhaF-TFE3 interaction also interrupted the phosphorylation of IRF3. In summary, this study has uncovered a previously unknown autotransporter-based bacterial strategy that targets a specific transcription factor to subvert innate immune responses, and thus has identified a new function for bacterial autotransporters.

Disclosure of Interest: None Declared
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Oral presentations: Cytokines and anti-viral defense

O65
IL-27 CONTRIBUTES TO ANTIVIRAL IMMUNE RESPONSES AT MATERNAL-FETAL INTERFACE DURING CONGENITAL INFECTION

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Abstract Content: Cytokines serve critical regulatory roles at the maternal-fetal interface during gestation. IL-27 is an immunoregulatory cytokine that is expressed to high levels at the maternal-fetal interface, yet its functional role during pregnancy remains unstudied. IL-27 can have both pro and anti-inflammatory immune impacts dependent upon context and has been previously shown to have a direct antiviral role in the skin, therefore we asked whether IL-27 signaling contributes to immune responses during congenital viral infection. Using an immunocompetent model of Zika virus infection, we evaluated infection outcomes in the presence or absence of immunoregulatory cytokine IL-27. We blocked IL-27 signaling through administration of IL-27 neutralization antibody prior to and throughout congenital Zika virus infection. We then assessed gross fetal morphology and viral loads in the fetuses and placentas and compared with isotype antibody control-treated pregnancies. We found significantly higher levels of fetal pathology during Zika virus infection in IL-27-neutralized mice than in IL-27 competent dams, as determined by total resorption of fetus. Yet, we observed similar ZIKV titers in the fetuses of IL-27-neutralized and control-treated dams. We demonstrate that IL-27R is expressed on immune cells at the maternal-fetal interface suggesting that fetal-pathology could be dependent on immune-mediated dysfunction. Additionally, we found that the placental tissues of IL-27-

neutralized dams had significantly higher ZIKV titers relative to the placental tissues of infected control dams, suggesting a potential antiviral role for IL-27 in the placenta. Overall, our data support an active role for IL-27 in facilitating antiviral immune responses at maternal-fetal interface and reveal a critical player of immunoregulation during congenital viral infection.

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O66

TYPE I AND III INTERFERON DRIVE NON-REDUNDANT AND AIRWAY SITE-SPECIFIC ANTIVIRAL IMMUNE RESPONSES IN HUMAN METAPNEUMOVIRUS INFECTION

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Abstract Content: Human metapneumovirus (HMPV) is a leading cause of acute respiratory infection. Although nearly every person is infected during childhood, re-infections occur often throughout life, highlighting difficulty in building long-term immunity. Early host responses to HMPV are poorly characterized, and further understanding could identify important therapeutic targets. Type I (IFN-α/β) and III (IFN-λ) interferons display potent antiviral activity against many respiratory viruses. However, their functions in HMPV infection remain largely unknown. Here, we identify distinct roles for type I and III IFN in HMPV. Mice lacking IFN-α/β receptor ($IFNAR^{-/-}$) exhibit less disease and reduced lung inflammatory cytokine levels, but no difference in lung HMPV titer. In contrast, mice lacking IFN-λ receptor ($IFNLR^{-/-}$) show moderate clinical disease, high lung cytokine levels, and increased lung HMPV titer. IFN-driven control of virus replication is airway site-dependent, as both $IFNAR^{-/-}$ and $IFNLR^{-/-}$ show increased HMPV titer in the nose. Non-redundant functions of type I and III IFN are supported by an upper respiratory tract-restricted HMPV infection model, in which $IFNAR^{-/-}$ mice display higher HMPV titers in the nose while $IFNLR^{-/-}$ mice show greater propensity for HMPV spread to the lung. Site-specific IFN-driven innate immune responses were also observed. Flow cytometry analysis suggests M2 macrophage loss with HMPV infection is coordinated mainly by type I IFN in the lung, but both type I and III in the nose. Additionally, IFN-specific differences were noted in epithelial cell, dendritic cell, and monocyte populations in the lung, but not nose. These data suggest type I IFN is necessary for HMPV pathogenesis, while IFN-λ is required to limit HMPV lung replication. Differences in virus control and immune responses in upper vs. lower airways of $IFNAR^{-/-}$ and $IFNLR^{-/-}$ mice suggest type I and III IFN exert non-redundant antiviral activity through site-specific signaling.

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O67

HSV-1 DEGRADES MULTIPLE CGAMP CHANNELS TO EVADE EXTRACELLULAR CGAMP RESPONSES

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Abstract Content: The presence of viral DNA outside of the nucleus is detected by the pattern recognition receptor cGAS during infection. cGAS then generates the cyclic dinucleotide cGAMP which activates the central hub of DNA sensing – STING. This results in the production of proinflammatory cytokines, primarily type I interferons, driving antiviral responses. Classically, cGAMP was thought to solely act within the cytosol, as its negative charge makes it membrane impermeable. Recently four channels (SLC19A1, SLC46A2, P2X7 and LRRC8A:C/E) have been shown to import and export cGAMP allowing movement between cells. Implicating extracellular cGAMP in defence against viral invasion; T cell proliferation; natural killer cell recruitment; vaccine efficacy; cancer clearance; metastasis and immunotherapy. Highlighting a new function for cGAMP as an extracellular cytokine.

We theorised if exogenous cGAMP functions within the innate immune response then viruses would act to evade this mechanism. We screened viruses for the capacity to degrade cGAMP channels during infection. Double stranded DNA (dsDNA) viruses were seen to degrade multiple cGAMP channels during infection. While no viruses with RNA genomes were seen to reduce cGAMP channel abundance. Remarkably Herpes Simplex Virus-1 (HSV-1) degraded three of the four known cGAMP channels - P2X7, SLC46A2 and LRRC8A:C. Therefore, we chose HSV-1 as a model virus to study the effects of exogenous cGAMP during viral replication.

By immunoprecipitating LRRC8C from HSV-1 infected cells and preforming mass spectrometry-based proteomics we identified UL56 the gene potentially responsible. Overexpression of UL56 confirmed it causes LRRC8A:C degradation. Unexpectedly we found UL56 also causes degradation of the P2X7 and SLC46A2 channels. Using the reverse approach, we see HSV-1 lacking the UL56 gene (HSV-1-ΔUL56) is unable to degrade cGAMP channels. This proves that UL56 is responsible for degradation of all three cGAMP channels. Mechanistically UL56 contains PPXY domains that act as a platform for recruitment of NEDD-4 family ubiquitin ligases. We show mutation of the PPXY domains to AAXA prevents degradation of cGAMP channels by UL56. Both when UL56-AAXA is over expressed or upon infection with AXAA mutated virus (HSV-1-AAXA). Consistent with degradation mediated by NEDD-4 ligases, chemical inhibition of the proteosome rescued LRRC8A:C abundance.

To show UL56 inhibits cGAMP import we developed in *vitro* models of cGAMP uptake for LRRC8A:C, SLC46A2 and P2X7. Either by activating channels endogenously expressed by 293 cells (LRRC8A:C) or by stably overexpressing the channels (P2X7/SLC46A2) to allow increased cGAMP uptake. In this system import of cGAMP can be blocked with channel specific inhibitors or by overexpression of UL56. Showing functionally UL56 prevents cGAMP from being imported into cells and so preventing IFN-β production. In *vivo* work comparing WT-HSV-1 to mutants (HSV-1-ΔUL56 and HSV-1-AXAA) unable to degrade cGAMP channels is ongoing. We show for the first time that a virus antagonises cGAMP channels to avoid the effects of exogenous cGAMP. Adding to the recent and exciting body of literature describing the role of cGAMP as a signalling molecule between cells.

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Oral presentations: ILCs in Cancer and Metabolism

O68

REGULATION OF ILC2S BY ADIPOKINE ADIPONECTIN

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Abstract Content: ILC2s are present in metabolically active organs such as adipose tissue and play a critical role in the maintenance of metabolic homeostasis at tissue and systemic levels. However, the mechanisms underlying the development and activation of ILC2s remain poorly defined. Here we show that IL-33, a potent ILC2 activator, stimulates phosphorylation of AMPK at Thr^{172} via TAK1 in primary ILC2s, which provides a feedback mechanism to inhibit IL-33-induced NFκB activation and IL-13 production. Treating ILC2s with adipokine adiponectin or adiponectin receptor agonist, AdipoRon, activated AMPK and decreased IL-33-stimulated activation of ILC2s. In contrast, adiponectin deficiency promotes ILC2 development and activation, leading to upregulated thermogenic gene expression in adipose tissue of cold exposed mice. ILC2 deficiency or blocking ILC2 function by neutralization of the IL-33 receptor with anti-ST2 diminished the suppressive effect of adiponectin on cold-induced adipose thermogenesis and energy expenditure. Furthermore, depleting adiponectin receptor 1 (AdipoR1) and AdipoR2 in ILC2 increased resident ILC2 population in adipose tissue and substantially drove favorable metabolic phenotype. Taken together, our study uncovers a novel mechanism underlying the hormonal control of ILC2 and reveals that adiponectin is a key regulator of ILC2 function via AMPK-mediated negative regulation of IL-33 signaling.

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O69

EICOSANOID METABOLISM AND FUNCTION IN HUMAN INNATE LYMPHOID CELLS

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Abstract Content: Lacking rearranged antigen receptors, innate lymphoid cell (ILC) activation is independent of antigens but is instead driven by signals derived from the tissue microenvironment. For ILC2, these signals have been shown to include members of the eicosanoid family of lipid mediators, which can be either potent activators or inhibitors, as seen for the prostanoids prostaglandin D2 (PGD2) and PGE2, respectively. Moreover, stimulation of human ILC2 with tissue alarmins such as IL-25, IL-33 and TSLP leads to endogenous production of PGD2, which is crucially required for human ILC2 activation and type 2 cytokine production. Eicosanoid metabolism and function across human ILC subtypes, however, remains poorly understood. The aim of our study was to delineate this aspect of human ILC biology. Analysis of previously generated RNA-seq data of ILC2 isolated from the lung and blood of asthmatics before and 24h after allergen-challenge revealed several previously unknown transcripts involved in eicosanoid-synthesis. Using mass-spectrometry we confirmed the production of PGD2 from alarmin-stimulated human tonsil ILC2s. We also detected production of PGE2, its downstream metabolite PB2 and PGE1, suggesting an active PGE-synthesis pathway in human ILC2. We additionally detected substantial PGE2-production from human tonsil ILC3. Exogenous PGE2 had a stimulatory function on human ILC3, promoting the production of IL-17F and IL-22. These data suggest analogous functions of PGD2 on ILC2 and PGE2 on ILC3, and a dichotomic effect of PGE2 on ILC2 and ILC3, raising the possibility for a role of prostanoids in ILC2-ILC3 plasticity. Understanding the role of endogenous and exogenous prostanoids in ILC regulation has important therapeutic implications for conditions such as allergy and asthma.

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O70

THE NK CELL RECEPTOR NKG2D CONTRIBUTES TO TUMOR PROGRESSION IN MUCOSAL CANCERS BY DRIVING IL-17A-MEDIATED INFLAMMATION

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Abstract Content: NKG2D is an activating immunoreceptor expressed on NK cells, innate lymphoid cells and T cells. Ligands to NKG2D are expressed predominantly on transformed and stressed cells and NKG2D/NKG2D ligand engagement typically leads to target cell death. Thus, this signaling pathway plays a key part in anti-tumor immunity identifying NKG2D as a promising candidate for immunotherapy. Indeed, several clinical trials to test NKG2D as a potential chimeric antigen receptor (CAR) to specifically eliminate NKG2D ligand-expressing tumor cells are in progress.
Although NKG2D can exert a protective function in cancer, it can also paradoxically promote inflammatory disorders. For example, aberrant expression and activation of NKG2D-expressing effector cells has been shown to result in tissue damage and autoinflammation. These dual functions can establish NKG2D-expressing cells which can drive cancer progression through their ability to induce tissue damage and sustain inflammation. Exactly how NKG2D orchestrates these two outcomes, however, is not fully understood.
Here, we study how NKG2D contributes to cancer-promoting inflammation in a mouse model of intestinal cancer. We found that intestinal tumor progression was delayed in NKG2D-deficient mice and that this was associated with a marked reduction of IL-17A-producing lymphocytes. Surprisingly, this reduction was due to a decrease of IL-17A-producing γδT cells. In NKG2D-sufficient mice, a subset of mucosal γδT cells co-expressed NKG2D and IL-17A, suggesting that these cells promote tumorigenesis by secreting the pro-inflammatory cytokines IL-17A in an NKG2D-

dependent manner. We extended our findings to an additional tumor site and show that blocking of NKG2D reduced IL-17A production in the pre-metastatic lung of a mammary tumor model. Collectively, our findings uncover how expression of the NKG2D receptor γδT cells drives tumor cell establishment and progression highlighting that immune subset specific expression of NKG2D receptor may be a key determinant of the outcome of signaling via this pathway.

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O71

DISTINCT PATHWAYS REGULATE HUMAN ILC PROLIFERATION AND FUNCTION

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Abstract Content: Dynamic changes in energetic pathways govern lymphocyte maturation, activation, and long-term persistence. In the past decade, immunometabolism studies have principally focused on characterizing T cells, resulting in the identification of specific metabolic programs for T cell activation and differentiation. Naïve T cells reside in a resting basal metabolic state, while upon activation T cells become preferentially glycolytic and take up glutamine to replenish TCA cycle intermediates during cell proliferation and cytokine production. By contrast, long-lived memory T cells exhibit a metabolic switch to fatty acid metabolism, mitochondria fusion and increased respiration. Whereas changes in metabolic processes in T cells have been well studied, our knowledge of innate lymphoid cell (ILC) metabolism is limited. We embarked to investigate the metabolic requirements of circulating human group 2 ILCs (ILC2s) and natural killer (NK) subsets at steady state and upon activation. We demonstrated that CD56Dim (NKDim) cells, which are highly cytotoxic, have fused mitochondria and enhanced metabolism compared with CD56Br (NKBr) cells, which are less cytotoxic but strong cytokine producers. Upon activation, we observe an increase in mitochondrial fusion in NKBr cells andparadoxically mitochondrial fission and depolarization in NKDim cells. The latter effect impaired interferon-γ production, but rescue was possible by inhibiting mitochondrial fragmentation, implicating mitochondrial polarization regulates NK cell function. Moreover, we could associate mitochondrial fusion and polarization with enhanced survival and function in mature NKDim cells, including memory-like CD57+NKG2C+ subsets in latently *human cytomegalovirus*-infected individuals, supporting mitochondrial remodeling as a central regulator of mature NK cell fitness. By comparing circulating human NK cells with 'naive' ILC2s we found that the latter have an unexpected metabolic profile with a higher level of oxidative phosphorylation (OXPHOS) than NKDim and NKBr cells. Accordingly, ILC2s are severely reduced in individuals with mitochondrial disease(MD) caused by genetic mutation in complex I leading to impaired OXPHOS. Metabolomic and nutrient receptor analysis revealed that ILC2 use branched chain amino acids and arginine to sustain high level OXPHOS at steady state. Following activation with interleukin-33 (IL-33), ILC2s became highly proliferative,relying on glycolysis and mammalian target of rapamycin (mTOR) to produce IL-13 while continuing to fuel OXPHOS with glutamine to maintain cellular fitness and proliferation, suggesting that function and proliferation are metabolically uncoupled in human ILC2s. Altogether our findings provide a working model for understanding how ILC metabolism conditions their biology. Moreover, the peculiar metabolic features of steady-state and cytokine-activated ILCs might represent potential targets for the development of new therapeutic strategies.

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O72

CITRACONATE IMPROVES MITOCHONDRIAL RESPIRATION DURING MACROPHAGE ACTIVATION BY REDUCING ACOD1-MEDIATED ITACONATE SYNTHESIS

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Abstract Content: *cis*-Aconitate decarboxylase (ACOD1, also known as IRG1) converts the tricarboxylic acid (TCA) cycle intermediate *cis*-aconitate to itaconate during macrophage activation and links innate immunity with metabolism. Genetic ablation of ACOD1 in mice usually exacerbates inflammation at the organismal level. However, increased ACOD1 activity has been associated with decreased mitochondrial respiration, a protumorigenic state, and immune paralysis, suggesting that pharmacologic inhibition of ACOD1 will have certain clinical applications. At the Cytokines 2021 meeting, we reported that citraconate (a naturally occuring isomer of itaconate) is a competitive inhibitor of ACOD1 that is predicted to bind to its active site. We have now investigated the effect of citraconate-mediated ACOD1 inhibition on mitochondrial respiration during macrophage activation. As expected, LPS/IFNγ stimulation reduced basal respiration, maximal respiration, and spare respiratory capacity of differentiated THP1 cells. Adding 25 mM itaconate to the medium reduced maximal respiration and spare respiratory capacity further, whereas 1 mM itaconate had no effect. In contrast, both 1 and 25 mM citraconate tended to normalize maximal respiration and spare respiratory capacity, likely by reducing endogenous itaconate accumulation. In a next step, we identified a citraconate analog, here referred to as Compound A, as a substantially more active competitive ACOD1 inhibitor. Compared to citraconate, Compound A demonstrated markedly lower Ki (0.2 vs. 38 µM) in a cell-free assay with recombinant ACOD1. When added to LPS/IFNg-stimulated dTHP1 cells, Compound A had a lower 50% inhibitory concentration (IC_{50}; 1.8 µM vs. 30.4) but a similar 50% cytotoxic concentration (CC_{50}; 67.9 vs. 66.2 mM) and thus a substantially higher selectivity index (SI; 37600 vs. 2180). These results underscore the impact of pharmacologic inhibition of endogenous itaconate synthesis on mitochondrial respiration in macrophage activation and provide further evidence that citraconate constitutes a promising scaffold for the development of pharmacologically optimized ACOD1 inhibitors.

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O73

HETERODIMERIC IL-15 (HETIL-15) IMMUNOTHERAPY SYNERGIZES WITH A FATTY ACID METABOLISM MODULATOR, REVERSING THE METABOLIC DYSFUNCTION OF TUMOR INFILTRATING CD8+T CELLS AND LEADING TO TUMOR ERADICATION IN TNBC MOUSE MODEL

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Abstract Content: **Introduction:** Metabolic fitness and T cell survival are crucial in anti-tumor responses because nutrients are often scarce and other regulatory molecules may be unfavorable in the tumor microenvironment leading to T cell dysfunction, stress, and apoptosis. Tumor-infiltrating cytotoxic $CD8^+$T cells often acquire an altered state of differentiation referred to as “exhaustion” and, as a result, they fail to control tumor growth. IL-15 is a cytokine that induces the differentiation, proliferation and cytotoxic function of $CD8^+$ T and NK cells. We have produced the native heterodimeric form of IL-15 (hetIL-15) which has advanced in clinical trials due to its anticancer activities in many different mouse cancer models. The objective of this study was to study the effects of hetIL-15 immunotherapy after locoregional administration in triple negative breast cancer models (TNBC), to evaluate the metabolic profile of the tumor infiltrating T cells and to assess any potential synergy using Fatty Acid Metabolism Modulators (FAMM) to enhance T cell metabolism.
Study design and methods: We studied the therapeutic efficacy of hetIL-15 immunotherapy in combination with a FAMM in the murine EO771 orthotopic breast cancer model. We monitored the effect of the treatment on metabolism and mitochondrial function of the tumor-infiltrating immune cells by flow cytometry, Mitotracker, 2-NBDG and/or Bodipy staining, and Seahorse flux analysis of isolated tumor-infiltrating lymphocytes.

Results: hetIL-15 peritumoral administration monotherapy resulted in complete regression in 40% of the treated animals and increased survival. We demonstrated that tumor infiltrating cytotoxic $CD8^+$T and NK cells increased in hetIL-15 treated tumors and showed enhanced activation and proliferation. Metabolic flux analysis of the tumor-infiltrating cytotoxic $CD8^+$T cells from treated mice confirmed a rise in oxygen consumption rate (OCR) with substantial increase of spare respiratory capacity, which supports an activated/non exhausted phenotype of these hetIL-15 treated effector cells. Since induction of fatty acid (FA) catabolism improves the tumor-infiltrated $CD8^+$T cells' ability to slow tumor progression, we combined hetIL-15 immunotherapy with FAMM. Combination therapy resulted in increased, mitochondrial function, FA uptake and OCR, revealing a more metabolically active phenotype compared to the tumor-infiltrating $CD8^+$T cells from hetIL-15 group. In addition, combined treatment of IL-15 immunotherapy and FAMM resulted in complete eradication of the tumors in 85% of mice.
Conclusions: Our results indicate that hetIL-15 synergizes with metabolic reprogramming of T cells to achieve superior antitumor efficacy and complete cures. We suggest that metabolic reprogramming of tumor-specific $CD8^+$T cells might represent a strategy to promote survival in the metabolically hostile TME as part of approaches to enhance the clinical efficacy of immunotherapy.

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O74

IMMUNE REGULATOR LGP2 TARGETS UBC13 TO MEDIATE WIDESPREAD INTERFERENCE WITH K63-POLYUBIQUITINATION, IRF3 AND NFKB ACTIVATION

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Abstract Content: The production of type I interferon (IFN) is essential for cellular barrier functions and innate and adaptive antiviral immunity. In response to virus infections, IFN and antiviral target gene expression is triggered by RNA sensor proteins RIG-I and MDA5, that stimulate a mitochondria-localized signaling apparatus to activate the IKK-family serine kinases that stimulate master transcription regulators IRF3 and NFkB. TRAF family lysine 63 (K63)-ubiquitin ligase proteins are essential for antiviral responses, and we have demonstrated that a third RNA receptor, LGP2, acts as a feedback inhibitor of TRAF-mediated K63-Ub that can interfere with diverse immune responses that feature NFkB and IRF3. Disruption of LGP2 expression in cells results in earlier and overactive transcriptional responses to virus or dsRNA. K63-Ub chains activate a range of cellular immune and inflammatory signaling pathways beyond the mammalian antiviral response, and the ability of LGP2 to interfere with these wide-ranging targets invited further inquiry into the mechanism by which LGP2 could regulate a growing list of K63-Ub mediated pathways. Results demonstrate that LGP2 inhibits K63-Ub by association with and sequestration of the K63-Ub conjugating enzyme (E2), Ubc13/UBE2N. The LGP2 helicase subdomain, Hel2i, mediates protein interaction that engages and inhibits Ubc13/UBE2N, affecting control over a range of K63-Ub ligase proteins, including TRAF6, TRIM25, and RNF125, all of which are inactivated by LGP2. These findings establish a previously unrecognized and unifying mechanism for LGP2-mediated negative regulation that can modulate a variety of K63-Ub signaling pathways.

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O75

REGULATION OF TLR4 AND TYPE I INTERFERON GENE INDUCTION RESPONSES BY THE ELONGATOR COMPLEX

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Abstract Content: Regulation of TLR4 and type I interferon gene induction responses by the ELongator complex

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The evolutionarily conserved Elongator complex acts by modifying of transfer RNA (tRNA) molecules at the wobble base position, which is required to ensure the fidelity and efficiency of translation of mRNA codons ending in –AA. However, the functional role Elongator plays in the innate immune response has yet to be thoroughly assessed or elucidated. Using CRISPR/Cas9 we generated immortalised bone-marrow derived macrophages (iBMDMs) lacking Elp3, the catalytic subunit of the complex, to investigate Elongator's function in the innate immune response. Unbiased quantitative proteomic analysis of Elp3 $^{-/-}$ iBMDMs displayed an downregulation of proteins required for type I interferon and antiviral signalling, and also impaired LPS-dependent IFNβ and IFN-stimulated gene (ISG) expression. Surprisingly, the IFNβ autocrine feedback loop (signalling via IFNAR & the ISGF3 complex) mediated-ISG expression was abolished in Elp3 KO iBMDMs similarly to LPS stimulation. Both LPS and IFNβ-mediated STAT1 phosphorylation and activation was abrogated in Elp3 KO iBMDMs. In contrast, IFNγ stimulated STAT1 phosphorylation and IRF1 induction were unimpaired in Elp3 KO iBMDMs. In contrast to TLR4 stimulation, STING-mediated IFNβ induction and IRF3 phosphorylation was unaffected by the absence of Elp3. Furthermore, Elp3 KO iBMDMs failed to induce type I IFN in response to Influenza A infection. This evidence suggests a previously undiscovered and novel mechanism of regulation of type I IFN responses and antiviral innate immunity by the evolutionaril conserved Elongator complex.

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O76

MAPKAPK5AS1 LNCRNA REGULATES IFNG LEVELS BY AN M6A DEPENDENT MECHANISM IN ACTIVATED T CELLS

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Abstract Content: Interferon-gamma (IFNγ) is secreted predominantly by activated lymphocytes and it is strongly associated with the Th1 response. This cytokine plays an important role in inducing and modulating an array of immune responses and it has been described to be involved in the development of systemic autoimmune disorders such as type 1 diabetes (T1D) or celiac disease (CD).
Autoimmune disease-associated noncoding SNPs, which have been described to often locate in tissue-specific regulatory elements, are emerging as key factors in epitranscriptomic regulation. In the present study, we found that the lncRNA *MAPKAPK5AS1* regulates *IFNG* induction in activated T lymphocytes by an m^{6}A-dependent mechanism. The SNP rs3177647, located within the lncRNA and very close to an m6A motif, is associated to both, T1D and CD, and presents allele-specific m6A methylation, with the risk allele for both diseases being more methylated. We observed that when activated, T cells present lower amounts of m6A eraser *ALKBH5*, which in turn reduces the levels of *MAPKAPK5AS1* inducing *IFNG* expression and secretion. Additionally, we found, that at basal stages, *MAPKAPK5AS1* is bound to GAPDH and to the 3'UTR of *IFNG* mRNA inhibiting its translation. In turn, when T cells are activated, m6A dependent degradation of *MAPKAPK5AS1* releases GAPDH from *IFNG*. In this scenario, GAPDH engages glycolysis and *IFNG* mRNA is translated and secreted. Moreover, we observed that T cells with reduced *MAPKAPK5AS1* levels are able to induce cell death and activate the STAT1-CXCL10 axis when co-cultured with intestinal or pancreatic cells confirming the involvement of this lncRNA in the autoimmune characteristic tissue damage.

In brief, our observations, shed light on how a noncoding m^{6}A-QTL influences autoimmune disease phenotype by IFNγ secretion, mediating predisposition to inflammation and autoimmunity, opening the door to novel therapeutic approaches for these disorders based on lncRNA and m6A regulation.

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O77

IL-33 ACTIVATES CD73-EXPRESSING CELLS PROMOTING TUMORIGENESIS DURING COLITIS-ASSOCIATED COLORECTAL CANCER

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Abstract Content: ***Background.*** Colorectal cancer associated to chronic colitis (CAC) has a different pathogenesis compared to sporadic or familial colorectal cancer (CRC) and represents the major complication of inflammatory bowel disease (IBD). It is now well-established that IL-33 and its receptor, ST2, are important factors in the pathogenesis of IBD. Emerging evidence also suggests its critical role in epithelial proliferation and the potential contribution to inflammation-driven tumorigenesis that can lead to CRC. The aim of our study was to characterize the precise contribution of IL-33/ST2 axis in the azoxymethane (AOM)/dextran sodium sulfate (DSS) model of colitis-associated CRC.
Methods. C57/BL6 wild-type (WT), *Il33*$^{-/-}$, T1/*St2*$^{-/-}$ and *Nt5e*$^{-/-}$ mice were given a single dose of AOM (10 mg/kg) followed by two cycles of 3% DSS for 7d in drinking water. Vehicle-treated WT mice served as controls and were sacrificed at the same time points. Another group of WT followed the same protocol and received CD73 inhibitor i.p. treatment or vehicle. Disease Activity Index (DAI), as well as endoscopic, stereomicroscopic and histological evaluations of colons were performed. IHC, immunofluorescence (IF), qPCR, and ELISA were done on full-thickness colons of WT for IL-33 and ST2 localization and identification, as well as mRNA and protein expression, respectively. RNA-Seq was performed on whole tissues from AOM/DSS treated WT, *Il33*$^{-/-}$ and T1/*St2*$^{-/-}$. qPCR analysis was done on isolated polyps from WT, *Il33*$^{-/-}$ and *T1/St2*$^{-/-}$ for *Nt5e* (CD73) and adenosine pathway targets.
Results. *Il33*, *Illrl1*(ST2L), and *Illrl1*(sST2) mRNA transcripts, as well as IL-33 and total ST2 proteins were dramatically elevated in AOM/DSS-treated WT mice vs. controls. IHC and IF of treated WT mice revealed localization of IL-33 to the colonic epithelium and to cells within the polyp LP morphologically consistent with stromal and mast cells. Little to no staining for IL-33 was present in controls. Using IF, IL-33 co-localized with sub-epithelial myofibroblast markers Actin and Vimentin, or with mast cell markers Tryptase and MCPT1. AOM/DSS treatment in *Il33*$^{-/-}$ and T1/*St2*$^{-/-}$ mice resulted in a significant decreased polyp number and size *vs.* WT, with colonoscopy revealing the development of protruding lesions with abnormal vascular patterns, suggesting tumorous lesions in WT mice, while all deficient mice showed their absence with a more impressive mucosal inflammation, likely due to reduced epithelial proliferation and repair caused by the deficiency of IL-33 signaling. RNA-Seq identified a significant reduction of *Nt5e* and adenosine pathway targets in *Il33*$^{-/-}$ and T1/*St2*$^{-/-}$ *vs.* WT. qPCR on isolated polyps confirmed this observation. AOM/DSS-treated *Nt5e*$^{-/-}$ showed a significant decreased polyp number and size *vs.* controls. Therapeutic inhibition of CD73 produced similar results.
Conclusions. Our results suggest that the IL-33/ST2 axis promotes tumorigenesis in colitis-associated CRC through the activation of CD73. Further studies are underway to determine mechanisms of action that support these findings.

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O78

LUNG IMMUNOPATHOLOGY DUE TO INFLUENZA A INFECTION IS DEPENDENT ON THE ONCOSTATIN M PATHWAY IN C57BL/6 MICE

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Abstract Content: The RNA virus Influenza A (IFA) is a common seasonal respiratory infection that typically causes a transient illness in healthy individuals, however significant numbers of patients (particularly the young and elderly) are affected by severe IFA-infections/pneumonia. Marked immunopathology due to IFA is generated in large part by overzealous inflammatory mechanisms such as elevated pro-inflammatory molecules (IL-6, TNFa, IL-1, IL-33). However, the role of the gp130 cytokine Oncostatin M (OSM) in severe acute lung inflammation due to virus infection is not well studied. OSM functions broadly on structural cells (epithelial, smooth muscle and fibroblasts) rather than immune/hematopoietic cells, due to its selective receptor expression (OSMRβ/gp130). Overexpression of OSM in mouse models induces downstream elevation of IL-6, TNF and IL-33 and immunopathology including inflammatory cell accumulation and tissue remodeling. Here, we first show that OSM is significantly elevated (protein in bronchoalveolar lavage fluid (BALF) and mRNA by RT-PCR in total lung homogenates at days 2-7 post infection) in C57Bl/6 mice infected with Influenza A H1N1. In examining scRNAseq (10x Genomics) of collagenase-digested single cell suspensions generated from lungs taken at day 7 post infection, OSM mRNA was expressed highly by neutrophils and interstitial macrophage /monocyte lineage cells but much lower in resident alveolar macrophages and other immune cells. To assess functional roles of the OSM pathway, we compared wild type (WT) to OSMRβ-/- female mice at Day 7 post infection (n=5-8/group) using a high dose (4000 *pfu*, LD80) of IFA PR8, and examined immunopathology. OSMRβ-/- mice showed markedly reduced inflammatory parameters including lower KC/CXCL-1 and neutrophil accumulation ($p<0.01$) in BALF. OSMRβ-/- mice also showed reduced measures of epithelial barrier dysfunction including significantly lower serum albumin in BALF ($p<0.05$), and significantly lower passage of lung-instilled FITC-labeled Dextran to plasma ($p<0.05$). Histopathology assessment of fixed lung tissue taken from mice at day 7 post infection (Ashcroft scoring) showed significant reduction of inflammatory infiltrate ($p<0.01$) and alveolar wall thickness ($p<0.05$) down to near baseline in OSMRβ-/- mice, whereas WT mice showed extensive immunopathology to this high dose of IFA PR8. This occurred despite no reduction of IFA *pfu* nor of OSM ligand levels detected in the OSMRβ-/- strain compared to WT. This suggests the reductions in immunopathology were not a function of reduced IFA *pfu* replication nor of IFA ability to elevate OSM in the OSMRβ-/- mice, but rather a deficiency in the downstream inflammatory pathways engaged by the OSM Receptor activation in this model. The results indicates a key role of OSM/OSMRβ in control of severe inflammation induced by high IFA viral load in mice. (Supported by the Canadian Institutes for Health Research).

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O79

REGULATION OF TAU PATHOLOGY BY THE INTERLEUKIN-1 RECEPTOR ACCESSORY PROTEIN

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Abstract Content: **Background:** Increasing evidence suggests neuroinflammation is associated with tau hyperphosphorylation and aggregation which are known to form neurofibrillary tangles and are a major pathological hallmark of many tauopathies including Alzheimer's disease (AD). However, the precise mechanisms of how brain inflammation drives tau pathology remain unclear. Previously, we have reported that tau pathology is induced in neurons through microglia-derived IL-1 and the activation of the IL-1 receptor type I (IL-1R1)-p38 mitogen activated protein kinase (p38 MAPK) pathway. Our data also suggests that pathologically modified tau (pTau) could serve as a danger signal and further activate IL-1 and nuclear factor-kB (NF-kB) in human microglia, suggesting a bi-directional relationship between pro-inflammatory response in microglia and tau pathology. It is unclear how IL-1 signaling is regulated in a cell-specific manner and at the receptor level. Transduction of IL-1/IL-1R1 signaling happens through the requirement of IL-1 receptor accessory protein (IL-1RAcP). In response to IL-1b stimulation, IL-1R1 engages IL-

1RAcP and activates NF-kB (primarily in microglia) or p38 MAPK (primarily in neurons). Besides, genome-wide association studies have revealed a strong association between several single nucleotide polymorphisms in *IL1RAP* and elevated pTau in cerebrospinal fluid and increased brain atrophy in AD. In this study, we aimed to determine if IL-1RAcP deficiency (microglial, neuronal and global) has any effect on tau pathology, neurodegeneration, and memory.
Methods: We generated CX3CR11Cre/IL-1RAcP$^{fl/fl}$ mice to achieve myeloid/microglial knock out of IL-1RAcP. We also used two additional mice lines which were deficient for neuron-specific isoform of IL-1RAcP (IL-1RAcPb) and global IL-1RAcP knockout. These mice were aged up to two years and challenged with a dose of LPS and sacrificed after 24 h. Detergent soluble hippocampal lysates were prepared and probed for antibodies to different phosphorylated epitopes of tau (AT8, AT180 and PHF-1), total tau and GAPDH. Fixed brains were cryoprotected in 30% sucrose, free-floating sagittal sections cut and processed with specific antibodies to detect phosphorylated tau. Fluorophore conjugated secondary antibodies were used to visualize sections. Currently, we are completing the Western blot/immunofluorescence analyses for IL-1b, cleaved caspase-1, and p38 MAPK. Behavioral and cognitive tests are also underway for the IL-1RAcP and IL-1RAcPb knockout mice.
Results: The CX3CR11Cre/IL-1RAcP$^{fl/fl}$ mice injected with LPS did not show any significant changes in their pTau levels compared to the vehicle injected group. The LPS injected IL-1RAcP global knockout mice displayed significantly lower levels of AT8 and AT180. In contrast, deletion of neuron-specific isoform of IL-1RAcPb appears to increase tau phosphorylation on AT8 and AT180 but decrease phosphorylation on the PHF-1 sites. Total tau levels (Tau5) also appear to significantly increase in IL-1RAcPb knockout mice. Further analyses of these data are currently under progress.
Conclusion: Our data suggests that global deletion of the IL-1RAcP decreases tau pathology in an LPS model of systemic inflammation. On the other hand, deletion of neuron-specific isoform of IL-1RAcPb appears to increase overall tau levels. Deletion of IL-1RAcP in myeloid/microglial cells appear not to affect the pTau levels.

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O80

INTESTINAL INTERFERON-LAMBDA RECEPTOR 1 EXPRESSION AND RESPONSES ARE SIGNIFICANTLY DECREASED IN PEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS

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Abstract Content: Interferon-lambdas (IFN-λs) are important cytokines that modulate immune responses and promote mucosal healing by acting through a unique IFN-λR1/IL-10RB heterodimeric receptor that is highly expressed on intestinal epithelial cells and subsets of immune cells. Mouse colitis models demonstrate *Ifnlr1* deficiency leads to exacerbated inflammation, while Ifn-λ treatment promotes gut barrier integrity and healing in wildtype mice. With limited data on whether IFN-λR responses are dysregulated in human inflammatory bowel disease (IBD), we determined if IFN-λR1 expression and IFN-λ responses were altered in intestinal biopsies from children with IBD. We identified a new monoclonal antibody clone that accurately stains IFN-λR1 protein in human intestinal tissue and used it for measuring IFN-λR1 levels by immunohistochemistry with biopsy samples from children without IBD (n=8), Crohn's disease (n= 7), or ulcerative colitis (n=10). Fresh patient biopsies were also cultured *ex vivo* for 24hr in media +/- IFN-λ3 and gene expression was quantified by RT-qPCR. We found a significantly lower percentage of epithelial and immune cells positive for IFN-λR1 in children with IBD compared to non-IBD controls ($p<0.01$). IFN stimulated gene transcript expression also differed upon IFN-λ3 stimulation of non-IBD and IBD patients' biopsies cultured *ex vivo*. Paired patient gut microbiome analyses identified specific microbe species that correlated with changes in IFN-λ receptor expression. Together, our findings suggest that pediatric IBD patients may have impaired induction of

critical IFN-λ-mediated antimicrobial responses and protective anti-inflammatory pathways. This work supports the strategy to restore and promote IFN-λ signaling as a therapy for pediatric IBD.

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O81

CYTOKINE-INDUCED EPIGENETIC REWIRING OF PATHOGENIC FIBROBLASTS IN PATIENTS WITH CROHNS DISEASE

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Abstract Content: In Crohn's disease (CD), transmural inflammation can affect any part of the gastrointestinal tract. There is no cure for CD, and for more than half of patients standard-of-care biologic therapy is ineffective. This leaves many patients with the only option of surgically removing affected bowel parts, which comes with a significant reduction in quality of life.
In addition to leukocytes, tissue-resident fibroblasts have been recently associated with inflammation in Crohn's disease. Fibroblasts are long-lived cells that interact with leukocytes in the tissue, making them an appealing candidate for driving chronicity of disease by sustaining a pro-inflammatory tissue microenvironment. We hypothesised that epigenetic modification is a key component of a persistent pathogenic fibroblast state.
Using single-cell RNAseq and ATACseq, we profiled the transcriptomic and epigenetic states of fibroblasts isolated from surgical resection specimen of therapy-refractory patients with CD. This demonstrated distinct epigenetic rewiring of inflammation-associated fibroblasts ('inflammatory fibroblasts'). Assessing the response of intestinal fibroblasts to a panel of CD-associated cytokines, we found that inflammatory fibroblasts are the main cytokine-responsive fibroblast phenotype in the tissue of patients with CD. Follow-up *in vitro* experiments reveal that a core set of cytokines is capable of training the pathogenic inflammatory fibroblast state, which persists over months in culture once induced. Strikingly, small molecule epigenetic modifiers that inhibit class I histone deacetylases reverse this pathogenic fibroblast state.
Together, our results reveal a distinct epigenetic footprint of a persistent and pathogenic pro-inflammatory fibroblast state in patients with CD, highlighting it as a novel mechanism to maintain chronicity of disease. Reversal of this state by HDAC inhibitors reveals the potential of epigenetic modulation in breaking chronicity, opening an alternative avenue for therapeutic intervention in CD.

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O82

TELOCYTE TSLP INITIATES THE ILC2-TUFT CELL SMALL INTESTINAL CIRCUIT IN RESPONSE TO FEEDING

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Abstract Content: The small intestine represents a critical interface for absorption of nutrients that becomes dysregulated in food allergy, an increasingly prevalent condition associated with enhanced type 2 immunity. Thymic stromal lymphopoietin (TSLP) is a key driver of type 2 inflammation and one of three alarmin cytokines along with IL-25 and IL-33 expressed at barrier tissues. TSLP regulates intestinal immunity and inflammation in *Trichuris muris* (*T.muris*) helminth infection, although the precise cell sources of TSLP in the intestine remain unclear. Here, we use a novel TSLP reporter mouse strain to identify populations of subepithelial fibroblasts designated telocytes in villi and peri-cryptal trophocytes as prominent sources of small intestine TSLP. Food feeding enhanced TSLP in the small intestine and activated type 2 cytokine production by lamina propria ILC2s. Telocytes and trophocytes express receptors for glucagon-like peptides 1 and 2 (Glp-1, Glp-2), which are processed from proglucagon mRNA in enteroendocrine L cells and secreted in response to nutrients. Glp-2 agonist activated ILC2s by a TSLP-dependent

mechanism that initiated increased tuft cell prevalence in intestinal epithelia, which was attenuated by deletion of TSLP in stromal cells and in TSLP receptor-deficient mice. Thus, telocyte TSLP relays epithelial nutrient signaling from epithelial L cells to ILC2s to initiate increased numbers of tuft cells. Culture of intestinal ILC2s *in vitro* revealed synergistic activation by combination of TSLP and IL-25. *T. muris* infection required both TSLP and IL-25 for clearance *in vivo*, corroborating synergism in these pathways for optimal ILC2 activation. We propose a model where telocyte TSLP couples peptide hormones linked with absorption and metabolism with IL-25-mediated amplification of a quality control chemosensory circuit poised to detect intestinal perturbations from irritants consumed with food.

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O83

GROUP 3 INNATE LYMPHOID CELLS PROGRAM A DISTINCT SUBSET OF IL-22BP-PRODUCING DENDRITIC CELLS DEMARCATING SOLITARY INTESTINAL LYMPHOID TISSUES.

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Abstract Content:
Type 3 innate lymphocytes (ILC3s) are tissue resident cells that are deeply integrated into the biology of the gut, associated with steady-state functions such as tissue regeneration and nutrient absorption. Indeed, by continuously producing the cytokine interleukin (IL)-22, ILC3s contribute to the homeostatic function of the intestinal epithelial cells, such epithelial stem cell self-renwal and regulation of lipid and carbohydrate absorption.
In the small intestine, $CCR6^+$ ILC3s are the major source of IL-22 production and are restricted to solitary lymphoid structures such as cryptoatches (CPs) and isolated lymphoid follicles (ILFs). CPs are evolutionary ancient lymphoid structures that develop postnatally and consist of a cluster of some hundreds ILC3s. Following microbial colonization of the gut, CPs mature into ILFs by recruiting B cells. ILFs are thought to be sites for T cell-independent Ig production, whereas CPs are believed to be regulatory hubs for ILC3 activation.
Mononuclear phagocytes (MPhs) are strong regulators of ILC3 function. In particular, IL-1b and IL-23 produced by different intestinal MPhs populations drive ILC3 production of IL-22. In CPs and ILFs, ILC3s are surrounded by a population of $CD11c^+$ mononuclear phagocytes: CP and ILF-associated MPh (CIA-MPh). The developmental origin of these cells and any functional properties remain unknown.
In this study, we aim to characterize the development and function of CIA-MPhs. Using multidimensional flow cytometry, confocal microscopy, adoptive transfer strategies and bulk RNA sequencing (RNAseq), we could show that CIA-MPhs are a distinct cDC subset that is $CD103^-$ $CD11b^{low}$ $MHC\text{-}II^+$ and expresses high levels of Lysozyme-M and PLET-1. We refer to this novel cDC subset as CP/ILF-associated DC or CIA-DC. Single-cell-RNA seq of intestinal cDCs confirmed that CIA-DCs are a transcriptionally distinct subset of intestinal cDCs that is closely related to cDC2s.
The differentiation of CIA-DC in the intestinal lamina propria required the presence of ILC3, as CIA-DC failed to differentiate RORgt deficient mice. We could show that CPs and ILF resident $CCR6^+$ILC3s were able to program the differentiation of CIA-DC precursors via LTa1b2 signals.
In depth analysis of the Bulk and scRNA-seq revealed that CIA-DC were highly enriched in genes associated with the regulation of immune responses. In particular, exclusive among all lamina propria leukocytes, CIA-DC expressed *Il22ra2*, the gene encoding IL-22BP, a soluble decoy receptor of IL-22. Thus CIA-DCs are, seemingly, the regulatory counterpart of CPs and ILF resident $CCR6^+$ILC3s.
Analysis of mice lacking CIA-DC-derived IL-22BP exhibited diminished expression of epithelial lipid transporters as well as reduced lipid resorption, showing that IL-22 regulation by CIA-DCs is important to regulate body fat homeostasis.
Our data provide first insights into ILC3 programming of DC and the design principles of an immunoregulatory checkpoint, associated with CPs and ILFs, necessary to control nutrient absorption.
This study was published end of 2020: Guendel et al, Immunity, 2020 Nov 17;53(5):1015-1032.e8 (doi: 10.1016/j.immuni.2020.10.012).

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O84

EOMES+ NK CELL SUBSETS ACCUMULATE IN THE INTESTINAL LAMINA PROPRIA DURING INFLAMMATION

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Abstract Content: Innate lymphoid cells (ILCs) are essential for the maintenance of intestinal homeostasis. Previous studies have shown an altered composition of ILC subsets during inflammatory bowel disease (IBD), potentially contributing to chronic inflammation. However, the identity of ILCs accumulating in the inflamed mucosa remains elusive. In this study, we have analyzed intestinal samples from IBD patients combining flow cytometry and multi-omic single cell approaches. This analysis revealed an enrichment of several Group 1 ILC clusters marked by CD56 and CD94 expression as well as *EOMES* gene activity, resembling various NK cell maturation stages rather than any previously described ILC1 populations. By analyzing dynamic transcriptional changes of the intestinal ILC compartment occurring over time in the *Helicobacter hepaticus* induced colitis model, we could show that colitis progression was similarly characterized by a marked increase of infiltrating circulating NK cells, but not ILC1 or ILC3, in the large intestinal lamina propria. Collectively, these data suggest that the imbalance between group 1 ILCs and ILC3s associated to progression of intestinal inflammation might result from accumulation of NK cell populations rather than from conversion of ILC3 towards ILC1, as previously suggested. Tackling this imbalance of NK cells and ILC3s could open up new possibilities for the treatment of IBD.

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O85

TH2 CELL-DERIVED IL-4 INDUCES THE EXPRESSION OF TYPE 2 ALARMINS TO ACTIVATE PULMONARY ILC2S

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Abstract Content: Th2 cells and type 2 innate lymphoid cells (ILC2s) are the central mediators of type 2 immunity. Despite the similarities in effector responses, unlike Th2 cells, ILC2 activation relies on the type 2 alarmins. Type 2 alarmins, including thymic stromal lymphopoietin (TSLP), interleukin (IL)-33, and IL-25, are produced by airway epithelium upon exposure to the allergen. Although several studies have been published about the crosstalk between ILC2s and Th2 cells, Th2 cell-mediated activation and expansion of ILC2s remain poorly understood. It is well known that CD4 T cells can produce IL-2 to drive ILC2 expansion. However, IL-2 alone is not sufficient for activating steady-state ILC2s. Here we show that mice passively injected with in vitro differentiated OT-II Th2 cells followed by OVA intranasal (*i.n.*) challenge activate the ILC2s. Moreover, mice challenged *i.n.* with IL-4 alone show a similar phenotype. Additionally, both models showed a significant increase in TSLP and IL-33 levels. Interestingly, TSLPR KO (*Crlf2*$^{-/-}$) mice challenged *i.n.* with IL-4 showed normal ILC2 activation, but ILC2s from IL-33 trap (*Il33* KO) mice were not activated by the same IL-4 *i.n.* challenge. These results indicate a direct role of IL-4 in the IL-33-dependent activation of ILC2s. Although the ILC2s from IL-33 trap mice show an activation phenotype following injection of OT-II Th2 cells, the neutralization of IL-25 significantly inhibited the activation of ILC2s. Together, these results indicate that IL-25 and

IL-33 have redundant roles in Th2 cell-induced ILC2 activation. Furthermore, neutralization of IL-4 significantly inhibited all three of the type 2 alarmins and thus ILC2 activation. Overall, our results reveal a correlation between production of the IL-4-induced type 2 alarmins (TSLP, IL-33, and IL-25) and CD4 T cell-mediated activation of ILC2s. This research was supported by the Intramural Research Programs of NIAID and NHLBI, NIH.

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O86
ROLE FOR ILC2-DERIVED LEUKAEMIA INHIBITORY FACTOR (LIF) IN ACUTE TYPE 2 LUNG CHALLENGE.
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Abstract Content: Group 2 innate lymphoid cells (ILC2s) are a significant source of type 2 cytokines such as IL-5, IL-13 and IL-9 and play an integral part in asthma and acute type 2 lung damage. In this study, we have investigated the expression of LIF by ILC2s and its role in acute type 2 lung challenge. Gene expression data suggest that group 2 innate lymphoid cells (ILC2s) and group 3 innate lymphoid cells (ILC3s) are primary haematopoietic sources of LIF. *In-vivo*, acute lung challenge with IL-33 or ragweed pollen extract upregulated LIF in the bronchiolar lavage (BAL) fluid, but this increase was abrogated in ILC2-deficient transgenic mouse models, indicating that ILC2 are required for LIF upregulation. Indeed, IL-33-stimulated ILC2s upregulated LIF expression *in-vitro.* We next identified that the LIF receptor is expressed in the lung by plasmacytoid dendritic cells (pDCs) and a subpopulation of endothelial cells. Notably, we observed that pDC infiltration into the lung correlated with ILC2-derived LIF production and was absent in ILC2-deficient mice and mice treated with anti-LIF neutralizing antibodies. Importantly, intranasal treatment of naïve ILC2-deficient mice with rmLIF was sufficient to induce pDC infiltration. In ongoing studies to investigate the role of ILC2-derived LIF in acute type 2 lung challenge, we have conditionally deleted *Lif* in ILC2s and *Lifr* in pDCs. Collectively, our data indicate that ILC2-derived LIF is a regulator of a rapid ILC2-pDC axis. Future studies will examine whether ILC2-derived LIF might be a potential modulator of viral-induced asthma where pDCs have been shown to be important.

Disclosure of Interest: None Declared
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Oral presentations: Cytokines and host physiology/homeostasis
O87
INTERFERON RECEPTOR GENE DOSAGE DETERMINES DIVERSE HALLMARKS OF DOWN SYNDROME
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Abstract Content: Down syndrome is caused by trisomy 21, the most common chromosomal abnormality in the human population and a leading cause of intellectual and developmental disability. Down syndrome is characterized by a distinct neurocognitive profile, immune dysregulation, atypical morphogenesis, and increased prevalence of multiple co-occurring conditions. Despite significant research efforts, the mechanisms by which an extra copy of chromosome 21 cause these effects remain largely unknown. Here we demonstrate that triplication of four interferon receptors encoded on chromosome 21 is necessary for multiple hallmarks of Down syndrome. Using whole blood transcriptome analysis, we demonstrate that overexpression of four interferon receptors associates with chronic interferon hyperactivity and systemic inflammation in people with trisomy 21 (N=400). To define the contribution of interferon receptor overexpression to Down syndrome phenotypes, we used genome editing to correct interferon

receptor gene dosage in mice carrying triplication of a large genomic region orthologous to human chromosome 21. Normalization of interferon receptor copy number attenuated a lethal antiviral response, prevented heart malformations, ameliorated developmental delays, improved cognition, and normalized craniofacial anomalies. Therefore, interferon receptor gene dosage determines major traits of Down syndrome, indicating that trisomy 21 elicits an interferonopathy amenable to therapeutic intervention with immune-modulatory strategies.

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O88

ROLE OF ENDOGENOUS RETROVIRUSES IN THE CONTROL OF TISSUE THRESHOLD OF ACTIVATION AND RESPONSES TO THE MICROBIOTA

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Abstract Content: All multicellular organisms are meta-organisms which have evolved to facilitate a symbiotic dialogue between the host and the microbiota. This is especially important at barrier tissues such as the skin, where communication between the microbiota and commensal-specific T cells is essential to promote protective immune responses, including antimicrobial defense and wound repair. However, our knowledge regarding the tissue imprinted cues that regulate the homeostatic immune relationship with the microbiota remains limited. Integrated retroviral elements, known as endogenous retroviruses (ERVs), comprise up to 8% of the human genome. We have previously shown that responses to a new skin commensal was controlled by reactivation of ERVs and associated type I interferon (IFN) responses. However, the class of interferon responsible, as well as the critical cellular partners involved in the orchestration of these responses remained unknown. We first focused on determining the cellular target of type I IFNs by topically associating an IFN-stimulated gene reporter mouse. Monocytes, DC1s and CD8 T cells were all responsive to type I IFNs in the skin. We further found that DC1 migration into the ear-draining lymph node is impaired in the absence of type I IFN signaling. This suggested type I IFNs may promote CD8 T cell responses to the microbiota in part by supporting DC migration to lymph nodes for antigen presentation. Accordingly, mice with a DC1-intrinsic deletion of the type I IFN receptor are being generated. We next assessed the cellular origin of type I IFN. Surprisingly, neither IFN-beta, nor IFN-alpha were detectable by RNA-seq, but rather IFN-kappa was the only type I IFN expressed in the skin. Contrary to reports demonstrating IFN-kappa is produced by keratinocytes during autoimmune inflammation, we find that during homeostasis, immune cells, in particular dendritic cells and Langerhans cells, express the highest levels of IFN-kappa in the skin. Using an IFN-kappa-deficient mouse model we found that CD8 T cell responses to the microbiota are significantly impaired, phenocopying the impairment observed in IFNAR-deficient animals. Thus, we conclude that immune cell-derived IFN-kappa supports CD8 T cell responses to the microbiota, in part by driving DC1 migration to draining lymph nodes. This work further establishes a novel role for IFN-kappa as a homeostatic type I IFN, capable of inducing protective immune responses to the microbiota. Collectively, this work explores how a type I IFN-dependent network promotes a multi-kingdom dialogue at the skin barrier site.

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O89

A NEWLY IDENTIFIED TRANSCRIPT OF THE LNCRNA, HOXA11OS, PROMOTES GUT HOMEOSTASIS THROUGH REGULATION OF IMMUNOMETABOLISM

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Abstract Content: Recent reports have suggested that long noncoding RNAs (lncRNAs) play a role in regulating intestinal epithelial barrier integrity. Indeed, a number of studies have demonstrated that lncRNA expression correlates with disease progression in ulcerative colitis (UC). Genome-wide association studies have identified over 200 risk loci associated with IBD with approximately 80% of disease-associated single nucleotide polymorphisms (SNPs) found in noncoding regions, including lncRNA loci. Given that these studies have primarily relied on experimental models of colitis and expression analysis in human tissue, it remains unclear what physiological role lncRNAs play in the development of UC

To identify lncRNAs associated with UC, we have profiled differentially expressed lncRNAs in over 1000 colon biopsies from UC and healthy patients. LncRNAs were then triaged based on overlapping sequences in IBD risk loci, disease severity and conservation between human and mouse. Based on these criteria, we have identified HOXA11AS in humans and its murine ortholog, HOXA11os. Using Oxford Nanopore sequencing on colon samples, we have identified a novel isoform of HOXA11os, predominantly expressed in the distal colon.

HOXA11os is highly expressed in the distal colon and is downregulated during intestinal inflammation. HOXA11os-deficient mice are highly susceptible to experimental models of colitis compared to wild-type littermate controls. Suggesting, HOXA11os plays an important role in maintaining intestinal homeostasis. Similar to mice, the human ortholog, HOXA11AS is highly abundant in the colon of healthy patients and is undetectable in the colon of patients with severe UC. Chromatin Isolation by RNA Purification (ChIRP) coupled with Mass Spectrometry, revealed HOXA11os binds to TCA-cycle proteins in the mitochondria. RNAscope analysis confirmed HOXA11os localizes to the mitochondria. Mechanistically, lamina propria cells from HOXA11os deficient mice displayed a lower Oxygen consumption rate (OCR) when compared to cells from WT littermate controls. Suggesting, HOXA11os plays a role in maintaining mitochondrial respiration. In summary, we have identified a previously unknow signaling axis by which HOXA11os can regulate metabolic pathways and restrain intestinal inflammation.

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Oral presentations: Cytokines and cardiovascular system

O90

THE RNA BINDING PROTEIN ARID5A MEDIATES IL-17 SIGNALING AND DISEASE PATHOGENESIS IN AUTOIMMUNE GLOMERULONEPHRITIS

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Abstract Content: The global burden of chronic kidney disease is substantial and growing. Antibody-mediated glomerulonephritis (AGN) is caused by an abnormal response to renal autoantigens, which is in part caused by exaggerated IL-17 downstream signaling. IL-17 induces inflammatory cytokines and chemokines that drive the kidney inflammatory response, and also the kidney damage biomarker lipocalin-2 (Lcn2, NGAL). The molecular signaling mechanisms by which IL-17 drives downstream genes leading to AGN are still not well understood. AT-rich interactive domain-containing protein 5a (Arid5a) is an unusual RNA binding protein (RBP) that can translocate to the cytoplasm, where it can bind to and stabilize target mRNA transcripts, including regulators of Lcn2 such as CCAAT/Enhancer binding proteins (C/EBPs). Arid5a can also compete with RBPs for RNA occupancy, such as the endoribonuclease Regnase-1 (MCPIP1, *Zc3h12a*). We found that Arid5a expression was dramatically increased in kidney during AGN, especially in renal tubular epithelial cells (RTECs). Therefore, to define the role of *Arid5a* in AGN in detail, we

generated *Arid5a*$^{-/-}$ mice by CRISPR/Cas9 and subjected them to an autoantibody-induced model of AGN. *Arid5a*$^{-/-}$ mice, like *Il17ra*$^{-/-}$ mice, were fully resistant to AGN, showing impaired infiltration of inflammatory cells into the kidney. Using bone marrow chimeras, we showed that Arid5a drives pathology by acting in non-hematopoietic cells, consistent with its prominent expression in RTECs. To define the underlying molecular mechanisms by which Arid5a mediates AGN pathology, we employed a human renal tubular epithelial cell line (HK-2) that is highly responsive to IL-17. Upon IL-17 stimulation, Arid5a bound directly to many target mRNAs involved in control of translation, as well as to the translation initiation complex. Accordingly, Arid5a was essential for efficient translation of IL-17-activated transcription factors including CEBP/beta and CEBP/delta, as well as their downstream genes that drive AGN pathology. Surprisingly, however, Arid5a$^{-/-}$ mice were resistant to infections that require IL-17 signaling, such as candidiasis. Thus, Arid5a is a novel regulator of cytokine-induced global translation, and may be an attractive target for autoimmune therapy by virtue of its selective role in IL-17-dependent autoimmunity but not infection control.

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O91

SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS REVEAL AN IL-1B MEDIATED VSMC PHENOTYPIC SWITCH DURING VASCULITIS AND CARDIOVASCULAR INFLAMMATION.

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Abstract Content: NLRP3 activation and IL-1β production are implicated in Kawasaki Disease (KD) pathogenesis, however a detailed description of the molecular networks and cellular subsets involved in this process is lacking. Here, we used single-cell RNA sequencing and spatial transcriptomics to characterize the cellular landscape of vascular tissues in a murine model of KD vasculitis. We observed infiltrations of innate and adaptive immune cells associated with increased expression of *Nlrp3*, *Il1b and Il18*. Monocytes, macrophages and dendritic cells were the main sources of IL-1β. Fibroblasts and vascular smooth muscle cells (VSMCs) expressed high levels of IL-1 receptor, while lymphocytes expressed high levels of IL-18 receptor. VSMCs in vasculitis lesions underwent a phenotypic switch, with upregulation of inflammatory mediators and fibroblast markers, and a downregulation of genes involved in contractile functions. Genetic inhibition of IL-1β signalling on VSMCs efficiently attenuated the phenotypic switch of VSMCs and the development of cardiovascular lesions during murine KD. In addition, pharmacological inhibition of NLRP3 prevented the development of cardiovascular inflammation. Our results unravel the cellular diversity involved in IL-1β production and signalling in KD cardiovascular lesions and demonstrate that therapeutic strategies targeting NLRP3 might be beneficial for human KD.

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O92

PI3K-DELTA SHAPES TH1 AND TH2 IDENTITY THROUGH COORDINATION OF IL-2 SIGNALING, FOXO1 INACTIVATION AND EPIGENETIC REMODELING

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Abstract Content: Activated PI3Kδ syndrome (APDS) is a primary immunodeficiency caused by heterozygous activating mutations in PI3Kδ, resulting in dysregulated immunity, recurrent respiratory infections and allergic diseases. Cytokine producing helper CD4 T cells are critical in orchestrating adaptive immunity; the coordinated differentiation of these cells into distinct effector lineages is instrumental in maintaining balanced immune responses. Given that APDS patients show both an inability to clear respiratory pathogens (Th1) and development of allergic

diseases (Th2), we hypothesized that hyperactivated PI3Kδ (Pik3cd^{E1020K}) may alter Th1 versus Th2 differentiation. Using in vitro polarization of murine naïve CD4 T cells, we observed increased production of IFNγ from Pik3cd^{E1020K} cells under Th1-inducing conditions and marked increases in IL-4 and IL-13 production under Th2 conditions. However, Pik3cd^{E1020K} Th2 cells also expressed high levels of T-bet and aberrantly produce the Th1 cytokine IFNγ. We confirmed this dysregulation in vivo; Pik3cd^{E1020K} CD4 cells show marked alterations following house dust mite (HDM) induced allergic airway inflammation, a powerful Th2 driven model, with impaired Th2 and inappropriate increased Th1 differentiation. scRNA-seq analysis of HDM induced Pik3cd^{E1020K} immune cells showed strong enrichment of IFNγ response gene signatures across multiple populations, including CD4, CD8, NK cells and neutrophils, highlighting a global switch towards type I immunity under type II inducing conditions. Using in vitro differentiated cells, we linked this unstable Th1 and Th2 differentiation to increased responsiveness to IL-2, with inhibition of IL-2 restoring normal patterns of differentiation in Pik3cd^{E1020K} CD4 T cells. Downstream of IL-2, we found increased phosphorylation and inactivation of the transcription factor Foxo1 in Pik3cd^{E1020K} CD4 T cells; RNA-seq revealed altered Foxo1 regulated gene signatures in Pik3cd^{E1020K} CD4. Forced expression of Foxo1 in Pik3cd^{E1020K} Th1 and Th2 limited excessive IFNγ and T-bet expression and rescued altered differentiation, whereas CRISPR-mediated targeting of Foxo1 recapitulated phenotypes of Pik3cd^{E1020K} T cells. Finally, RNA-seq analysis of in vitro polarized Pik3cd^{E1020K} Th1 and Th2 cells showed increased expression of numerous Foxo1 inhibited genes, including *lmna*, encoding a nuclear envelop protein that interacts with repressed chromatin via lamin associated domains, as well as enrichment of methionine salvage pathway genes, including *mat2a*, which catalyzes the generation of the universal methyl donor S-adenosyl methionine (SAM); SAM levels have been shown to influence histone methylation patterns. Accordingly, Pik3cd^{E1020K} CD4 exhibit altered H3 trimethylation, including H3K27 and H3K4 trimethylation, in a Foxo1-dependent manner. Together these data suggest that balanced regulation of PI3Kδ plays a critical role in shaping Th1 and Th2 lineage identity through integrating cytokine signaling, transcription factor activity and global chromatin reorientation.

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Oral presentations: ILCs in Inflammation and Pathology

O93

EARLY-LIFE RESPIRATORY INFECTION SUPPRESSES LUNG RESIDENT GROUP 2 INNATE LYMPHOID CELLS AND BRONCHIOALVEOLAR STEM CELLS RESULTING IN ABNORMAL LUNG DEVELOPMENT

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Abstract Content: Normal healthy lung development can be disrupted by specific respiratory tract infections in early life. These infections can cause permanent deleterious changes to lung structure and function and increase the likelihood of developing chronic lung diseases. However, the mechanisms involved remain poorly understood. Using experimental mouse models of early-life bacterial and viral respiratory infections, we show that infection decreased the number of group 2 innate lymphoid cells (ILC2) in the lungs. This was associated with a concomitant decrease in the number of bronchioalveolar stem cells (BASC) that reside at bronchioalveolar duct junctions and replenish both airway and alveolar epithelial cells after injury. Furthermore, these infections resulted in impaired alveolar structure and lung function. Diphtheria toxoid-mediated depletion of ILC2 during the neonatal period resulted in a decrease in BASC, whilst pulmonary administration of recombinant interleukin (IL)-33 increased BASC numbers in neonates but not adults. Naïve neonatal mice had 5-fold greater numbers of both ILC2 and BASC than naïve adult mice. t-distributed stochastic neighbour embedding (tSNE) analysis of lung ILC revealed distinct clustering between both ages, with neonates showing higher expression of the IL-33 receptor ST2 and ICOS. Neonates had increased numbers of functional IL-5^{+} and IL-13^{+} ILC2 compared to adult mice. Our studies reveal differences in ILC2 in neonatal and adult mouse lungs, and that a lack of these cells in early life alters the number of pulmonary stem cells. Enhancing the function of these cells during early-life development could reduce the risk of developing chronic lung diseases.

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O94

ZBTB46 DEFINES AND REGULATES GROUP 3 INNATE LYMPHOID CELLS THAT PROTECT THE INTESTINE

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Abstract Content: RORgt is a lineage-specifying transcription factor expressed by immune cells that are enriched in the gastrointestinal tract and orchestrate immunity, inflammation, and tissue homeostasis[1-15]. However, fundamental questions remain regarding the cellular heterogeneity among these cell types, the mechanisms controlling protective versus inflammatory properties, and their functional redundancy. Here, we define all RORgt$^+$ immune cells in the intestine at single cell resolution and unexpectedly identify a subset of group 3 innate lymphoid cells (ILC3s) expressing Zbtb46, a transcription factor that specifies conventional dendritic cells (cDCs)[16-20]. Zbtb46 is robustly expressed by CCR6$^+$ lymphoid tissue inducer (LTi)-like ILC3s that are developmentally and phenotypically distinct from cDCs, and expression is fine-tuned by lineage-specifying transcription factors, microbiota, and cytokines during homeostasis or chronic intestinal inflammation. Zbtb46 functions to restrain inflammatory properties of ILC3s, including OX40L-dependent expansion of Th17 cells and exacerbated intestinal inflammation following enteric infection. Finally, Zbtb46$^+$ ILC3s are a dominant source of IL-22, and selective depletion of this population renders mice susceptible to enteric infection and associated intestinal inflammation. These results define an unexpected transcription factor shared between cDCs and ILC3s, a cell-intrinsic function for Zbtb46 in restraining pro-inflammatory properties of ILC3s, and a non-redundant role for Zbtb46$^+$ ILC3s in orchestrating intestinal health.

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O95

NOTCH-DEPENDENT IL9 SIGNALING IN ILC2 REGULATES FIBROSIS

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Abstract Content: Background: Innate lymphoid cells are critical modulators of mucosal immunity, inflammation, and tissue response. Previously, we found that dermal and circulating ILC2s were significantly elevated in patients with SSc compared to controls. Whether the increased ILC2 number serves as a prognostic marker for SSc remains elusive. Furthermore, recent evidence indicates that ILC2 cells promote collagen synthesis by fibroblasts in fibrotic conditions, but relatively little is known about the underlying cellular and molecular mechanisms.
Objectives: 1. Perform a long-term follow-up study to correlate peripheral ILC2 counts and fibrosis progression in patients with SSc; 2. Find signaling pathways regulating fibrosis by ILC2s via single-cell multi-omics strategy; 3. Validate candidate genes in ILC2 *in vitro* and *in vivo* using CRISPR-Cas9 knock-out tools.
Methods: Clinical patient data of a 5-year follow-up study of 49 SSc patients; **Skin, lung and liver fibrosis model**; **Single-cell RNA sequencing (10x Genomics)**; **Spatial transcriptomics sequencing via Slide-seq**; **Single-cell proteome profiling via CyTOF**; **Generating ILC2 knockout using CRISPR-Cas9 tools; Adoptive Transfer of ILC2 cells; Confocal and light-sheet microscopy imaging** were performed to visualize ILC2s and fibrotic tissue remodeling.
Results: The lung function of SSc patients was inversely correlated while skin stiffness was positively correlated with circulating ILC2 counts during a prospective observation period of 5 years. Furthermore, patients with higher ILC2 counts showed persistent activity of fibroblasts as assessed by fibroblast activation protein (FAP) positron emission tomography/computing tomography (PET/CT) and showed a significantly diminished survival rate compared to SSc

patients with low ILC2 counts. On a molecular level, ILC2 derived IL-9 was identified as a key driver in this setting as mice with specific knockout of IL-9 in ILCs were protected from fibrosis in various fibrosis models; Adoptively transferred WT ILC2s but not *IL9*$^{-/-}$ ILC2s mediated bleomycin-induced lung fibrosis in *Rag2/il2rg*$^{-/-}$ double knockout mice; IL9^{+} and IL9R^{+} ILC2 cells were increased in skin and lung biopsies of SSc patients; *In vitro* co-culture assays suggested that a cell/cell contact-dependent interaction between ILC2s and fibroblast is required to activate Notch signaling in ILC2s. Notch signaling positively regulates the IL-9/IL-9R axis by increasing IL-9R expression on ILC2s inducing a positive feedback loop that leads to persistent activation of ILC2s. In addition, Notch-dependent elevation of IL-13 levels in ILC2s leads to persistent activation of fibroblasts and matrix remodeling.
Conclusions: Circulating ILC2s can serve as a prognostic marker for disease progression in SSc; Furthermore, Notch-dependent IL9-IL9R autocrine signaling in ILC2s is indispensable for fibrosis. Therefore, Notch signaling in ILC2s might be a novel therapeutic target for intervening fibrosis.

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O96
GROUP 2 INNATE LYMPHOID CELLS SUPPRESS INNATE TYPE 3/17 RESPONSES IN HEPATIC FIBROSIS
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Abstract Content: Type 2 immunity can promote physiologic tissue remodeling yet excessive activation can also drive fibrotic disease. Group 2 innate lymphoid cells (ILC2s) are a dominant organizer of this immune flavor, but how ILC2 topography and local interactions dictate these responses are unknown. Using several mouse models of liver fibrosis, we used quantitative 3D imaging to define fibrosis-associated portal and periductal ILC2 accumulation in proximity to an expanded IL-33-producing fibroblast subset. However, ablation of IL-33 or IL-4/IL-13 had no impact on hepatic fibrosis. Unexpectedly, constitutive or inducible loss of ILC2s worsened carbon tetrachloride (CCl4)- or bile duct ligation-induced liver fibrosis. Mechanistically this occurred in part via suppression of innate IL-17A-producing lymphocytes, which also accumulated in periportal regions during fibrosis. Collectively, these data identify a novel role for ILC2s in the liver portal tracts as a negative regulator of the innate type3/17 immune response to hepatic damage and suggests resident-lymphocyte topographic crosstalk may be a critical determinant of liver health and disease.

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Oral presentations: Joint Plenary Session: Cytokines and ILCs - neuro/homeostasis context

O97
NEURONAL REGULATION OF NON-REDUNDANT FUNCTIONS OF INNATE LYMPHOID CELLS
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Abstract Content: Emerging studies indicate that cooperation between neurons and immune cells regulates anti-microbial immunity, inflammation, and tissue homeostasis. For example, a neuronal rheostat can provide excitatory or inhibitory signals that control tissue-resident group 2 innate lymphoid cell (ILC2) functions at mucosal barrier surfaces. ILC2s selectively express NMUR1, a receptor for neuromedin U (NMU), a prominent cholinergic neuropeptide that promotes ILC2 responses. Many functions of ILC2s can be shared with some adaptive lymphocytes,

including the production of type 2 cytokines, which promote anti-helminth immunity and allergic inflammation, and the release of amphiregulin (AREG), which contributes to tissue protection following exposure to infectious or inflammatory insults. These shared functions have provoked controversy as to whether ILCs and adaptive lymphocytes perform redundant versus non-redundant functions *in vivo*. In this study, we employ a newly developed transgenic mouse model that selectively targets ILC2s with high efficiency for depletion or gene deletion *in vivo* in the presence of an intact adaptive immune system. ILC2-specific deletion of *Areg* revealed that AREG production by ILC2s promotes non-redundant functions in anti-parasite responses and tissue protection following intestinal damage and inflammation. Notably, NMU expression levels were increased in inflamed intestinal tissues from both mice and humans. Further, NMU selectively induced AREG production in both murine and human ILC2s, indicating that neuronal regulation of non-redundant tissue-protective functions of ILC2s is an evolutionarily conserved mechanism that promotes intestinal immunity and tissue protection.

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O98

PD-1 REGULATES ILC3-DRIVEN INTESTINAL IMMUNITY AND HOMEOSTASIS

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Abstract Content: Programmed cell death 1 (PD-1) has been extensively studied in the context of cancer and chronic infection, however, its function in the maintenance of tissue homeostasis is less well understood. We found that a subset of intestinal group 3 innate lymphoid cells (ILC3) residing in the villi of the small intestine and colon constitutively expressed PD-1. PD-1 expression was dependent on the microbiota and was induced during inflammation in response to IL-23 but, conversely, was reduced by the activation of Notch ligand. PD-1$^+$ ILC3 exhibited both increased metabolic activity associated with augmented proliferation and IL-22 production compared with PD-1$^-$ ILC3. The loss of PD-1 signaling in ILC3 led to a reduced IL-22 production in a cell intrinsic manner. During inflammation, NCR$^-$ ILC3 PD-1 expression was increased while PD-1 deficiency led to increased susceptibility to colitis and failure to maintain gut barrier integrity. Collectively, our findings uncover a new function of the PD-1/PDL1 axis and highlight the role of PD-1 signaling in the maintenance of gut homeostasis.

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O99

MICROBIAL ENERGY METABOLISM FUELS A CSF2-DEPENDENT INTESTINAL MACROPHAGE NICHE

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Abstract Content: Macrophages (MPs) are essential regulators of intestinal homeostasis and comprise at least three subpopulations discriminated by the markers Tim-4 and CCR2. Tim-4$^+$ MPs primarily arise from fetal precursors, while CCR2$^+$ MPs develop postnatally from infiltrating monocytes. The gut microbiota and host factors are thought to synergistically guide intestinal MP development, although the exact nature, regulation, and location of such collaboration remain unclear.
Here, we identified an intestinal niche for CCR2$^+$ MPs that is dependent on the microbiota and the growth factor colony stimulating factor 2 (CSF2). Tissue-resident type 3 innate lymphoid cells (ILC3s), enriched in tertiary lymphoid organs

(TLOs), are the main source of CSF2. CCR2^{+} MPs, but not Tim-4^{+} MPs, accumulate in ILC3-containing TLOs. Consequently, the absence of CSF2, ILC3s, or the gut microbiota resulted in a loss of CCR2^{+} MP numbers. Furthermore, we found that CSF2 production required P2X7R-mediated NLRP3 inflammasome activation by microbiota-derived extracellular ATP. Germ-free mice showed a significant reduction in CCR2^{+} MP numbers that was restored by colonization with conventional, but not ATP synthetase-deficient, microbes. Luminal ATP levels increased upon diversification of the microbiota and dictated this turnover of CCR2^{+} MPs. Single cell RNA-sequencing of MPs isolated from TLOs and the remaining lamina propria revealed location-specific, CSF2-dependent differences in MPs that contribute to cellular fitness and host defense. In line with these data, *Csf2*$^{-/-}$ mice had reduced CCR2^{+} MP numbers following enteric infection, resulting in elevated pathology.
Collectively, our findings implicate a key role for commensal microbial energy metabolism in regulating TLO-resident, CSF2-producing ILC3s to educate monocyte-derived CCR2^{+} MPs for optimal host defense and immune homeostasis.

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Oral presentations: Human ILCs

O100

HUMAN UNCONVENTIONAL ILC2 SHOW IMMUNOLOGICAL MEMORY PROPERTIES

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Abstract Content: Group 2 Innate Lymphoid Cells (ILC2s) are mediators of type 2 immunity and play important roles both in homeostasis and during inflammation. ILC2s express the IL-7 receptor CD127 as IL-7 is critical for ILC development. Whereas ILC2s are abundant in barrier tissues, very few ILC2s are detected in the adult human intestine. Here, we show that adult human intestine contains an unconventional ILC2 population characterized by CD127-/lo CD45RO+. These unconventional CD127-/lo CD45RO+ ILC2s are not restricted to human intestine but are present in other healthy and inflamed human tissues, including dermis and nasal polyps from chronic rhinosinusitis patients. Single cell RNA sequencing of dermal ILCs suggested that the CD127-/lo CD45RO+ cells were previously activated ILC2s. Furthermore, purified dermal unconventional CD45RO+ ILC2s presented enhanced capacities to proliferate and produce IL-5 upon ex vivo stimulation with IL-25+TSLP compared to conventional CD45RA+ ILC2s. Altogether, these data suggests that CD127-/lo CD45RO+ ILC2s, present in healthy tissues, are experienced or memory ILC2s. In addition, mouse memory ILC2s are enriched in the CD127-/lo compartment and are the main source of IL-5 and IL-13 upon in vivo challenge in a mouse model of lung allergic inflammation. The identification of CD127-/lo ILC2s challenges our current definition of human ILC2s, and further characterization of their immune memory properties will expand our understanding of allergic diseases.

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O101

THE EFFECT OF TISSUE ENVIRONMENT ON THE EPIGENETIC SIGNATURES IN HUMAN INNATE LYMPHOID CELLS (ILC)

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Abstract Content: **Introduction:** DNA methylation can influence the transcriptional profile in immune cells, inducing or maintaining a specific cell phenotype and its effector functions. In mice, as well as in humans, ILCs show a heterogenetic phenotype across different tissues. The identity of murine innate lymphoid cells (ILC) is epigenetically imprinted by distinct DNA methylation patterns among the subpopulations. Thus, we hypothesized that unique epigenetic signatures, which depend on a specific tissue environment, also regulate human ILC subsets. **Objectives:** We aim to identify epigenetic signatures that define human ILC subpopulations in different tissue environments regarding their DNA methylation patterns. We hypothesize that the methylation status reflects the local signals received by the tissue-resident ILCs. **Methodology:** Mononuclear cells were isolated from human peripheral blood, tonsils, lymph nodes, and colon and lung biopsies. We enriched lineage-negative cells from lymph node and lung tissue using magnetic-activated cell sorting (MACS). For all tissues, the mononuclear cells were stained with surface antibodies and ILC1, ILC2, ILC3, and NK cells were separately collected via fluorescence-activated cell sorting (FACS). DNA libraries were prepared for bisulfite conversion and genome-wide DNA methylation profiling of tonsil- and blood-derived ILCs was performed. The data were analyzed bioinformatically to identify differentially methylated regions (DMRs) between each pair of ILC subsets. We chose DMR-associated genes from the top differentially methylated regions to obtain specific epigenetic signatures for each subpopulation. For the lung, colon, and lymph node tissue, the chosen signature candidate regions will be amplified and enzymatically converted. The methylation status of the target regions of each subset will be assessed via pyrosequencing and the epigenetic signatures will be compared across the different tissues. **Results:** We identified separate epigenetic signatures for ILC1, ILC2, and ILC3, comprising 10, 13, and 11 DMR-associated genes, respectively. Among the top DMR-associated genes, we encountered candidates with previously known associations to ILC function, such as Tbx21 in tonsil-derived ILC1s or Gata3 in blood-derived ILC2, thereby validating our experimental approach. Genes that had not been investigated in an ILC context were found among all three signatures. Based on the number of DMRs, ILC1 appear to relate epigenetically most closely to NK cells, whereas ILC2 seem most distant to the other subsets. ILC3 are closer to NK cells and ILC1 than to ILC2. We are currently investigating the characterized signatures in ILCs from gut, lung, and lymph nodes. We are analyzing whether the candidate genes are specific for their respective ILC subpopulation or if they are dependent on the microenvironment of the tissue niche. **Conclusion:** Collectively, we identified epigenetic signatures comprising several marker genes in human ILC1, ILC2, and ILC3. We will determine tissue-specific differences in the DNA methylation pattern of ILC subpopulations of the lung, the colon, and the lymph nodes.

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O102

PRO-INFLAMMATORY EPIGENETIC CHANGES CONVERT TYPE 2 ILCS TO ILC1-LIKE CELLS FOLLOWING ALLOGENEIC HSCT

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Abstract Content: Although allogeneic hematopoietic stem cell transplantation (alloHSCT) is the preferred treatment for a variety of blood malignancies, its use is limited by the development of acute graft-versus-host disease (aGvHD). ILC2s are immune cells that play an important role in maintaining homeostasis in mucosal tissues. Interestingly, previous work from our group and others has shown that ILC2 cells are significantly impaired in their ability to reconstitute after chemotherapy or stem cell transplantation. However, the mechanism for this finding has not been clear. Previous work from our group has shown that ILC2s in the GI tract are depleted by chemotherapy or radiation prior to alloHSCT, with the infusion of donor ILC2s improving improved clinical score and survival of recipient mice. Given that current studies demonstrate significant ILC plasticity, we evaluated the hypothesis that the significant decrease in ILC2 cells post-therapy is due to post-transplant induced epigenetic changes that convert ILC2 cells to either ILC1 or ILC3-like cells. To address this hypothesis, we used ChIP-seq, ATAC-seq and RNA

transcriptome to evaluate transcriptional regulation of ILC2 cells expanded ex vivo and evaluated in vivo and in vitro. Strikingly, single-cell, multiomic analysis of donor-derived ILC2s after alloHSCT revealed a previously unreported population of ILC1-like cells that differentiate from ILC2s in the SI LP ("exILC2s"). To recapitulate this transdifferentiation, we modeled skewing of ILC2s in vitro with IL-12 and IL-1b and observed a reduction in Type 2 LDRFs and the acquisition of proinflammatory Type 1 LDRFs consistent with the phenotype and function of IFN-γ-secreting exILC2s. Unlike their unmanipulated WT ILC2 counterparts, our in vitro-derived exILC2s failed to ameliorate aGvHD and instead accelerated morbidity and mortality. These data demonstrate, for the first time, that these cells fail to repopulate their protective niche after alloHSCT due to epigenetic reprogramming and pathological transdifferentiation. These findings provide a cuttting edge understanding how the plasticity of ILC2s contributes to mucosal dysregulation and the pathogenesis of aGvHD after alloHSCT. To inform new approaches for modulating innate lymphocyte cell plasticity in human disease, ongoing experiments are underway to define ILC1, ILC2, and exILC2 like cells in human alloHSCT recipients. Using single cell transcriptomics, peripheral ILCs are being compared between patients who remain stable after alloHSCT and those who experience an episode of aGVHD within the first 100 days following HSCT. We anticipate that coupled with our striking findings in the mouse small intestine, this work will inform novel stragtegies for modulating innate lymphocytes in human disease.

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Poster presentations

01. Cytokine induction/production

P1

RATIONAL ENGINEERING OF A FOLDING PATHWAY CHANGES MECHANISM AND CHAPERONE DEPENDENCY OF INTERLEUKIN SECRETION

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Abstract Content: Equipping engineered lymphocytes with suitable immune-activating cytokines and/or receptors for engineered cytokines is a promising avenue to maintain and control lymphocyte functionalities in desired ways. In our work we focus on the IL-12 family, that has several features that ideally qualify it for lymphocyte engineering approaches: The human IL-12 family contains four members, IL-12, IL-23, IL-27, and IL-35. Each is a heterodimeric cytokine and signals via heterodimeric receptors. These four cytokines with a unique structural setup are made up of only five subunits, with the four-helix bundle α-subunits being secretion-incompetent in isolation and the β-subunits being secreted alone. In the context of heterodimer formation, the β-subunit assists in assembly-induced folding of the α-subunit and subsequent release from the endoplasmic reticulum (ER). This biogenesis is accompanied by folding enzymes, so-called chaperones, and cytokine release is strictly regulated by the ER quality control system.
Cytokine engineering, here used for IL-23 (p19/p40), provides many opportunities for current major challenges in adoptive immunotherapy. Based on structural studies that revealed why the α-subunit of IL-23 fails to fold correctly in isolation (Meier et al., 2019), we applied *in silico* methods together with rational engineering approaches to design an autonomously folding- and secretion-competent human IL-23α/p19 protein. We succeeded in obtaining a highly stable human IL-23α/p19 variant by just three point mutations, including the introduction of a disulfide bond that stabilizes its first α-helix of its characteristic four-helix bundle fold. Stability of the variant is increased not only intracellularly but also of the purified subunit. Importantly, this mutant can still assemble with IL-12β/p40 with low-μM affinity – thus with higher affinity compared to the wildtype. Besides this, the optimized IL-23α/p19 mutant shows a different intracellular folding behaviour, which goes hand in hand with an altered cellular chaperone repertoire acting on it. This IL-23α/p19 mutant allowed us to address key questions concerning the IL-12 family: whether a molecular competition of IL-12 versus IL-23 formation exists in cells, as these heterodimers both contain the IL-12β/p40 as β-subunit, and why IL-12 family subunits have evolved for assembly-dependent instead of independent folding and secretion.

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P2

DEVELOPING A DEEP UNDERSTANDING OF THE IMMUNE RESPONSE IN HEPATITIS B VACCINATED NON-RESPONSIVE INDIVIDUALS USING A KNOWLEDGE-DRIVEN APPROACH

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Abstract Content: Background and Aims:
Despite the availability of efficacious vaccines against the hepatitis B virus (HBV), this virus still is critical for occupational infectious disease. Approximately 5% of the people vaccinated against HBV do not develop long-lived protective neutralizing antibodies. The present study focuses on the comprehensive analysis of human immune responses in different medical situations, especially in HBV-vaccinated individuals. This requires seamless integration of an enormous amount of diverse fine-grained data. The fine-grained definition and integration of diverse datasets can provide a common understanding of the human immune system and more profound insights into an individual's immune response.
Methods:
We collected heterogeneous datasets of 23 HBV-vaccinated individuals. Vaccination induced responses were analysed in single individuals after three months of Engerix-B vaccination by measuring the hepatitis B surface antigen-specific antibody levels (anti-HBsAg). Individuals were categorized, based on the anti-HBsAg levels into responders, non-responders, and low responders. The Blood samples were collected before and after vaccination and were used for longitudinal flow cytometry-based immunophenotyping of a broad range of immune cells. Furthermore, HLA-typing and whole-genome sequencing was performed and information about pre-existing conditions, medications, vaccinations, and living habits of the participants were collected. A data integration system is defined in terms of a unified schema datasets, and mappings. The unified schema, for deep immunoprofiling in vaccination, infectious diseases, and transplantation (ImProVIT) represents immune concepts, properties, and relationships present in the collected datasets, while the mappings define these concepts in terms of the datasets. The Unified Medical Language System (UMLS) is used to standardize the representation of the data and to enable entity alignment. A knowledge graph (KG) was created from the execution of the mappings over the datasets.
Results:
The unified schema ImProVIT states a common understanding of the human immune system in terms of 36 classes (concepts), 105 attributes (properties), 140 relations and 123 hierarchies of concepts. The modelled holistic profile enables the representation of study participant in terms of epidemiological information, antibody measurements, observed immune responses, and HLA allelic variants. All the biological assays that are applied on biosamples to measure the above-mentioned parameters are also modelled.
Conclusions:
The methodology used for defining the data integration system and creating the knowledge graph has allowed for a fine representation of the immune system profile of HBV-vaccinated individuals. It also enables the interaction of domain experts, doctoral students, and data scientists to create a knowledge graph to develop a common understanding of the human immune system. The unified schema ImProVIT enabled us to integrate data from other use cases focusing on aspects of the immune system and facilitates us to understand human immune responses in different medical conditions.

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P3

METHOTREXATE BLOCKS SLC46A2 MEDIATED TRANSPORT OF NOD1 AGONISTS AND INHIBITS PSORIASIS PROGRESSION

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Abstract Content: NOD1 is a cytosolic receptor activated by small peptidoglycan fragments from the bacterial cell wall. How these fragments, or muropeptides, gain access to the cytosolic is unclear. Recently, we identified the SLC46 family of transporters as mediators of this process in *Drosophila*. One member of this family, *Slc46a2* is expressed in a limited number of cell types, including keratinocytes in the skin epidermis, a critical barrier tissue. We have demonstrated that *Slc46a2* is critical for NOD1 activation by DAP-muropeptides in the murine skin, rapidly signaling a robust inflammatory response mediated by IL-1a. In epidermal keratinocytes, *Slc46a2, Nod1, Caspase-1,* and *Gasdermin-D* are required for DAP-muropeptide triggered membrane permeabilization and IL-1α release. Moreover, *Slc46a2* and *Nod1* are critical for skin inflammation in a model of psoriasis. Given the similarity to the Proton-Coupled Folate Transporter (SLC46A1), we hypothesized that SLC46A2 may also transport anti-folates and discovered that the anti-folate methotrexate potently inhibited the *SLC46a2*-dependent response to DAP-muropeptide, thereby identifying SLC46A2 as an important target for anti-inflammatory intervention.

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P4

NOVEL REGULATORS OF TYPE III CYOTOKINE EXPRESSION IN TYPE III INNATE LYMPHOID CELLS IDENTIFIED BY CRISPR SCREENS

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Abstract Content: Type III cytokines interleukin (IL)-22 and IL-17 are expressed by innate and adaptive immune cells to defend against extracellular pathogens, yet their dysregulation contributes to autoimmunity and malignancy. A deeper knowledge of IL-22/17 regulation is thus warranted to understand physiologic processes and disease pathogenesis, potentially revealing druggable targets. Towards this goal, we have implicated IL-17/22 regulators within a murine type III innate lymphoid cell (ILC3)-like model via genome-wide activation or inhibition CRISPR screens following IL-1β and IL-23 stimulation. Top candidates were enriched from several biological pathways, ranging from kinases to mRNA splicing co-factors. We identified previously known regulators, including IL-23 receptor components IL23R and IL12Rβ1, as well as novel potential regulators, including a nuclear protein called SON DNA Binding Protein (SON). Mechanistic studies of top verified candidates are underway, with SON demonstrating regulatory actions on IL12Rβ1. Our screens provide a diverse array of candidate factors that govern expression of type III cytokines by ILC3 lymphocytes, with potential for extrapolation to other cytokine-expressing cells.

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P5

CHARACTERIZATION OF THE COMPLEMENT SYSTEM IN CYTOKINE-DRIVEN NEUROINFLAMMATION

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Abstract Content: Introduction: Interleukin-6 (IL-6) and Type I Interferon (IFN-I) are vital mediators of the innate immune response to pathogens; however, chronic overproduction of IL-6 and IFN-I in the central nervous system (CNS) can pathologically contribute to multifarious neurological diseases. The complement system is also an indispensable effector of innate immunity, comprised of more than 30 soluble molecules that primarily work to opsonize pathogens for phagocytosis, instigate inflammation, and lyse cells (1). IL-6, IFN-I, and various complement factors have been shown to act synergistically under certain conditions, resulting in a positive feedback loop that mediates chronic inflammation in the brain (2). Here, we aimed to determine whether complement activation mediates

cytokine-driven disease using two transgenic mouse models displaying chronic overexpression of either IL-6 or IFN-I restricted to the CNS.
Methods: Brains of transgenic mice with either overexpression of IL-6 (GFAP-IL6) or IFN-I (GFAP-IFN) and their wildtype (WT) counterparts were probed for complement activation via transcriptomic analyses. Specific complement components were validated in GFAP-IL6 and GFAP-IFN brains via RT-qPCR and immunofluorescence. PMX205, a C5aR1 receptor antagonist, was chronically administered to GFAP-IL6, GFAP-IFN, and WT mice via the drinking water. The contribution of complement component C3 was also investigated by cross breeding the GFAP-IL6 and GFAP-IFN mice with C3 knockout mice. All murine models were evaluated by a clinical scoring system, weekly weights, and behavioural testing on the rotarod and balance beam. Brains from mice from both studies were examined for changes in gene expression, protein expression, and histology.
Results: GFAP-IL6 and GFAP-IFN mice have significantly increased relative gene expression of complement components C5aR1, C3, C4, C1q, and C6 compared to WT counterparts. Inhibition of C5aR1 resulted in unique alterations to histopathology and blood brain barrier permeability but did not significantly alter the clinical phenotype of GFAP-IL6 and GFAP-IFN mice. Homozygous knockout of complement C3 led to distinct changes in the phenotypical and pathological profile of GFAP-IL6 and GFAP-IFN mice.
Conclusion: Complement activation is implicated in IL-6 and IFN-I driven neuroinflammatory diseases. Inhibition of complement is a promising therapeutic target for diseases in which IL-6 and IFN-I play a pathological role.
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P6

TL1A PROMOTES DEVELOPMENT OF MULTI-CYTOKINE SECRETING TH9 CELLS

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Abstract Content: The TNF superfamily member TL1A is a costimulatory molecule that signals through its receptor DR3 on T lymphocytes. The Th9 subset of T lymphocytes secretes the pleiotropic cytokine IL-9 which has functions in allergic airway disease, helminth infections, and tumor immunity. TL1A increases IL-9 production from Th9 cells. However, its role in regulating other functions of Th9 cells is unknown. Here we demonstrate that TL1A increases expression of IL-9 and IL-13 as well as the frequency of IL-9 and IL-13 co-expressing cells in murine Th9 cell cultures through flow cytometric analyses. We also show that the *Il9* and *Il13* promoter and enhancer regions are differentially accessible in response to TL1A over a five-day culture period through chromatin accessibility assays. At the *Il9* locus, TL1A enhances binding of IRF4, BATF, and PPARg. Mechanistically, this is linked to decreased H3K9 tri-methylation and increased H3K4 tri-methylation at *Il9* enhancer region CNS2. At the *Il13* locus, TL1A enhances binding of BATF and PPARg at the *Il13* promoter alongside decreased H3K9 tri-methylation and increased H3K4 tri-methylation. We further show in an *in vivo* murine chronic model of allergic airway disease that blockade of TL1A signaling decreases the multi-cytokine Th9 cell population. Together, these data indicate that TL1A contributes to heterogeneity of IL-9-secreting T cell populations.

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P7

THE EPIGENETIC REGULATOR TET2 IS ESSENTIAL FOR VIRUS-STIMULATED HUMAN PDC DIFFERENTIATION FROM IFN-A PRODUCING CELLS TO ANTIGEN PRESENTING CELLS

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Abstract Content: Plasmacytoid dendritic cells (pDC) bridge the gap between innate and adaptive immunity by producing an abundant amount of type I interferon (IFN) in response to viruses by signaling through TLR9 and -7, respectively. pDC are potent producers of IFN-α in response to DNA and RNA viruses and subsequently mature into antigen presenting cells, although whether the same cell does both has been controversial. To evaluate epigenetic regulation of pDC activation and maturation, we investigated the role of DNA methyltransferase 1 (DNMT1) and Ten-eleven translocation methyl-cytosine dioxygenase 2 (TET2), which are commonly known as a gene silencer and activator, respectively. DNMT1 and TET2 proteins were quantified in PBMC from healthy donors using intracellular flow cytometry. In separate experiments, PBMC were stimulated with Herpes Simplex Virus (HSV) or Influenza-A virus (IAV) for 6- and 24h. PBMC were surface-stained for pDC markers BDCA-2, CD123, HLA-DR and CD11C (the latter to exclude myeloid DC) and co-stimulatory markers CD40, CD83, CD80 and CD86, then intracellularly for DNMT1, TET2 and IFN-α, and cells were acquired by flow cytometry. Our data demonstrated that DNMT1 and TET2 proteins were expressed in pDC freshly obtained from peripheral blood. Moreover, upon 6h incubation at 37ºC, TET2 was upregulated in both HSV- and IAV-stimulated pDC, both in pDC that produced IFN-a and the fraction of cells that did not. In contrast, levels of DNMT1 in pDC were not altered upon stimulation with either virus. Even though DNMT1 proteins were not elevated in virus-stimulated pDC, using Amnis ImageStream imaging flow cytometry, both TET2 and DNMT1 were found to be translocated into the nucleus at 6h in virus-stimulated pDC compared to mock-stimulated cells. To investigate the role of TLR9 signaling in TET2 upregulation in HSV-stimulated pDC, we pretreated the PBMC with or without a TLR9 inhibitory ODN and found that blocking of TLR-9 signaling inhibited both IFN-a production as well as HSV-induced TET2 upregulation. To determine whether IFN-a produced by pDC might be involved in upregulation of TET2, we treated virus-stimulated pDC with exogenous IFN-α for 6h and observed an upregulation of TET2 protein expression, which may indicate an involvement of autocrine or paracrine signaling by virus-induced IFN-a in TET2 upregulation. pDC upregulate co-stimulatory markers at 24 hrs, a timepoint when IFN-a production is already finished. To determine the effect of TET2 on pDC maturation, pDC were pre-treated with the TET2 inhibitor, BobCat339. We observed an inhibition of the expression of co-stimulatory markers CD40, CD80 and CD86 on virus-stimulated pDC in the BobCat339-treated samples; however, BobCat339 did not inhibit virus-stimulated IFN-α production in pDC. Overall, these results suggest that TET2 plays an essential role in the maturation of virus stimulated pDC, but not in induction of IFN-a production.

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P8

CGAS AND STING CONTRIBUTE TO THE INDUCTION OF INTERFERON-B RESPONSES IN ASTROCYTES EXPOSED TO RNA VIRUSES

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Abstract Content: Several RNA viruses can infect the central nervous system (CNS), which is typically associated with local inflammatory responses. During viral encephalitis, tissue-resident cells sense the virus through different pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), or the cGAS/STING axis, and mount innate immune responses. While it is well established that the DNA sensing platform cGAS/STING is necessary for sensing of DNA-encoded viruses, increasing evidence points towards a crucial role of cGAS/STING also during RNA virus infection. In order to address the function of cGAS and STING in CNS-resident cells, we generated primary astrocytes from newborn wild type (WT), as well as cGAS-deficient and STING-deficient mice. The resulting astrocytes had a purity of approximately 97%. Upon *in vitro* exposure of the astrocytes to DNA-

encoded herpes simplex virus type 1 (HSV-1) or RNA-encoded vesicular stomatitis virus (VSV) and tick-borne encephalitis virus (TBEV) the induction of interferon (IFN)-β responses was monitored in cell-free culture supernatants. WT astrocytes mounted basic, moderate and abundant IFN-β responses to HSV-1, VSV and TBEV, respectively. As expected, in STING deficient astrocytes HSV-1 induced IFN-β responses were completely abolished. Interestingly, in astrocytes lacking cGAS or STING VSV and TBEV exposure resulted in mildly and heavily diminished IFN-b responses, respectively, when compared with WT astrocytes. We detected increased STING expression in WT astrocytes upon HSV-1 and VSV infection, while TBEV infection did not induce such an effect. Furthermore, STING oligomerization was detected during HSV-1 and TBEV infection of WT astrocytes. Current investigations aim at identifying the source of cGAS or STING activation in the context of stimulation with RNA-encoded viruses, with one of the possible options being the release of mitochondrial DNA during the course of infection. Collectively, our data point towards an involvement of the cGAS/STING axis in astrocytes during RNA-encoded virus infection for the induction of innate immune responses.

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P9

EARLY IL-1 BLOCKING: GAMECHANGER IN COVID-19?

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Abstract Content: The attenuation of the hyperinflammated state has been thought as a measure to contain the pathophysiologies of severe COVID-19. Patients with autoinflammatory disorders like- adult onset still's disease, familial mediterranean fever and hyperinflammatory pathologies like- macrophage activation syndrome, multisystem inflammatory syndrome in children (MIS-C) and CAR-T mediated cytokine release syndrome have overlapping clinical manifestations with severe COVID-19 patients (1). As a result, cytokine inhibition approaches are being pursued for managing COVID-19. Initially, the concept of involvement of IL-1 in the pathophysiology of COVID-19 was sceptical and underappreciated, as IL-1 was not found to be highly activated in patients. But now, compelling evidences from different laboratories have shown IL-1 activation thru NLRP3/AIM2 inflammasome involvement in the pathogenesis of the disease with anakinra (blocker of IL-1α/ IL-1β) having a benefiting effect (1).
Concurrently, the SAVE-MORE trial guided by soluble urokinase plasminogen activator receptor (suPAR levels ≥ 6ng/ml), showed anakinra to have mortality benefits. suPAR, a biomarker indicative of neutrophil migration promoting calprotectin (S100A8/A9) and IL-1α, was activated earlier than CRP and IL-6 (1). With COVID-19 being characterised by hypercoagulability, IL-1α can play a cardinal role in coagulation phenomena too- by itself getting thrombin activated and inducing platelet productions (1). Interestingly, CAR-T therapy are associated with aberrant cytokine release along with neurological defects with IL-1 and IL-6 productions. Intriguingly, IL-6 blocker (tocilizumab) failed to protect mice from neurological damages, while IL-1 blocker (anakinra) protected mice both from cytokine release and neurotoxicity (1). With acute and long COVID-19 patients, known to manifest neurological symptoms and NLRP3 known to be implicated in neurological diseases like Alzheimer's - blocking IL-1 can be more efficacious than IL-6 in managing COVID-19(1). Increased incidents of type 2 diabetes in long COVID patients (2) can perhaps be explained by persistant IL-1β production from NLRP3 activation ensuing in pancreatic β cell dysfunctioning (3).Presence of autoantibodies to IL-1Ra (hyperphosphorylated form) in MIS-C (4) and in adults can neutralize the antagonist effect, leading to amplification of IL-1 signalling- further implicates IL-1 in disease pathogenesis.
The recent surge of fresh infections worldwide can be due to combinatorial environmental factors like- onset of spring season (pollen dissemination), air pollutants and low humidity. Pollens are known to induce NLRP3 activation producing IL-1, and down regulating antiviral IFN-λ response (5). Deforestations and increased industrialisation has increased fine particulate matters ($PM_{2.5}$) in air, leading to enhanced NLRP3 activation and lung inflammations (6)- which can might explain the recent surge in Delhi, Gurgaon, Beijing and Shanghai. Similarly, low relative humidity block antiviral ISGs activation, mucociliary viral clearance and predispose to inflammasome activation (7)- which can further explain the recent surge in these geographical areas. Hence, early IL-1 blocking, could be helpful in managing COVID.

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P10

IL-35 SUBUNITS ARE ANTI-INFLAMMATORY CYTOKINES

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Abstract Content: The IL-12 family consists of four strictly heterodimeric members which together contribute to a balanced immune response. IL-35 is the least understood member of the IL-12 family – yet plays an important role in limiting autoimmunity as well as anti-tumor responses. While sharing key features of the IL-12 family, IL-35 also differs in many ways: it is the only strictly inhibitory IL-12 cytokine, it is predominantly produced by a different set of immune cells, and signaling occurs via a variety of different receptor combinations. Although the biological functions of IL-35 qualify it as a highly attractive therapeutic target, our biochemical lack of knowledge on IL-35 currently limits further progress in the field.
In this study, we investigated the mode of secretion for IL-35 and found that its two subunits IL-12α/p35 and EBI3 can either be secreted in assembled or unassembled forms. Instead of a strictly heterodimeric protein – the hallmark of IL-12 family cytokines – IL-35 thus appears to be a compound cytokine. We thus established procedures to recombinantly produce the human IL-12α/p35 and EBI3 subunits from mammalian cells and investigated their effects in human PBMCs and an HDM induced allergy model of human monocyte derived macrophages. Stimulation with our recombinant proteins resulted in the downregulation of the pro-inflammatory cytokines IL-8, IL-1β, IL-6, and TNF-α. Surprisingly, while our recombinant subunits were able to reconstitute functional IL-12 and IL-27, they showed no sign of interaction to form IL-35, therefore further manifesting their independent character and the exceptional position of IL-35 in the IL-12 family. In summary, the results obtained in this study point towards a new mode of IL-35 secretion, which allows not only the heterodimeric IL-35 but also its subunits IL-12α/p35 and EBI3 to act as independent cytokines.

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P11

CCL3-CCR5 AXIS IMPROVE INNATE IMMUNE RESPONSES DURING SEPTIC PERITONITIS

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Abstract Content: Cecal ligation and puncture (CLP) caused septic peritonitis in wild-type (WT) mice, with approximately 30% mortality within 7 days after the procedure. Concomitantly, the protein level of intraperitoneal CCL3 was increased, with infiltration by CCR5-expressing macrophages into the peritoneum. CLP induced 70% mortality in CCL3-/- and CCR5-/- mice, which, however, exhibited a similar degree of intraperitoneal leukocyte infiltration as WT mice. Despite this, CCL3-/- and CCR5-/- mice exhibited impairment in intraperitoneal bacterial clearance, together with a reduction in the expression of intraperitoneal inducible NO synthase (iNOS) compared with WT mice. Thus, CCL3-CCR5 axis is crucial for optimal host defense against bacterial infection by activating bacterial killing functions of phagocytes, and by augmenting iNOS-mediated NO generation, with few effects on macrophage infiltration.

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P12

RECOGNITION OF CELL WALL TEICHOIC ACIDS DISPLAYED ON INTACT CELL WALLS OF LACTIPLANTIBACILLUS PLANTARUM BY MURINE MACROPHAGES LEADS TO IL-12 SECRETION

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Abstract Content: Selected lactic acid bacteria can stimulate macrophages and dendritic cells to secrete IL-12, which plays a key role in activating innate and cellular immunity. In this study, we investigated the roles of cell wall teichoic acids (WTAs) displayed on whole intact cell walls (ICWs) of *Lactiplantibacillus plantarum* in activation of mouse macrophages. ICWs were prepared from several lactobacilli using isolation from whole bacterial cells without physical disruption, and thus retaining the overall shapes of the bacteria. WTA-displaying ICWs of several *L. plantarum* strains, but not WTA-lacking ICWs of strains of other lactobacilli, elicited IL-12 secretion from mouse bone marrow-derived macrophages and mouse macrophage-like J774.1 cells. The ability of the ICWs to induce IL-12 secretion was abolished by selective chemical elimination of WTAs from ICWs, but was preserved by selective removal of cell wall glycopolymers other than WTAs. WTA-displaying ICWs, but not WTA-lacking ICWs, were ingested in the cells within 30 min. Treatment with cytochalasin D, an inhibitor of actin polymerization, abolished IL-12 secretion in response to ICW stimulation and diminished ingestion of ICWs. When overall shapes of ICWs were physically disrupted, the disrupted ICWs (DCWs) failed to induce IL-12 secretion. However, DCWs and soluble WTAs inhibited ICW-stimulated IL-12 secretion from macrophages. Taken together, these results show that WTAs displayed on ICWs with preserved bacterial shapes are key molecules in elicitation of actin remodeling and subsequent IL-12 secretion from macrophages after stimulation with ICWs of *L. plantarum*.

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P13

REGULATION OF MDA5 SIGNALING AND INTERFERON INDUCTION BY THE E3 UBIQUITIN LIGASE, HOIL1

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Abstract Content: Interferons (IFN) are critical mediators of the immune response to viral infections. Replicating viruses are detected within cells by RNA sensors, RIG-I and MDA5. RIG-I detects viruses such as Sendai virus (SeV) and vesicular stomatitis virus (VSV), whereas MDA5 detects viruses such as Theiler's murine encephalomyelitis virus (TMEV) and murine norovirus (MNoV). We previously demonstrated that HOIL1 was required for IFN induction and IRF3 phosphorylation during infection of cultured primary cells with viruses sensed by MDA5, but not during infection with viruses sensed by RIG-I. Furthermore, mice deficient in HOIL1 exhibited defective control of MNoV persistent enteric infection. HOIL1 is a component of the Linear Ubiquitin Chain Assembly Complex (LUBAC) that regulates multiple immune signaling pathways including NF-κB activation and cell death pathways. The other LUBAC components are HOIP and SHARPIN, and HOIP is the catalytic subunit that generates linear (methionine-1-linked) polyubiquitin chains. HOIL1, however, is also a functional E3 ligase that can ubiquitinate substrates using an unusual oxy-ester bond, but only a few HOIL1 substrates have been identified to date. Our new data revealed a novel requirement for HOIL1 E3 ligase activity in MDA5 signaling and IFN induction during RNA virus infection, through complementation of HOIL1-deficient mouse embryonic fibroblasts with catalytically inactive HOIL1. Signaling protein overexpression studies and oligomerization assays indicated that HOIL1 functions upstream of MAVS to regulate MDA5 signaling. Studies are ongoing to identify proteins that interact with and are ubiquitinated by HOIL1 to regulate MDA5 signaling.
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P14

TRANSCRIPTOMIC PROFILING OF MICROGLIA REVEALS ARTEFACTUAL SIGNATURES ASSOCIATED WITH CELL SORTING

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Abstract Content: Microglia are resident immune myeloid cells of the central nervous system (CNS) that respond to homeostatic changes. Due to the numerous roles of microglia in health and disease, it is critical to illuminate their transcriptomic signatures as a basis to describe their potential functional traits. Global gene expression studies on murine microglia rely on microglia cultured from neonatal mice (*in vitro*) or microglia sorted based on the transgenic expression of a reporter protein in Cx3cr1-Cre^{ERT2}TdTomato$^{wt/ST}$ adult mice (sorting). Another strategy is adoption of RiboTag, in which HA-tagged ribosomal protein 22 (Rpl22) is expressed in a Cre-mediated manner (Cx3cr1-Cre^{ERT2}Rpl22$^{HA/-}$) allowing isolation of ribosome-bound mRNA (RiboTag). Correspondingly, it is critical to determine whether different experimental strategies affect the read out of microglia transcriptomic profiles. We thus sought to perform side-to-side comparison of the transcriptomes of *in vitro* microglia, sorted microglia and microglial translatome isolated by the RiboTag method. To achieve this, *in vitro* cultured microglia and transgenic mice expressing the reporter protein or ribosomal HA-tags were challenged with either vesicular stomatitis virus (VSV) or PBS and RNA sequencing was performed. Within the differentially expressed genes of the control groups, we identified three clusters of microglia. Cluster I comprised genes such as *Cx3cr1, Hexb, P2ry12* and *Aif1* that were enriched under all three conditions. Interestingly, cluster II comprised genes exclusively enriched in sorted microglia, and which were less abundantly present in RiboTag and *in vitro* microglia. This included genes related to immediate early genes, chemokines, and cytokines and part of NF-kB signalling cascade. These gene signatures reflect a cell activation status. Reanalysis of published single-cell RNA-sequencing (scRNA-seq) data of pre-sorted CNS immune cells revealed that this activation signature was enriched in microglia populations when compared with other CNS cell subsets. Arguably, method-related artefacts should be neutralized if controls and test samples were prepared by the same approach. To evaluate this, we performed differential expression analysis between PBS and VSV treatment in *in vitro* microglia, RiboTag and sorted microglia. We identified the genes *Ccl4, Il1b, Ccl3, Tnf* and *Socs3* to be highly upregulated in sorted microglia and to be upregulated to a lesser extent in RiboTag and *in vitro* microglia, implying that identical processing does not necessarily normalize experimental artefacts. To circumvent these artefacts, we evaluated the usage of transcriptional inhibitors during the microglia isolation procedures. Reanalysis of scRNA-seq datasets that used a standard enzymatic protocol with or without transcriptional inhibitor revealed that the genes *Ccl4, Ccl3, Tgfb1* and *Nfkbiz* were significantly downregulated in cells treated with transcriptional inhibitor. Collectively, our data highlights that microglia acquire transcriptional alterations during cell sorting, while the RiboTag approach is less prone to such artefacts and more faithfully recapitulates *in vivo* transcriptional signatures of microglia. Where cell sorting of microglia is experimentally required, we propose the inclusion of transcriptional inhibitors during the tissue dissociation process.

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P15

DEVELOPMENT OF BEAD-BASED MULTIPLEX ASSAY PANELS FOR SIMULTANEOUS QUANTIFICATION OF PROINFLAMMATORY CHEMOKINES IN HUMAN AND MOUSE SAMPLES

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Abstract Content: Chemokines play pivotal roles in various processes such as immune surveillance, organ development, angiogenesis, and immune responses. Expression profiling of chemokines, especially those involved

in inflammation and immune disorders, is important for understanding various disease processes. BioLegend has developed two Multiplex Immunoassay Panels using fluorescence-encoded beads that can simultaneously detect 12 human and 8 mouse chemokines, respectively, in just 25ul of sample. Both human and mouse Panels include Eotaxin-2 (CCL24), MCP-3 (CCL-7), MCP-2 (CCL8), Fractalkine (CX3CL1), and MIP-2 (CXCL2). In addition, the human Panel contains reagents for measuring concentrations of I-309 (CCL1), MCP-4 (CCL13), MDC (CCL22), ELC (CCL19), BLC (CXCL13), SDF-1 (CXCL12), and PARC (CCL18), while the mouse Panel can quantify ITAC (CXCL11), Lymphotactin (XCL1), and MIP-1γ (CCL9) chemokines. Each antibody pair used in these Panels was thoroughly screened and finally selected using strict sensitivity, specificity, accuracy and reproducibility parameters. These LEGENDplex™ Multiplex Panels have higher sensitivity and wider dynamic range compared to traditional sandwich ELISA method. Both LEGENDplex™ Panels have been validated on relevant biological samples: mouse splenocytes, fibroblast, and Raw 264.7 cells and human PBMC under various stimulation conditions. The results obtained using these LEGENDplex™ Panels confirmed the expected stimulation-induced changes in concentrations. The LEGENDplex™ Chemokine Panels provide a convenient and versatile research tool to characterize multiple human and mouse sample types for biomedical research and drug discovery.

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P16

PIVOTAL ROLE OF THE CCL5/CCR5 AXIS IN ACCELERATED THROMBOLYSIS IN MURINE DVT MODEL

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Abstract Content: Deep vein thrombosis (DVT) formation has been thought to be caused by blood stagnancy, endothelial injury of the vein, and hypercoagulability. However, recent studies suggest that DVT is also closely related to inflammatory process. To better understand the pathophysiology of venous thrombosis, further studies of the relationship between inflammation and coagulation are needed. Venous thrombus resolves by a process of organization and recanalization that is similar to the formation of granulation tissue in wound healing. Here, we found that CCR5-deficience impaired thrombus resolution. We further examined the pathophysiological role of CCL5-CCR5 axis in the resolution of DVT on *Ccr5* knockout (KO) mice using inferior vena cava (IVC) ligated stasis-induced DVT model using C57BL/6 (WT) and KO mice. The thrombus masses were larger in KO mice than in WT ones. We examined the effects of CCL5-CCR5 axis on the gene expression of *Vegf*, *Plat* and *Plau* in peritoneal macrophages. CCL5 significantly enhanced these genes expressions and phosphorylation of ERK in the WT-derived macrophages. These observations implied that the ERK signal pathway regulated *Vegf*, *Plat* and *Plau* gene expression in macrophages. Indeed, the effects of CCL5 on *Vegf*, *Plat* and *Plau* gene expression in macrophages were abrogated by ERK inhibition. CCL5 could enhance the gene expression of *Vegf*, *Plat* and *Plau* in macrophages through activation of ERK signaling in a CCR5-dependent manner. These observations suggest that CCL5-CCR5 axis may contribute to resolution of venous thrombus.

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P18

STUDY OF CYTOKINE PRODUCTION BY ERYTHROBLASTS INDUCED FROM BONE MARROW CD34 PRECURSORS

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Abstract Content: Introduction. Erythroid nucleated cells (erythroblasts) have pronounced immunoregulatory properties in normal and pathological conditions. Erythroblasts provide feto-maternal tolerance, form the susceptibility

of newborns to infection, promote infiltration and tumor progression, reduce the production of pro-inflammatory cytokines by myeloid cells, the activation and secretion of cytokines by lymphocytes, promote the production of anti-inflammatory cytokines and differentiation of T cells into immunosuppressive T-regulatory and Th2 cells . In our work, we characterized the immunoregulatory potential of erythroblasts derived from bone marrow CD34+ cells, which can be considered as candidate cells for nonspecific cellular immunosuppressive therapy.
Materials and methods. The bone marrow of 6 adult donors was obtained using a trephine biopsy from the ilium. CD 34+ cells have been used to generate induced erythroid cells by SCF, TPO, Flt3 ligand, IL-3, IL-6, erythropoietin. CD 34-negative cells were used to isolate erythroblasts by positive magnetic selection for the CD 71 marker. The resulting cells were cultured for 72 hours, after which a conditioned medium was collected for cytokine analysis using the Bio-Plex Pro Human Cytokine 48-Plex Screening Panel. Cytokine production was analyzed using the CytokineExplore online tool (http://exabx.com/apps/cytokineexplore/).
Results. PLSDA (Partial Least Squares Discriminant Analysis) was used to analyze cytokine production and plotted the importance of variables/cytokines when selecting two groups, which quantifies a measure of the relative contribution or importance of each cytokine in distinguishing/separating two contrasting groups. All cytokines were divided into functional groups according to the generally accepted classification. It was shown that according to the production of chemokines, interleukins, growth factors, natural and induced erythroblasts are non-overlapping groups on the PLSDA plot. The most important cytokines contributing between groups are RANTES, MCP-1, IP-10 for chemokines, IL-18, IL-3? IL-12p70, IL-1 beta for cytokines, PDGF,SCF, SDF-1alpha for growth factors. The production of colony stimulating factors, interferons and TNF family cytokines did not differ between the two groups.
Discussion.
The overall spectrum of cytokines is the same in both types of erythroblasts, but there are differences in the amount of cytokines produced. Most of the cytokines in the conditioned media of erythroblasts are mediators that provide chemotaxis, migration, T-cell activation, and suppression of inflammatory responses. Induced erythroblasts produce lower amounts of these cytokines, which may be due to the lack of appropriate hematopoietic niches and microenvironment. Based on these data, it can be assumed that erythroid cells, with the help of soluble factors, are capable of autocrine regulation not only of erythropoiesis, but also of the activity of other hematopoietic cells. Cytokines contribute to the creation of a microenvironment capable of self-sustaining and attracting various cell populations.
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P19

ELEVATED LEVELS OF CXCL16 IN SEVERE COVID-19 PATIENTS: EFFECTS ON MORTALITY

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Abstract Content: Background and Aims
Genome-wide association studies have recently identified 3p21.31, with lead variant pointing to the CXCR6 gene, as the strongest thus far reported susceptibility locus for severe COVID-19. CXCL16 is synthesized as a transmembrane molecule that is expressed as a cell surface-bound molecule, and as a soluble chemokine. The CXCR6/CXCL16 axis mediates homing of T cells to the lungs in disease and hyper-expression is associated with localised cellular injury. The aim was to characterize the CXCR6/CXCL16 axis in the pathogenesis of severe COVID-19.
Methods
Plasma concentrations of CXCL16 collected at baseline from 115 hospitalized COVID-19 patients participating in ODYSSEY COVID-19 clinical trial (and controls) were assessed. Another cohort of samples (n 79) was used to see if the effect replicates. CXCL16 levels in plasma were determined with ELISA assay. Furthermore, whole-genome sequencing was conducted on all samples.
Results

We report elevated levels of CXCL16 in a cohort of COVID-19 hospitalized patients. We previously reported elevated levels of CXCL16 in a cohort of COVID-19 severe hospitalized patients (P-value<0.02). Importantly we report a significant effect of elevated CXCL16 on mortality (P-value<0.03) effect that is now replicated (mortality (P-value<0.0004)). Clinically, at 700pg/mL, the OR is 20.6, (p-value 0.04) essentially suggesting one has a ~25% mortality when CXCL16 levels are above ~700pg/ml. We also further characterize the role of the CXCR6 expression on CD8 T cells.

Conclusions

These latest findings further support the significant role of the CXCR6/CXCL16 axis in the immunopathogenesis of severe COVID-19 and warrant further studies to understand which patients would benefit most from targeted treatments.

Disclosure of Interest: S. Smieszek Employee of: Vanda Pharmaceuticals Inc.

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P20

INFLAMMATORY CYTOKINES AND PROCALCITONIN IN NEWBORNS FOLLOWING SPONTANEOUS DELIVERY AND ELECTIVE CESAREAN SECTION

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Abstract Content: Inflammation is considered to be a fundamental process accompanying physiological human birth and also plays role in perinatal pathologies. The goal of the study was to assess the concentrations of inflammatory cytokines and procalcitonin with respect to the mode of delivery and dynamics of inflammatory molecules in neonatal samples in first 48-72 hours of life.

The concentration of inflammatory molecules were measured using the Luminex ®xMAP multi-analyte profiling platform in cord blood and peripheral neonatal blood. Study groups included newborns delivered spontaneously (Spontaneous group) and via elective caesarian section (Elective group).

Cord blood concentrations of IL-6 and procalcitonin were significantly higher (p<0.0001) in Spontaneous group compared to Elective group. Neonatal blood concentration of TNF from Elective group was significantly higher compared to Spontaneous group (p=0.0077). The concentrations of procalcitonin and TNF significantly increased within the first 48 to 72 hours following either mode of delivery. IL-6, and IL-18 were significantly higher in neonatal compared to umbilical cord blood only in Elective group while the increase in Spontaneous group did not reach the statistical significance. The concentration of IL-1alpha, IL-1beta, IL-17A and IL-22 did not show any significant differences between the Spontaneous and Elective groups, including the differences between umbilical cord and neonatal blood.

Our findings show physiological changes of inflammatory cytokines following spontaneous and elective cesarean delivery. Mode of delivery should be taken into account when evaluating potential markers of neonatal sepsis and other inflammation-related pathologies.

Disclosure of Interest: None Declared

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LB-01

PROTEOMIC PROFILING OF EPIGENTIC MECHANISMS MEDIATING TRAINED IMMUNITY IN MACROPHAGES

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Abstract Content: Introduction: Tuberculosis (TB) is the leading cause of death in South Africa, and the bacilli Calmette-Guerin (BCG) vaccine is currently administered at birth in endemic areas to prevent TB transmission [1].

The BCG vaccine has been shown to be particularly immunogenic [2-3], and causes trained immunity, where after vaccination innate immune cells mount heightened immune responses to secondary unrelated pathogens [4]. Studies show that monocytes display epigenetic memory of BCG vaccination at the histone level for up to three months, characterized by increased TNF-alpha and IL-6 production upon secondary stimulation with other pathogens [5]. BCG-mediated trained immunity is incompletely understood. Mass spectrometry- based proteomics and bioinformatic analysis allow for global profiling of cytokines and histones mediating inflammatory cytokine expression which can provide insightinto mechanisms of sustained BCG mediated immunogenic signaling.
Methods: THP-1 macrophages were infected with BCG for 24 hours, and protein extracted for total proteomic analysis. Histones were isolated to assess global changes in posttranslational modifications, and total protein and histone isolates were assayed using tandem mass spectrometry (Q Exactive). Bioinformatic analysis allowed for discovery of differentially regulated cytokines and histone post translational modifications in BCG infected and uninfected macrophages. These data were cross-referenced with phospho-data previously obtained from BCG infected RAW macrophage to assess activation of histone modifiers.
Results: Bioinformatic analysis revealed decreased phosphorylation of four histone acetyltransferase (KAT) peptides, two demethylation (JmjC) peptides, a lysine demethylase peptide, and a lysine methyltransferase peptide, identifying mechanisms for robust upregulation in histone methylation after BCG infection. This data provides novel mechanisms and supports previously identified changes in methylation of histones at the promoters of TNF-alpha and IFN-gamma following vaccination [5]. Several cytokines were differentially regulated between infected and non-infected macrophages. Pathway analysis identified histone modifications with the ability to promote expression of cytokines identified.
Conclusion: Proteomic analysis allows for robust global profiling of cytokines and reveals a complex interplay of cytokine expression upon initial BCG infection. Our data implies that dynamic histone modification occurs upon BCG infection in macrophages and provides insight into epigenetic mechanisms that could mediate sustained immunomodulation, or trained immunity, in BCG vaccinated people.

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LB-02

IMMUNE PROFILE OF ACTIVATED NATURAL KILLER CELLS

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Abstract Content: Natural killer cells (NK cells) are innate lymphoid cells that function as both cytotoxic effectors and regulators of immune responses. NK cells are activated following the detection of abnormalities in target cells such as the loss of MHC class I expression, up-regulation of stress-induced ligands that occurs in response to infection or cancer, and activation of antibody-dependent cell-mediated cytotoxicity (ADCC) through IgG Fc receptor ligation. We analyzed the activity of ADCC of NK Cells on human epidermal growth factor receptor 2 (HER2) positive SKOV-3 cells with an anti-HER2 antibody, trastuzumab. Using the R&D Systems® FlowX Human NK Cell Killing Flow Cytometry Kit and the R&D Systems® Human XL Cytokine Luminex® Performance Assay 44-plex, NK Cells isolated and expanded from three separate donors were analyzed for Cytotoxicity, Granzyme B, Cytokine and Chemokine production. When cocultured with the target cell, the NK Cells produced a statistically significant quantity more

Granzyme B, Interferon-gamma (IFNγ), Granulocyte-macrophage colony-stimulating factor (GM-CSF), and several pro-inflammatory chemokines. This increase in pro-inflammatory markers correlated to increased expression of hLAMP-1/CD107a on the NK Cell surface as sign of NK Cell activation and with a decrease in target cell viability. Together, this data shows an in vitro method developed by R&D Systems® to study NK Cell activity and cytotoxicity. When run in parallel to Flow Cytometry cell surface analysis, R&D Systems® Luminex® immunoassays provides a clear picture of the NK Cell effector function.

Disclosure of Interest: M. Larson Shareholder of: Bio-Techne, Employee of: Bio-Techne, B. Astry Shareholder of: Bio-Techne, Employee of: Bio-Techne, S. Degese Shareholder of: Bio-Techne, Employee of: Bio-Techne, J. Rivard: None Declared, H. Mishra: None Declared, L. Peng Employee of: Bio-Techne, K. Flynn Shareholder of: Bio-Techne, Employee of: Bio-Techne, C. Goetz Shareholder of: Bio-Techne, Employee of: Bio-Techne, D. Bryant Shareholder of: Bio-Techne, Employee of: Bio-Techne
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LB-03

SINGLE CELL MULTI-OMICS REVEALS ENHANCED PRO-INFLAMMATORY CYTOKINE MEDIATED CELLULAR CROSSTALK IN THE NF1 MUTANT GLIOBLASTOMA TUMOR IMMUNE MICROENVIRONMENT

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Abstract Content: Glioblastoma Multiforme (GBM) is an aggressive primary brain tumor with a dismal 5-year survival rate of 5-6%. NF1 is a common driver mutation of GBM and is mutated in approximately 17% of cases. Large scale transcriptomic studies of patient GBM samples have correlated NF1 loss with increased tumor immune infiltration. The GBM tumor immune microenvironment (TIME) is composed primarily of tumor associated macrophages, and has lower levels of lymphocytes (T-cells, B-cells, NK-cells) and other myeloid populations (dendritic cells, myeloid-derived suppressor cells). Here, we aimed to uncover mechanisms by which NF1 loss promotes inflammation in GBM using genetically engineered mouse models of NF1 mutant and NF1 WT GBM. We characterized these models and found that the greater immune infiltration seen in the NF1 mutant human GBM was also present in the NF1 mutant mouse GBM. Next, we used CITEseq to dissect the activation states and communication networks in the GBM TIME. This revealed that NF1 mutant GBMs have significantly increased ligand-receptor mediated crosstalk between the tumor and TIME cells compared to NF1 WT GBMs. Increased cellular crosstalk in NF1 mutant GBMs occurred primarily through greater cytokine production and receptor expression by both tumor and immune cell populations. We show this increased cytokine secretion at the protein level as well in both mouse and human NF1 mutant GBMs using targeted proteomics assays. Among several pathways explored, enhanced TNFa signaling through NFKB was one mechanism responsible for the increased pro-inflammatory cytokine signaling in NF1 mutant GBMs. Increased pro-inflammatory cytokine signaling impacted the TIME, specifically the activation states of tumor infiltrating T-cell populations. Our work uncovers several cytokine signaling networks that promote inflammation in NF1 mutant GBMs with potential application for future immunotherapeutics.

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LB-04

MITO+ RBCS PHAGOCYTOSIS PROMOTES TYPE I IFN-DEPENDENT IL-1B RELEASE IN SLE MONOCYTES

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Abstract Content: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the breakdown of tolerance to endogenous nucleic acids (NAs) and the upregulation of Type I Interferon (IFN). However, blocking this cytokine pathway benefits only a fraction of patients, pointing to additional pathogenic players. Emerging evidence supports that mitochondrial dysfunction contributes to SLE pathogenesis and Type I IFN production. We have recently shown that antibody-mediated internalization of red blood cells (RBCs) carrying mitochondria (Mito+ RBCs), a hallmark of SLE, induces type I IFN production through activation of cGAS in macrophages. We now describe monocytes (Mo) undergoing erythrophagocytosis and co-expressing IFN-inducible genes (ISGs) and interleukin-1b (IL-1b) in patients with active disease. This phenotype is recapitulated *in vitro* upon internalization of Mito^{+} RBCs. While ISGs expression depends on RBC mitochondrial (mt)DNA activation of cGAS, IL-1b entails RBC mtRNA activation of RIG-I-like receptors (RLRs), which in turn induces Mo-derived mtDNA fragments to bind NLRP3. Importantly, the release of IL-1b requires Type I IFN signaling, which activates an unconventional secretory pathway.

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02. Cytokine signaling induction, attenuation and silencing

P21

TOWARDS BIOPHYSICAL AND STRUCTURAL CHARACTERIZATION OF ANTI-INFLAMMATORY IL-37 COMPLEXES WITH IL-18RA, SIGIRR AND IL-18BP

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Abstract Content: IL-37, a member of the IL-1 cytokine family, has been described as an anti-inflammatory cytokine which acts to suppress innate immunity, however the molecular mechanisms surrounding its activity have remained elusive.
Cellular and transgenic animal studies suggest that IL-37 engages IL-18Rα and SIGIRR to exert its anti-inflammatory functions. SIGIRR contains one immunoglobin-like domain, in this way resembling the soluble decoy receptor IL-18 binding protein (IL-18BP), which is also a proposed interaction partner of IL-37. Interestingly, previous studies have shown that increasing concentrations of IL-37 result in suppression of its anti-inflammatory activity, due to the unique dimerization propensity of IL-37 at higher concentrations. Mutation of Tyr85 (Y85A) at the dimer interface results in a constitutive monomeric state of IL-37 even at millimolar concentrations, promoting the anti-inflammatory potential of this cytokine.
Owing to the rather recent discovery of the interaction partners of IL-37, important challenges remain to characterize the biochemical, biophysical and functional interactions of IL-37 with its binding partners. Here we present an integrative structural biology approach to characterize IL-37 protein complexes with IL-18Rα, SIGIRR and IL-18BP. Currently, we have established an extensive molecular toolbox enabling us to complement the existing cellular assays and in vivo studies with biophysical and structural insights into the organization of the IL-37 receptor complexes. Using biophysical techniques, such as bio-layer interferometry (BLI), we characterize these interactions using purified proteins, in addition to providing valuable information about affinity and kinetics dictating these interactions. In addition, further elucidation of the monomeric activity of IL-37 by employing the Y85A mutant may facilitate therapeutic interrogation. Furthermore, detailed structural snapshots obtained from ongoing crystallization efforts will enable to pinpoint key residues at the interfaces between IL-37 and IL-18Rα, SIGIRR and IL-18BP. Collectively, we foresee that these insights may give rise to interesting opportunities for development of novel therapeutics targeting IL-1 cytokines and receptors.

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P22

RNA METHYLATION AND DEMETHYLATION IN IMMUNE/INFLAMMATORY RESPONSES

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Abstract Content: Whereas regulation at the level of gene transcription is well established, post-transcriptional control of mRNA is also a vital component in immune responses. Several immune mRNAs were recently shown to carry a methylation at the *N6* position of adenosine, m^6A. m^6A is added to specific RNA by methyltransferase enzymes termed 'writers', which can be reversed by demethylases 'erasers'. Diverse 'readers' can bind to m^6A directly or to sequence structures influenced by this modification. While RNA modifications are emerging as a key regulatory step in adaptive immune response, much is yet to be learned, including whether epitranscriptomic marks are implicated in structural cell function. Beyond their well-established role in providing structural support to organs, epithelial and mesenchymal cells are actively involved in immune responses, and tissue-specific immunity is become increasingly appreciated. Indeed, epithelial cells play an active role in Sjogren's syndrome (SjS) pathogenesis. Although this condition is mediated by autoreactive B and T cells, pro-inflammatory triggers maintain a feed-forward process that sustain inflammation, resulting in epithelial cells damage and pathology. To assess whether m^6A machinery components are differentially expressed in SjS patients *vs* controls, we retrieved RNA-seq data from salivary gland epithelial cells (SGEC) that were sorted from minor salivary gland (MSG) biopsies of 5 SjS patients and 4 controls. We noticed a significant increase in *METTL14* expression (component of the m^6A writer complex) among the 495 differentially expressed genes. *METTL14* mRNA total counts positively correlated with the lymphocytic infiltration. We then investigated whether pro-inflammatory triggers similarly increase *METTL14* expression in SGEC cultured *in vitro*. Although SGEC can be activated by different cytokines/immune triggers present in SjS salivary glands (SG), our choice to specifically investigate the effects of Poly(I:C), IFNa and IFNg on SGEC relied on the over-representation of the IFN signaling pathway in the SGEC of SjS patients. Indeed, Poly(I:C), IFNa and IFNg -stimulated SGEC increased the expression of *METTL14* mRNA as seen in SGEC from SjS. Accordingly, such alterations may affect the whole RNA methylome and hence differentially regulate the expression of immune-related genes implicated in disease pathogenesis which is currently under evaluation.In contrast to SGEC, the expression of m^6A machinery components is not modified in renal epithelial cells. Collectively, posttranscriptional regulation in epithelial cells through m^6A marks represents a previously unidentified paradigm of autoimmune inflammation.

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P23

FROM THE CRADLE TO THE GRAVE: MECHANISTIC INSIGHTS INTO CYTOKINE REGULATION BY MRNA DECAY

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Abstract Content: Regulation of mRNA stability by RNA-binding proteins is indispensable for precise control of immune responses. How and when the inflammatory mRNAs are selected for degradation are fundamental questions that remain incompletely understood. We are addressing these questions using inflammation as a model; inflammatory responses are characterized by transient stabilization of cytokine mRNAs followed by their marked destabilization. The time parameters of such stability profile are specific for each mRNA and decisive for efficient yet not destructive immune responses. Essential factor in inflammatory mRNA decay is the RNA-binding protein TTP (tristetraprolin; gene name *Zfp36*). TTP binds to 3' UTRs of target mRNAs and facilitates their degradation by recruiting deadenylation and decapping enzymes. How TTP regulates the gene-specific timing of its target degradation remains elusive. To address this question, we employ unbiased proximity based labeling screens in combination with genetic approaches. Not only is our data in line with previous reports of TTP interaction with ribonucleoprotein granules, known hubs for mRNA storage and mRNA decay, but further uncovers its so far, less known involvement in the nucleus and translation. Our data indicate that TTP determines the fate of its target mRNAs as early as after their

transcription. Moreover, association of TTP with its target mRNA is required for the subsequent interaction with the RNA degradation machinery located in P-bodies.

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P24

SOCS3HIGH IMMATURE NATURAL KILLER CELLS WITH DECREASED GRANZYME AND INCREASED WNT EXPRESSION PROMOTE TUMOR PROGRESSION IN TRIPLE NEGATIVE BREAST CANCER

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Abstract Content: Natural Killer (NK) cells are cytotoxic lymphocytes that accumulate within the TME and are generally considered to be anti-tumorigenic. Surprisingly, single cell RNA sequencing (scRNA-seq) and functional analysis of multiple TNBC tumors revealed a unique subcluster of $CD11b^{-}CD27^{-}$ $Socs3^{high}$ immature NK cells in TNBC subset. These tumor NK cells express a reduced cytotoxic granzyme signature and are responsible for activating Wnt signaling mediated in TNBC cells. In support, we found that depletion of NK cells or Wnt ligand secretion from NK cells by LGK-974 in TNBC models decreased tumor progression. Additionally, combinatorial targeting of NK cell number or function sensitizes anti-PDL1 or chemotherapy response in TNBC. TNBC patient tumors revealed that increased numbers of $CD56^{bright}$ NK cells are significantly correlated to TNBC patients with poor Overall Survival (OS), suggesting their abundance could serve as a potential biomarker to stratify aggressive TNBC subsets for targeted treatment. Together, our findings unravel the identity and paradoxical function of pro-tumorigenic NK cell subsets that may be exploited in the future development of diagnostic and therapeutic strategies to improve outcome for aggressive TNBC patients.

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P25

COMPUTATIONAL MODELING OF STAT ACTIVATION FEATURES THAT PREDICT GENE EXPRESSION PROFILES

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Abstract Content: Integration of complex cytokine cues is complicated by the overlapping usage of signal transducers and activators of transcription (STATs) by diverse cytokines. In macrophages, IL-6 and IL-10 activate common STATs (STAT1 and STAT3) but typically induce pro- versus anti-inflammatory responses, respectively. Regulators mediating cytokine-specific STAT dynamics have been described, such as the negative regulator SOCS3, which inhibits IL-6, but not IL-10 signaling, contributing to distinct phospho-STAT3 duration in response to these cytokines. However, we lack a systematic understanding of the mechanisms that shape dynamic features of STAT activation and the degree to which these features encode STAT functional specificity. This lack of fundamental understanding impedes our ability to effectively target cytokine-specific STAT-dependent inflammatory responses. We have developed a computational workflow to predict cytokine-induced gene expression from STAT phosphorylation dynamics, that allows us to identify the mechanistic underpinnings of specific functional responses. Our work utilizes a mechanistic model of IL-6 and IL-10-induced signaling to simulate STAT3 and STAT1 activation behaviors that recapitulate diverse macrophage responses to combinatorial cytokine stimuli. Secondly, we use machine learning to predict cytokine-induced global gene expression patterns from our model-simulated STAT trajectories. The integration of our mechanistic and machine learning models allows us to determine gene sets that can be predicted by specific STAT activation features (e.g., early peak or response duration), and to identify proteins in our signaling network that control

these predictive features. This novel pipeline will enhance our knowledge of cytokine-specific gene expression while contributing to efforts to therapeutically target select sets of pathology-associated STAT target genes.

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P26

IDENTIFICATION OF A NOVEL INTERLEUKIN-9 RECEPTOR COMPLEX IN NON-HEMATOPOIETIC CELLS

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Abstract Content:
Beyond cell-cell contact, communication within a microenvironment or at a distance requires the secretion of cytokines that bind to specific receptors on responsive cells. Interleukin (IL)-9 is a cytokine with pleiotropic effects not only on immune cells that contribute to the progression of tumor and autoimmunity, but also on organ structural cells including airway and intestinal epithelial cells involved in the development of inflammatory and allergic diseases. Typically, IL-9 receptor (IL-9R) functions as a complex of two subunits – the α-chain (IL-9Rα) and a common γ (γc) chain, that is shared with other cytokine receptors. Although structural cells express IL-9Rα, they lack expression of the γc chain. It is still not clear how IL-9 transmits a signal through a single IL-9Rα chain in non-hematopoietic cells. This study aimed to explore the possibility that like the type II IL-4Rα, the IL-9Rα chain can pair with the IL-13Rα1 chain, forming a type II IL-9R and can mediate IL-9 responses in epithelial cells. We demonstrated that the IL-9Rα /IL-13Rα1 complex was detected *in situ* (at distances < 40 nm) in mouse tracheal epithelial cells (mTECs) from naïve mice using proximity ligation assay. Genes including *Muc5ac, Scgb1a1*, *Spdef* and *Bpifb1* are upregulated in mTECs upon IL-9 stimulation both *in vitro* and *in vivo*. *Muc5ac* is also upregulated after exposure to IL-9 *in vitro* in mTECs from mice which lack the γc chain, providing further evidence of a type II IL-9R on epithelial cells. Collectively, our results identify a putative receptor complex - a type II IL-9R composed of IL-9Rα and IL-13Rα1, providing a mechanism for responses of organ structural cells to IL-9.

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P27

THE AIM2 DNA SENSOR PROMOTES GASTRIC INFLAMMATION AND TUMORIGENESIS THROUGH INFLAMMASOME-DEPENDENT AND -INDEPENDENT MECHANISMS

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Abstract Content: Chronic gastric inflammation can be triggered by pathogens such as *Helicobacter pylori,* perpetuated by overactivation of the immune system and lead to a cascade of precancerous lesions resulting in gastric cancer (GC). Importantly, the key regulators of the immune system that promote gastritis and GC are not well understood. The pattern recognition receptor absent in melanoma 2 (AIM2) is a cytosolic DNA sensor which contributes to the pathogenesis of numerous autoimmune and chronic inflammatory diseases as well as cancers. There is emerging evidence that the roles of AIM2 are context-dependant, but remain largely ill-defined. Canonically, AIM2 has been reported to function in the inflammasome complex, however there are increasing reports of inflammasome-independent roles of AIM2. Considering the significance of AIM2 in innate immune responses, we investigated whether it contributed to the pathogenesis gastric inflammation and cancer.
We found *AIM2* expression has a strong negative correlation with GC patient survival and is significantly upregulated in patient tumors. Using the well-established *gp130*$^{F/F}$ mouse model of GC, we observed high levels of *AIM2* in tumor-bearing mice compared to WT counterparts. *Gp130*$^{F/F}$ mice lacking *Aim2* had significantly decreased tumor burden,

however, inflammation or inflammasome activation was unaffected, suggesting the existence of a novel inflammasome-independent pro-tumorigenic mechanism for AIM2 in GC. Specifically, we identified a decrease in the migration of AIM2-deficient primary mouse gastric epithelial cells and AIM2 knockdown patient organoids. Critically, interaction with a microtubule regulatory protein, end binding protein 1 (EB1) was found to be important for mediating the migratory function of AIM2, with its expression also significantly higher in GC tumors. These data support changes in epithelial cell migration and motility as the underlying mechanism of the pro-tumorigenic role of AIM2 in GC.
Our investigation at earlier stages of pre-cancerous gastritis revealed a different role for AIM2. AIM2 is elevated in *H. pylori*-positive patient biopsies compared with negative samples, and in mice gavaged with *H. felis* compared to control broth. Inflammation was less severe in *H. felis* treated *Aim2*$^{-/-}$ mice compared to WTs, showing lower immune cell infiltrates and decreased mucosal thickness. Additionally, *H. felis*-driven proliferation and apoptosis in both epithelial and immune cells was attenuated in *Aim2*$^{-/-}$ stomachs. This data suggests a driving role for AIM2 in inflammation triggered by *H. pylori* infection which, unlike in later in disease, occurs via the inflammasome activation. This is evidenced by higher levels of active Caspase I in *H. pylori*-positive patient samples and decreased levels of inflammasome mediated pro-inflammatory cytokines, IL-1B and IL-18 in *Aim2*$^{-/-}$ mice.
Taken together, this work uncovers novel, inflammasome-dependant and independent pathogenic mechanisms for AIM2 in gastric disease and cancer which may pave the way for novel and improved strategies for GC patient outcomes through the use of AIM2 as a biomarker to identify patients which may benefit from the development of therapeutic targeting of AIM2.

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P28

SYSTEMIC IL-15, IFN-GAMMA, AND IP-10/CXCL10 SIGNATURE ASSOCIATED WITH EFFECTIVE IMMUNE RESPONSE TO SARS COV-2 IN BNT162B2 MRNA VACCINE RECIPIENTS

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Abstract Content: BACKGROUND: Early responses to vaccination are important for shaping both humoral and cellular protective immunity. Dissecting innate vaccine signatures may predict immunogenicity to help optimize the efficacy of mRNA and other vaccine strategies. We characterize the cytokine and chemokine responses to the BNT162b2 mRNA (Pfizer/BioNtech) vaccine in antigen-naïve, in previously COVID-19)-infected individuals and in immunocompromised individuals including patients with hematological malignancies, a population at high risk of developing severe disease upon SARS-CoV-2 infection.
METHODS: The different cohorts were studied following BNT162b2 mRNA vaccination. Anti-Spike antibody was measured by ELISA. Effectiveness of Ab response was measured by neutralization assays to a panel of variants. Systemic innate responses were measured at the day of each vaccination and 1 day later (NCT04743388) using the Mesoscale Discovery platform.
RESULTS: Transient increases in IL-15 and IFN-gamma levels early after boost correlate with Spike antibody levels, supporting their use as biomarkers of effective humoral immunity development in response to vaccination. We identified a systemic signature including increases in IL-15, IFN-gamma, and IP-10/CXCL10 after the 1st vaccination, which were enriched by TNF-alpha and IL-6 after the 2nd and 3rd vaccination. In previously COVID-19-infected individuals, a single vaccination resulted in both strong cytokine induction and antibody titers, similar to the ones observed upon booster vaccination in antigen-naive individuals. In contrast, although vaccination elicited an innate cytokine signature featuring IFN-gamma, IL-15 and IP-10/CXCL10, most patients with hematological malignancies showed a diminished systemic cytokine response. In patients who failed to develop antibodies, the innate systemic response was dominated by IL-8 and MIP-1a with significant attenuation in the IFN-gamma, IL-15 and IP-10/CXCL10 signature response. Changes in IFN-gamma and IP-10/CXCL10 at priming vaccination and IFN-gamma, IL-15, IL-7 and IL-10 upon booster vaccination correlated with the Spike antibody magnitude and were predictive of successful antibody development in this cohort.

CONCLUSION: These data identified a cytokine hub featuring IFN-gamma, IL-15 and IP-10/CXCL10 characterizing a response to the COVID-19 BNT162b2 mRNA vaccination. These data identify a cytokine signature as correlating with successful antibody development in naïve persons as well as in patients with hematological disease treated with cell therapies. These approaches provide a tool to connect innate signatures associated with protective adaptive responses to vaccines.

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

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Abstract Content: Cells integrate extracellular cues through biochemical signaling pathways to induce the appropriate transcriptional programs. The signaling pathways that drive cytokine-induced gene expression are often depicted by a dozen or so landmark phosphorylation and transcriptional events, evoking the perplexing problems of how cytokines utilize common STAT transcription factors (TFs) to induce distinct functional programs, and to what extent cooperating pathways support response diversity. We hypothesized that in reality, thousands of dynamic post-translational modifications orchestrate the activation or repression of cytokine-specific genetic programs. Our phosphoproteomic analysis of macrophage responses to IL-6 and IL-10, two cytokines that are dependent on the overlapping usage of STAT1 and STAT3, identified 2,300 cytokine-specific phosphosites and 716 cytokine-induced transcription factor phosphosites. To systematically identify STAT-cooperating TFs that support the expression of specific cytokine-regulated gene sets, we developed an interdisciplinary strategy that uses global, temporally-resolved phosphoproteomic and transcriptomic data, together with statistical modeling and analysis of STAT-adjacent TF-motifs. We are currently experimentally validating multiple candidate STAT-cooperating TFs, which have motifs that are enriched in unique gene sets, and which are phosphorylated in a cytokine-specific manner. Our ability to identify causal links between phosphorylation events and cytokine-driven functional specificity betters our fundamental understanding of how extracellular cues are integrated and is a step towards identifying and manipulating the biochemical events required for healthy versus pathology-associated gene expression.

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TOWARDS STRUCTURAL AND FUNCTIONAL STUDIES OF IFN-LAMBDA1-4 USING PROTEIN ENGINEERING AND MOLECULAR DYNAMICS SIMULATIONS

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Abstract Content: Type III interferons (IFN-λ1-4) are an important class of cytokines which have antiviral and antiproliferative properties. Their shared receptors– IFN-λR1 and IL-10Rβ - are both expressed only at barrier tissues, like the lungs and liver, making type III interferons attractive therapeutic candidates. However, the clinical use of type III interferons is still limited, partially due to their lower activity relative to type I interferons and issues with large-scale production (IFN-λ4). To explain and overcome these issues, we use a combination of protein engineering and molecular dynamics to study the structural and functional behavior of the IFN-λs. Using directed evolution to overcome

the low affinity of the complex, we engineered a high-affinity version of IL-10Rβ to enable structural studies for all four wild-type IFN-λ ternary complexes. As part of these efforts, we apply other protein engineering strategies to express and isolate unmodified IFN-λ4 to fully characterize and explain its functional differences with IFN-λ1-3 based on protein structure. In parallel, we use molecular dynamics simulations to model the behavior of these currently unsolved protein complexes. Using trajectory and free energy data, we identify predicted differences in behavior between engineered type III interferons and their wild-type counterparts as well as find potential hot spots of protein-protein interaction that can be leveraged for engineering. Together, this work aims to paint a comprehensive portrait of type III interferon structure, function, and signaling to characterize their role in human health and disease and enable the development of more effective IFN-λ-based therapeutics.

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UPPER RESPIRATORY TRACT MICROBIOME – BRAIN LOOP: ORCHESTRATE LONG COVID

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Abstract Content: Long-Covid is characterised by myriad of clinical manifestations which persist for many months post infections, regardless of the severity of the initial infection. Intriguingly, the aetiology of long Covid has been poorly understood. Upper respiratory tract (URT) has been the initial colonisation ground for many respiratory viruses including SARS-CoV-2, as diseased patients are detected by nasal swabs. The adult URT is known to colonise primarily- *Staphylococcus*, *Corynebacterium*, *Pseudomonas* amongst others. Reports also state that COVID-19 patients have high predominance of these bacteria in blood in early (2 days) and late (14 days), stages (post admission) . Notably, the olfactory and taste sensors are located in the URT, and being regulated by bacterial derived metabolites (*Staphylococcus*/ formyl peptides, *Pseudomonas*/ acyl-homoserine lactone, etc) by engagement to fMLPR and T2R receptors respectively. More accumulation of signalling molecules are known to impair signal transduction by endocytosis of receptors. Hence enrichment of these bacteria can lead to enhanced production of these secondary metabolites and overall dampening of sensory pathways. Indeed, in Covid and Long Covid-19, smell/taste sensations have been known to be frequently impaired. Notably, dogs can detect COVID by sniffing VOCs and *Staphylococcus*, *Corynebacterium*, *Pseudomonas* are also known to emit characteristic VOCs .
After initial seeding of SARS-CoV2 in URT, the virus can either pass on to brain or lower respiratory tract (lungs).Though not intact virus particle, but shedded S1 protein and inflammatory cytokines have been known to cross BBB, which can be attributed to varying neurological disorders . As metabolites of gut microbiota (SCFA, bile acids, tryptophan metabolites, etc) are known to modulate tight junctions and compromise permeability of BBB , the URT metabolite (acyl-homoserine lactone) is also known to increase permeability of epithelial junctions . Hence, URT metabolites perhaps might be able to modulate BBB functioning. BBB is the endothelial layer separating circulating system and CSF of CNS. With endothelial cells lining all the organs, it could be epigenetic changes in endothelial cells responsible for persistent symptoms in many organs in long COVID. Substantiating it, neuropilin-1 (co-receptor for SARS-CoV-2), known for maintaining endothelial functionality is activated by TGFb (activated in COVID) in ILC2 cells, promote Th2 phenotype. Fatigue- another common long COVID manifestation can also be caused by oxygen starvation by microclots in tissues, generated by endothelial damage.
The gut-brain axis is two way communications between gut and brain in maintaining homeostasis and controlling inflammations. However, perhaps in COVID, it's the brain-gut axis which plays an important role in transmitting signals from brain to gut by nervous systems, ensuing in gut dysbiosis and flaring up the inflammatory state.
Hence hierarchically, it could be initial URT dysbiosis-leading to enrichment of specific bacteria and further production of certain metabolites, which alter the BBB and endothelium- culminating in autoinflammation and gut dysbiosis , leading to persistent inflammatory states- defined as long COVID.

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A NITROALKENE COMPOUND SCREEN FOR INHIBITORS OF STING-DEPENDENT INFLAMMATION

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Abstract Content:
The STING signaling pathway is a central mediator of inflammation in several autoimmune and autoinflammatory diseases such as STING-associated vasculopathy with onset in infancy (SAVI). Currently, there is an unmet need for clinically available therapeutics targeting STING for the resolution of STING-dependent inflammation.

Here, we screened 56 nitroalkene compounds for their potential to inhibit STING-dependent inflammation. We determined the *in vitro* therapeutical potential of the nitroalkene compounds by measuring their ability to suppress CXCL-10 release, type I IFN release, $NF_{\kappa}B$/IRF activation and STING signaling. The inhibitory actions were assessed in relation to cytotoxicity and this enabled to us identify lead nitroalkene compounds. Finally, we demonstrated *in vivo* modulation of STING-dependent inflammation by a lead nitroalkene compound in lung, liver, spleen, heart and plasma.

In conclusion, we establish that nitroalkene compounds can target and inhibit STING-dependent inflammation. Thus, nitroalkene compounds show a high therapeutical potential for treating autoimmune and autoinflammatory diseases where STING is a central driver.

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PD-L1-DIRECTED PLGF/VEGF BLOCKADE SYNERGIZES WITH CHEMOTHERAPY BY TARGETING CD141+ CANCER-ASSOCIATED FIBROBLASTS IN PANCREATIC CANCER

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Abstract Content: Pancreatic ductal adenocarcinoma (PDAC) has a poor 5-year overall survival rate. Patients with PDAC show limited benefits after undergoing the available chemotherapy or immunotherapy modalities. Herein, we reveal that chemotherapy upregulates placental growth factor (PlGF), which directly activates cancer-associated fibroblasts (CAFs) to induce fibrosis-associated collagen deposition in PDAC. Patients with poor prognosis had high PlGF/VEGF expression and an increased number of PlGF/VEGF receptor-expressing CAFs, leading to enhanced collagen deposition. We also developed a multi-paratopic VEGF decoy receptor (Ate-Grab) by fusing the single-chain Fv of atezolizumab (anti-PD-L1) to VEGF-Grab to target PD-L1-expressing CAFs. Ate-Grab exerted anti-tumor and anti-fibrotic effects in a murine orthotopic PDAC model via PD-L1-directed PlGF/VEGF blockade. Furthermore, Ate-Grab synergized with gemcitabine by relieving desmoplasia. Single-cell RNA sequencing identified a novel subset of CAFs, the $CD141^{+}$ CAF population, as responsible for the therapeutic effects of Ate-Grab. Overall, our results elucidate the mechanism underlying chemotherapy-induced fibrosis in PDAC and highlight a combinatorial therapeutic strategy for desmoplastic cancers.

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MOLECULAR ANALYSIS OF INTERLEUKIN-11 RECEPTOR VARIANTS

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Abstract Content: Interleukin-11 (IL-11) is a member of the IL-6 family of cytokines with pro- and anti-inflammatory properties. IL-11 binds to its specific α-receptor, the IL-11 receptor (IL-11R) and signals via a homodimer of the β-receptor gp130 (classic signaling). Importantly, soluble forms of the IL-11R (sIL-11R) are generated by proteolytic cleavage and bind IL-11. The resulting sIL-11R/IL-11 complex is also able to induce signal transduction, a pathway that is called trans-signaling.
Several mutations within the *IL11RA* gene have been identified, and some of these are found in patients with craniosynostosis, a disease characterized by the premature closure of one or more cranial sutures. However, for most of the mutations no associations with pathological conditions are known. We analyzed the five IL-11R variants V15M, G231D, R261H, A370V and R395W, located in the extracellular part of the receptors, all for which at least one homozygous individual is listed in the genome aggregation database (gnomAD). We analyzed the expression, maturation, cell surface levels, proteolysis and biological activity.
Our results show that all variants are biologically active and able to activate intracellular signaling in response to IL-11. In contrast to the other variants, G231D and R261H displayed impaired protein maturation, reduced cell surface expression and reduced proteolytic cleavage by the proteases ADAM10 and RHBDL2, leading to a reduced capacity for IL-11 trans-signaling.
Our results suggest that a reduced IL-11R level at the cell surface is sufficient for normal IL-11 signaling, which might explain why no phenotype has been described for individuals carrying these mutations.

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P35

CHARACTERISING IL-23 RECEPTOR BINDING AND SIGNALLING ACTIVATION USING ENERGY TRANSFER AND LUCIFERASE COMPLEMENTATION

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Abstract Content: Interleukin-23 (IL-23) is a pro-inflammatory cytokine involved in the defence against pathogens such as *M. tuberculosis*. However, the cytokine has also been implicated in the development of several autoinflammatory conditions, with variants of the gene being protective against Psoriasis and Crohn's disease. The IL-23 cytokine and its receptor (made up of a complex of the single transmembrane domain containing proteins IL23R and IL12Rβ1) have been targeted by drug discovery resulting in the successful development and licensing of anti-IL-23 therapeutics. Despite these advances the exact mechanism of cytokine binding and receptor activation remains poorly understood, with contradicting reports postulating activation mechanisms of both ligand-induced dimerisation and conformational change. This study used the proximity-based techniques of NanoLuciferase Bioluminescence Resonance Energy (NanoBRET) and NanoLuciferase Binary Technology (NanoBiT) to measure the affinity of IL-23 for its individual full-length receptor components and the heteromeric complex formed between them, expressed in live cells. The technology also allowed the measurement of complex formation between the receptor components and the measurement of changes in the arrangement of the *N*-terminal domains of the receptor upon cytokine binding. These approaches demonstrated that non-activated complexes of IL23R and IL12Rβ1 formed in the absence of IL-23, with these complexes forming a high affinity (27 pM) binding site for the cytokine. Subsequent cytokine binding induced a change in the relative positions of the *N*-terminal domains of the receptor accompanied by induction of signalling. Finally, it was demonstrated that semi-active cytokine independent complexes could be formed through constraining the *N*-terminal domains of pre-formed receptor complexes using high affinity NanoBiT complementation.

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CAUGHT IN THE ACT: NOVEL TSLP NANOTOOLS FOR STRUCTURE-FUNCTION STUDIES

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Abstract Content: Thymic Stromal Lymphopoietin (TSLP) is an IL2-family cytokine crucially important for mediating type 2 immunity at barrier surfaces and it has been linked to widespread allergic and inflammatory diseases of the airways, skin, and gut. Over the last two decades TSLP has been extensively studied in the scope of atopic and chronic inflammatory diseases. Furthermore, TSLP-mediated signaling has recently emerged as a novel molecular player in non-allergen induced conditions linked to Th1, Th9 and Th17 immunity responses. Together with broadening its pathophysiological profile these findings imply that the microenvironment of this pleiotropic cytokine might define the direction of its inflammatory response depending on the type of inflammation involved. Efforts to develop biologics that antagonize TSLP signaling have recently reached fruition with the clinical approval of Tezepelumab, a monoclonal antibody targeting TSLP, for the treatment of patients with severe asthma. While several other agents remain in different phases of clinical trials mostly for potential treatment of allergic conditions, high quality structural insights into TSLP/TSLPR complex and new knowledge about TSLP's specific function in the inflammatory response continues to highlight the need of developing potent agents with antagonistic properties against TSLP signaling.
With the aim to create novel tools to further interrogate the function and mechanism of activation of TSLP, we have developed and identified a selection of anti-TSLP camelid-derived single domain antibodies (VHHs). Fifteen nanobodies from four different CDR families were selected based on their CDR differences, expressed in *E.coli* and purified, resulting in good yields of the recombinant protein and having high purity and good stability. Furthermore, biophysical characterization by biolayer interferometry showed that nanobodies bind TSLP with low nanomolar affinity, and comparable fast on-rates coupled to distinctly variable off-rates. Additionally, cellular studies showed antagonistic effects in TSLP-mediated STAT5 signaling. To structurally characterize these nanobodies and their modes of binding to TSLP, we determined several structures of TSLP-VHH complexes by X-ray crystallography at high resolution.
Collectively, our structural studies of TSLP-VHH complexes, complemented by an array of biochemical/biophysical/cellular studies, provide insights into the mode of action of the developed anti-TSLP VHH and open new avenues for antibody-based therapeutic lead development.

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ADIPOCYTE SPECIFIC IL1R SIGNALING DRIVES ATHEROSCLEROSIS VIA REGULATION OF PRO-INFLAMMATORY MOLECULES EXPRESSION

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Abstract Content: Adipose tissue inflammation has been implicated in various chronic inflammatory diseases and cancer. Perivascular adipose tissue (PVAT) surrounds the aorta as a fourth layer and was previously shown to regulate the function of endothelial and vascular smooth muscle cells in the aorta. Moreover, PVAT contains various immune cells and therefore could contribute to the inflammatory environment in the aorta during atherosclerosis development via the cross-talk with adventitia. IL1 plays an important pathogenic role in atherosclerosis and inhibition of IL1b has been proven to suppress cardiovascular inflammation in the CANTOS trial.
Here we decided to address the role of IL1R signaling in adipocytes using adipocyte specific ablation of IL1R. We found that *Il1r$^{fl/f}$Ldlr$^{-/-}$ AdipoCre+* fed with Western Diet for 16 weeks developed significantly less atherosclerosis compared to *Il1r$^{fl/f}$Ldlr$^{-/-}$ AdipoCre^{-}* controls. Gene expression analysis of isolated adipocytes revealed a significant reduction of several chemokines, including *Ccl2, Ccl5, Ccl7* as well as IL6. Immune cell composition

analysis by Flow cytometry and scRNA sequencing demonstrated a limited presence of various inflammatory cells in PVAT of*Il1r*$^{fl/f}$*LdlR*$^{-/-}$ *AdipoCre*$^{+}$, which in turn limits their accumulation in the aorta.
Taken together our data suggest a novel mechanism controlling inflammation in atherosclerosis implying that IL1R signaling directly regulates the production of pro-inflammatory chemokines and cytokines by adipocytes thereby controlling immune cell recruitment to PVAT, which in turn contribute to the enhanced inflammation in the aortic wall and atherosclerosis.

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CROSS-TALK BETWEEN IL-6 TRANS-SIGNALLING AND AIM2 INFLAMMASOME/IL-1B AXES BRIDGE INNATE IMMUNITY AND EPITHELIAL APOPTOSIS TO PROMOTE EMPHYSEMA

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Abstract Content: Emphysema is a major debilitating component of chronic obstructive pulmonary disease, developing mainly in association with inhalation of infectious agents and/or noxious particulate matter found in cigarette smoke (CS). This results in the triggering of potent dysregulated innate immune responses, leading to chronic pulmonary inflammation, alveolar apoptosis and the irreversible damage/destruction of lung structure and function. However, the key regulators of innate immunity and their role in the susceptibility and progression of emphysema remain ill-defined. In this study, we utilise spontaneous *gp130*F/F and CS-induced mouse models of emphysema, as well as biopsies from emphysematous patients to investigate whether innate immune inflammasome complexes comprising the adaptor ASC, Caspase-1 and specific pattern recognition receptors (PRRs) promote the pathogenesis of emphysema. Results show that the expression (mRNA, protein) of ASC and Caspase-1 was indeed upregulated in these models, along with inflammasome-associated PRR and DNA sensor, AIM2. This upregulation of AIM2 coincided with the preferential production of mature downstream effector cytokine IL-1β over IL-18. This result was supported by the genetic blockade of ASC, AIM2 and the IL-1 receptor, as well as therapy with AIM2 antagonistic suppressor oligonucleotides in *gp130*F/F mice, which improved the emphysematous phenotype by preventing elevated alveolar cell apoptosis. Blockade of AIM2 genetically or with inhibitors also protected mice exposed to CS from pulmonary alveolar apoptosis. Interestingly, the functional requirement for AIM2 in driving this apoptosis within the lung epithelium was independent of its expression in hematopoietic-derived immune cells and activity on pulmonary inflammation. This study also reports that the upregulated pulmonary expression of AIM2 in emphysema was dependent on the upstream cytokine IL-6 and it's trans-signalling axis (via soluble IL6-R), which can be selectively blocked with a sgp130 antagonist. Collectively, this study suggests a novel cross-talk between the AIM2 inflammasome/IL-1β and IL-6 trans-signalling axes, paving the way for novel therapeutic strategies for the treatment of emphysema without compromising the immune system.

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MECHANISM OF IL-1A-INDUCED ALTERATION OF THE GUT MICROBIOME IN EXPERIMENTAL CROHN'S DISEASE LIKE ILEITIS

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Abstract Content: Crohn's disease (CD) is a complex, chronic inflammatory bowel disease (IBD), with a rising incidence during the past decades. Although the cause of the disease is still unknown, an exaggerated immune response to the gut microbiota in individuals with a genetic predisposition has been postulated as a possible causative mechanism. Available treatments, including biological therapies, such as anti-TNF and anti-adhesion molecule medications have improved the quality of life of patients with CD, but they tend to lose efficacy with time. IL-1α, a major component of the IL-1 family of cytokines, is considered a key regulator in the pathogenesis of inflammatory diseases including IBD. We have recently discovered that specific blockade of IL-1α using a monoclonal antibody (FLO1) in a spontaneous model of CD-like ileitis i.e., SAMP/YitFc (SAMP) mice, markedly suppressed intestinal inflammation by a mechanism(s) involving specific alterations of the gut microbiome. We showed that IL-1α neutralization is associated with taxonomic divergence of the intestinal microbiota and that there is a predictive relationship between IL-1α neutralization and the presence of specific bacterial species such as *Mucispirillum Schaedleri* and *Lactobacillus salivarius*. We hypothesize that IL-1α exerts a unique regulatory role in the function of the intestinal microbiome. These functions might be mediated through a direct effect on the gut microbial ecosystem, or modulation of antimicrobial peptides, such as α-defensins, or both. To test our hypothesis, we studied the expression of antimicrobial peptides in intestinal organoids (EnOs) from ileal tissue of SAMP mice stimulated with or without recombinant Il-1α **(**rIL-1α) for 6 hours. We found a reduced RNA expression of the Paneth cells markers Def-rs1 and Lyz; and goblet cells marker Muc2 in organoids stimulated with rIL-1α. Additionally, we tested the effects of IL-1α on purified bacterial isolates from human fecal samples collected by our Cleveland Digestive Diseases Research Biorepository Core. Bacteria were cultured overnight in brain heart infusion (BHI) broth and the resulting cultures used to prepare pre-inocula with an initial optical density of 600nm (OD600nm) of 0.2, and then transferred in a 96 well plate for stimulation assay. Bacteria were incubated with 100ng/ml of recombinant IL-1α or controls (BHI only). OD600nm was measured at 20 min intervals for 16 hours. Interestingly, we found that the growth rate of strains belonging to *Escherichia coli*, *Klebsiella pneumoniae* and *Shigella flexneri* species was promoted after incubation with rIL-1α. Studies using specific bacterial strains that were found altered in SAMP mice after anti-IL-1α therapy are in progress.
Taken together those results suggest that IL-1α might affect the gut microbiome in SAMP mice by altering Defa-rs1 and other antimicrobial peptides leading to a consequent impaired host defense, or it might have a direct flora-modifying function affecting the intestinal microbiome. Defining the specific bacterial strains that could functionally mediate the anti-inflammatory effects of anti–IL-1α therapy, and the underlying immunological pathways that are affected may lead to the identification of novel targets for effective therapeutic interventions, based on the interaction(s) between IL-1α, the host mucosal immune system and the gut microbiome.

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P40

INTRACELLULAR DOMAINS OF TYPE I AND III INTERFERON RECEPTORS ENCODE DIFFERENCES IN JAK-STAT SIGNALING MAGNITUDE

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Abstract Content: Signaling by type I and III interferons (IFNs) produces differential STAT phosphorylation, gene transcription and antiviral responses, despite using the same JAK-STAT cascade. As it has previously been shown that ligand binding affinities and receptor expression levels are insufficient to explain these differences, we hypothesized that molecular determinants are encoded within the receptors' intracellular domains to differentiate type I and III IFN signaling. To test this hypothesis, we engineered synthetic, heterodimeric type I and III IFN receptors that

can be stably expressed to the same level and respond to the same ligand. By directly comparing the signaling capabilities of our synthetic receptors, we have found that the type I receptor can still produce a greater signaling magnitude than type III, and we have identified that this signaling strength is driven by a specific <100aa sequence within the intracellular region of IFNAR2. When inserted into IFNLR1, this sequence allows the type III receptor to phosphorylate STAT1/2 as strongly as the type I receptor. In contrast, inserting other intracellular sequences from IFNAR2 into IFNLR1 has no effect on type III signaling. Altogether, our work pinpoints narrow regions within the intracellular domains of type I and III IFN receptors that encode different signaling magnitudes independently of receptor expression levels or ligand binding affinities.

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EFFECTS OF JAK INHIBITOR AND STAT6 INHIBITOR ON IL-13-INDUCED CHANGES IN AIRWAY EPITHELIAL CELLS

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Abstract Content: INTRODUCTION: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a disease frequently characterized by mucosal eosinophilia likely influenced by elevated levels of type 2 cytokines, notably IL-13, and CCR3 ligands of the eotaxin family. The cytokine-activated Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway has an important role in the control of immune responses.
OBJECTIVES: We aim to evaluate inhibitory effects of JAK inhibitor and STAT6 inhibitor on IL-13-induced eotaxin-3 responses in airway epithelial cells (BEAS-2B and primary human sinonasal epithelial cells [SNEC]) and identify possible mechanisms of action in airway epithelium .
METHODS: BEAS-2B and SNEC from inferior turbinate (IT) scrapings from control and CRSwNP patients were cultured with IL-13 (5ng/ml) with or without Fligotinib, JAK inhibitor (10-1000nM) and AS1517499, STAT6 inhibitor (10-1000nM) for 48 hours. The effects of those inhibitors on IL-13-induced effects were measured by ELISA, qRT-PCR, and pH imaging.
RESULTS: Fligotinib and AS1517499 dose-dependently suppressed IL-13-mediated eotaxin-3 mRNA expression and protein secretion from BEAS-2B cells and SNEC ($p<0.05$). In addition, using intracellular pH imaging, we found that IL-13 induced intracellular pH alkalinization that profoundly affected eotaxin-3 gene expression and was blocked by Fligotinib and AS1517499 ($p<0.05$).
CONCLUSION: IL-13 potently stimulates eotaxin-3 production in airway epithelial cells and is strongly inhibited by JAK inhibitor and STAT6 inhibitor. Inhibition of IL-13-induced eotaxin-3 by JAK inhibitor and STAT6 inhibitor may provide therapeutic benefit in CRSwNP via a novel H^+/K^+-dependent mechanism.

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LONG NON-CODING RNAS INVOLVED IN THE SIGNAL TRANSDUCTION OF IL-6-TYPE CYTOKINES

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Abstract Content: Interleukin 6 (IL-6) plays important roles in liver physiology and disease, e.g. as an inducer of acute phase proteins. It is also involved in hepatocarcinogenesis. Hepatocellular carcinoma (HCC), the main form of primary liver cancer, is the fourth leading cause of cancer-related deaths worldwide. HCC is an inflammation-driven

cancer, with the TNF and IL-6 families of cytokines playing key roles in maintaining a chronic inflammatory state within the liver.
We are interested in the involvement of (short and long) non-coding RNAs in IL-6-mediated signal transduction, with a focus on liver cells. We previously found miRNAs like miR-146b-5p to be differentially regulated by IL-6-type cytokines in primary hepatocytes and hepatoma cell lines which therefore can contribute to the signaling events elicited by this family of cytokines. In an independent approach we have identified miRNAs able to modulate this signaling pathway by targeting the expression of key signaling players like gp130, Jak1, and/or STAT3.
We have now identified hundreds of long non-coding RNAs (lncRNAs) to be differentially expressed downstream of IL-6 signaling in HepG2, HuH7, and Hep3B hepatoma cells. The analysis of time series transcriptomics data revealed that 26 lncRNAs are commonly regulated in the three cell lines (p-value < 0.01). Clustering and dynamic regulatory network analyses have allowed us e.g. to predict association to an immune signature or metabolic functions of previously poorly characterized lncRNAs such as AC008875.3 and TUBA5P, respectively. Ongoing studies include the qPCR validation of selected, differentially expressed lncRNAs as well as their expression modulation (antisense oligonucleotides, overexpression) in order to assess their possible function(s) in the context of IL-6 signal transduction. The analysis of antisense and divergently transcribed non-coding RNAs to transcripts of key protein coding players of the JAK/STAT signaling pathway (e.g. SOCS3-DT) further pinpoints to a possible role of potentially *cis* (and/or *trans*)-acting lncRNAs as so far understudied components of this well-known signaling pathway.

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MULTIVALENCY ENHANCES THE SPECIFICITY OF FC-CYTOKINE FUSIONS

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Abstract Content: Interleukin (IL)-2 has potential as a therapy in cancer and autoimmunity but is limited in effectiveness by its specificity toward desired immune populations. IL-2 muteins may have improved cell type selectivity through altered receptor-ligand binding kinetics. Here, we analyze the response of immune cells to a panel of IL-2 muteins in monomeric and dimeric Fc fusions using tensor factorization. We find that dimeric muteins have considerably altered selectivity profiles. We then dissect the mechanism of altered specificity in dimeric ligands using a multivalent binding model and show that the enhanced selectivity by dimeric muteins arises due to multivalent ligands' avidity for cells based on the abundance of their target receptors. Finally, we utilize this model to inform the design of tetravalent IL-2 muteins with potentially enhanced selectivity for regulatory T cells beyond what is achievable using mono- or bivalent variants. In total, we show that multivalent cytokines have unique selectivity profiles, that modeling can help to aid in their design, and that tensor factorization provides an effective approach to visualize ligand responses across diverse cell populations.

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THE DNA SENSOR CGAS, A NEW PLAYER IN THE IMMUNE RESPONSE OF MEGAKARYOCYTES AND PLATELETS

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Abstract Content:
Megakaryocytes are giant bone marrow cells responsible for the production of blood platelets. Besides having a key role in haemostasis, platelets interact with many cell populations and are important players in immune responses. They contribute to microbial clearance, tissue damage and autoinflammation. Platelets express immune receptors, including pattern recognition receptors that detect microbial molecules. Upon activation, these receptors induce the secretion of immune mediators and modulate platelet's function. Platelets were long ago suggested to be activated by DNA, yet the receptor involved and the biological implication of this activation are still not fully defined.

The cyclic GMP-AMP Synthase (cGAS) is the primary DNA sensor of most cells and is ubiquitous. cGAS activation by DNA leads to the production of cGAMP, a second messenger that binds to and activates the adaptor protein STING to induce the production of type-I Interferon and other pro-inflammatory cytokines. By sensing both microbial- and self-DNA, the cGAS-STING pathway underpins immune response to infections and drives pathological inflammation in several chronic inflammatory diseases.

In this study, we made the exciting discovery that the proteins from the cGAS-STING axis are expressed and functional in bone marrow megakaryocytes. Upon activation, the cGAS-STING axis allows the production of immune mediators such as CCL5 and type-I IFN, both critical in the regulation of hematopoiesis. We also show for the first time that blood platelets express a functional cGAS proteins able to produce cGAMP upon DNA stimulation, and regulate the inflammatory response of platelets. Taken together, our data unveil a new and intriguing role for the cGAS-STING axis in the megakaryocyte's lineage. Given that megakaryocytes and platelets interact with several cell populations in a variety of immune responses, our study represents a significant area for future investigations in the field of infectious diseases and other related cGAS-driven inflammatory syndromes.

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INTERFERON INDUCED ISGYLATION IS DIFFERENTIALLY REGULATED AS INDUCED-PLURIPOTENT STEM CELLS DIFFERENTIATE TO EPITHELIAL CELLS

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Abstract Content: Background & Aims: Epithelial cells in multiple organs, including the lung and liver, are among the first cells to encounter viruses. Type I and III Interferons (IFN) activate an antiviral response in epithelial cells through the upregulation of interferon-stimulated genes (ISG). Following IFN stimulation, increased expression of ISG15 and subsequent ISGylation are observed in epithelial cells as a component of the antiviral response.
Approach and Results: To study the regulation of ISGylation, we utilized patient samples and *in vitro* cell culture models including induced pluripotent stem cells (iPSCs), primary epithelial cells, immortalized cell lines, and epithelial cells differentiated from iPSCs (iPCS-EC). Protein and mRNA expression were measured following treatment with type I and III IFN. When compared to epithelial cells, we observed a lack of ISGylation and several other notable aspects of the ISGylation pathway in iPSCs. These include a lower baseline expression of the ISGylation activating enzyme, UBE1L, a lack of IFN-induced expression of the ISGylation conjugation enzyme, UBE2L6, and an attenuated activation of transcription factor STAT1. ISGylation was observed in iPSCs following increased expression of UBE2L6. Furthermore, epigenetic mechanisms regulate ISGylation by downregulating UBE1L expression as iPSCs differentiate to epithelial. Intriguingly, transformed epithelial cell lines, demonstrated weak ISGylation following IFN treatment.
Conclusions: These data revealed that proteins involved in ISGylation demonstrate differential regulation in a STAT1-dependent (UBE2L6) and STAT1-independent (ISG15) manner. The inhibition of STAT1 in iPSCs, subsequently inhibited IFN-induced UBE2L6 expression and ISGylation specifically. In transformed epithelial cell lines, we observed lower levels of ISGylation. These findings have significant mechanistic implications to the development of a functional ISGylation pathway in epithelial cells as part of an effective antiviral response.

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THE ROLE OF THE INFLAMMASOME ADAPTOR ASC IN GASTRIC TUMOURIGENESIS

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Abstract Content: Inflammasomes are key regulators of innate immunity in chronic inflammatory and autoimmune diseases, however, their role in inflammation-associated tumourigenesis remains unclear. In gastric cancer (GC), the third leading cause of cancer associated death worldwide, we have previously discovered a pro-tumourigenic role for the key inflammasome adaptor apoptosis-associated speck-like protein containing a CARD (ASC) in the *gp130*$^{F/F}$ spontaneous mouse model of intestinal-type GC [1]. Specifically, we identified interleukin 18 (IL-18) as the major downstream effector cytokine; and furthermore, that the pro-tumourigenic activity of the ASC inflammasome predominantly resides within the tumour epithelium. However, the identity of the specific pattern recognition receptor(s) (PRRs) that activate tumour-promoting inflammasomes during GC is unknown.
We have investigated the role of the best-characterised inflammasome-associated PRR, nucleotide-binding domain, and leucine-rich repeat containing receptor, pyrin domain-containing (NLRP) 3, in GC. In gastric tumours of *gp130*$^{F/F}$ mice, genetic ablation of NLRP3 did not lessen the development of gastric tumours [2]. Similarly, cellular processes associated with tumourigenesis in the gastric mucosa, namely, proliferation, apoptosis, and inflammation, were comparable between *gp130*$^{F/F}$ and *gp130*$^{F/F}$:*Nlrp3*$^{-/-}$ mice. Furthermore, inflammasome activation levels, determined by immunoblotting and immunohistochemistry for cleaved Caspase-1, which along with ASC is another integral component of inflammasome complexes, were unchanged in *gp130*$^{F/F}$ and *gp130*$^{F/F}$:*Nlrp3*$^{-/-}$ gastric tumours. Overall, we concluded that NLRP3 does not play a major role in promoting inflammation-drive gastric tumourigenesis.

To identify candidate PRRs that activate the ASC inflammasome in the *gp130*$^{F/F}$ mouse model, we employed a proteomics approach in which ASC was isolated in *gp130*$^{F/F}$ mouse tumour samples using an immunoprecipitation method. Proteins associating to ASC were also isolated and subsequently identified using mass spectrometry, using a method called rapid immunoprecipitation mass spectrometry. Consequent computational analysis has led to the identification of several candidate PRRs whose expression profile is being validated in GC patient cohorts and *gp130*$^{F/F}$ mice. We are also investigating the functional requirement of these PRRs for inflammasomes in GC using CRISPR knockdown of these PRRs in human GC cell lines, followed by analyses to determine the effect of the CRISPR knockdown on cellular processes associated with tumourigenesis, as well as inflammasome activation.

Overall, identifying PRRs that activate the ASC inflammasome in gastric tumourigenesis provides the potential for the development of PRR-directed targets for inhibitors for use as anticancer agents in GC.

[1] Deswaerte V, et al. *Cancer Res*, 78:1293-1307, 2018
[2] West AJ, et al. *Front Oncol*, 12: 830350, 2022

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P48

CYTOKINE LEVELS IN PLASMA-DERIVED EXOSOMES ARE UNDERESTIMATED AS CONTRIBUTORS TO CYTOKINE PROFILES IN CANCER PATIENTS AND HEALTHY DONORS

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Abstract Content: Small extracellular vesicles or exosomes (exo) play a key role in cell-to-cell signaling. They are produced by all cells, circulate freely and are present in all body fluids. Evidence suggests that biologically active cytokines are present on the surface and/or in the lumen of exo. We measured cytokines in exo isolated by

ultrafiltration/size exclusion chromatography (SEC) from pre-cleared plasma of 30 patients with head and neck cancer (HNC) and 10 healthy donors (HDs). Paired untreated and detergent-treated (0.5% Triton X-100) plasma samples and isolated detergent-treated exo (TrX-exo) were tested for cytokine levels by multiplex immunoassays (Luminex, 65-plex). The results showed that HNC patients' plasma contained a larger variety and significantly higher levels of cytokines than HDs' plasma. Cytokine levels in TrX-exo from HNC patients' plasma were also significantly higher than those in TrX-exo from HD's plasma. Paired detergent-treated plasma and TrX-exo samples tested for selected cytokines by Luminex gave concordant results. However, Luminex performed on paired untreated and detergent-treated plasma samples showed significantly discordant results. Levels of most cytokines were significantly elevated ($P<0.0001$) in the detergent-treated vs. untreated plasma. These data suggest that in untreated plasma, Ab-based assays detect only soluble cytokines and miss cytokines carried by exo in the vesicle lumen. Permeabilization of exo is necessary for the Ab-based detection of total cytokines present in plasma, i.e., soluble plus exo associated cytokines. Importantly, our in vitro data also show that exo-associated cytokines are biologically active in assays such as wound healing or proliferation. In HNC patients with the different stage disease, cytokine levels in detergent-treated plasma correlated with the disease stage. As cytokines are usually tested by multiplex immunoassays in fresh, untreated plasma, their levels are underestimated and inaccurate. Correlations of the cytokine presence/levels with disease states should be performed in detergent-treated plasma to account for exosome associated cytokines.

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P49

CHARACTERIZATION OF CONSERVED NONHUMAN PRIMATE CELL SPECIFIC-TRANSCRIPTOMIC RESPONSE TO INTERLEUKIN-15

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Abstract Content: Interleukin 15 (IL-15) is an immunomodulatory cytokine that can direct immune cell activation, regulate lymphocyte trafficking, and support T cell effector functions. Recently, IL-15 has been linked with vaccine protection to SIV infection in rhesus macaques (RM) receiving cytomegalovirus (CMV)-based vector vaccine expressing SIV antigens (RM-CMV/SIV vaccine). We applied a systems biology approach, including functional genomics analyses, to define the immune parameters of RM-CMV/SIV vaccine protection. Our studies identified a whole blood transcriptomics signature of immune programming linked with IL-15 signaling. However, the response to IL-15 in primates is not defined. To address this knowledge-gap, we used both bulk and single cell (sc) RNA sequencing (RNAseq) to characterize the response to IL-15 treatment in RM and Mauritian-origin cynomolgus macaques (CM). We analyzed whole blood collected over a 21-day course from RMs and CMs treated with increasing doses of recombinant purified heterodimeric IL15/IL15Rα (rRh-Het-IL-15) at three time-points (days 0, 3, and 7), and scRNA-seq was performed on corresponding RM PBMCs isolated from whole blood at days 0, 3, 7 and 10 post IL-15 administration. Additionally, bulk mRNAseq and scRNAseq were performed on cultured RM PBMCs treated with a single dose of either 4 or 40 ng/ml of rRh-Het-IL-15 and collected at 5 time-points post treatment. We applied co-expression analyses and integrated bulk mRNAseq and scRNAseq cell-specific transcriptome analyses that defines the whole blood and cell-specific response to IL-15 in vivo, ex vivo, and in vitro. We show that IL-15 imparts both innate and adaptive immune response activation across myeloid cells, NK cells and T cells while suppressing gene expression within B cell activation and proliferation pathways across RMs and CMs. Further, we found that the IL-15-induced innate immune genes are specifically expressed in T cell subsets and remain up-regulated over time. Data set integration to map IL-15-response gene expression across whole blood and single cells reveal cell-specific gene signatures linked to IL-15. Together, these data identify a conserved transcriptomic IL-15 response across two primate species, thus informing clinical studies of CMV vector-based HIV vaccine efficacy in human trials and further supporting the use of IL15 as a therapeutic for HIV.

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LB-05

DUXC-FAMILY TRANSCRIPTION FACTORS INHIBIT THE TRANSCRIPTIONAL RESPONSE TO INTERFERON GAMMA

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Abstract Content: DUXC-family transcription factors are endogenously expressed at the cleavage stage of development in placental mammals and help drive an initial burst of zygotic gene transcription. The human ortholog, DUX4, was discovered due to its causative role in facioscapulohumeral muscular dystrophy (FSHD), and DUX4 expression in cancers is associated with immune evasion and can suppress Type-II interferon gamma (IFNγ)-induced MHC Class-I protein expression. We expand this research to show that DUX4 suppresses the transcriptional response to IFNγ, Type-I interferon beta (IFNβ), double-stranded (ds) RNA, and dsDNA treatments. A transcriptionally silent C-terminal portion of the DUX4 protein interacts with STAT1, reduces its occupancy at interferon stimulated gene (ISG) promoters, and abolishes Pol-II recruitment, thus preventing ISG upregulation. A short, conserved motif in the C-terminal activation domain (CTD) of DUX4 is required to suppress ISG upregulation, and phosphorylation of STAT1 Y701 enhances interaction with DUX4. This phenomenon occurs in multiple endogenous contexts, such as when DUX4 is spontaneously expressed in FSHD myoblasts and when a CIC-DUX4 fusion protein containing the DUX4-CTD is expressed in Kitra-SRS sarcoma cells. The mouse ortholog, Dux, also interacts with STAT1 and suppresses the transcriptional response to IFNγ, suggesting an evolved role of the DUXC family in modulating immune signaling pathways with implications for early development in placental mammals, cancer, and muscular dystrophy.

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LB-06

POLY(DA:DT) ACTIVATES THE NLRP1 INFLAMMASOME IN UNPRIMED HUMAN KERATINOCYTES

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Abstract Content: Cytosolic DNA can activate innate immune signaling pathways including inflammasomes, which catalyze caspase driven IL-1 family cytokine release and inflammatory cell death. In human keratinocytes, the canonical DNA inflammasome sensor AIM2 is not expressed in resting cells. We show that transfection of the synthetic nonlinear dsDNA poly(dA:dT) in unprimed keratinocytes elicits a NLRP1-dependent inflammasome in an AIM2 and STING independent manner. In contrast, conventional linear dsDNA ligands such as HT-DNA were unable to stimulate NLRP1. Keratinocytes transfected with poly(dA:dT) demonstrated a phenotype strikingly comparable to poly(I:C) dsRNA transfection, but we were unable to isolate a poly(dA:dT)-derived dsRNA intermediate sufficient to activate an inflammasome. Instead, poly(dA:dT) induction of the NLRP1 inflammasome occurred through an indirect mechanism that correlated with oxidative nucleic acid damage and global cellular stress. Finally, multiple broad spectrum MAPK-related kinase inhibitors abrogated the poly(dA:dT)-induced NLRP1 inflammasome. Altogether, our study reinforces the notion that poly(dA:dT) stimulates multiple pattern recognition receptors, and our identification of NLRP1 as an additional poly(dA:dT) target highlights its utility as a therapeutic vaccine adjuvant. Furthermore, studies utilizing poly(dA:dT) as an AIM2 inflammasome agonist should take into account it may cross-react with NLRP1.

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LB-07

TUFT CELLS INHIBIT PANCREATIC INJURY THROUGH IL-25 SYNTHESIS AND SECRETION

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Abstract Content: Background & Aims: In response to injury, pancreatic acinar cells transdifferentiate into ductal cells (ADM) as a protective mechanism that promotes tissue repair. We have found that ADM does not strictly consist of a homogeneous population of ductal cells, but contains differentiated cell types typically rare or absent from the pancreas, like tuft cells (TCs). TCs are solitary chemosensory cells normally found in hollow organs throughout the respiratory and digestive tracts. We have found that both oncogenic KRAS or pancreatitis induce TC formation in the pancreas. TC secretion of cytokine IL-25 has been reported to play a role in helminth clearance in the intestine, but the role of TCs and TC-derived IL-25 in pancreatitis has not been defined.
METHODS: Genetically engineered mouse models (GEMMs) lacking (KO) either POU2F3, the master regulator transcription factor for TC formation, or Interleukin (IL-) 25 specifically in the pancreas were generated. Adult mice and controls were given pancreatitis using the cholecystokinin ortholog caerulein and the pancreas was collected to conduct histological studies. CYTOF mass spectrometry was conducted to profile the immune infiltrate.
RESULTS: Induction of pancreatitis in mice lacking TCs resulted in more severe injury and greater tissue loss, as compared to controls. Pancreatitis derived TCs express IL-25 and IL-25 ablation also resulted in enhanced injury as well as edema. Both TC and IL25 KO mice displayed enhanced infiltration of macrophages, activated fibroblasts, and t-regulatory cells in the injured tissue.
CONCLUSIONS: Tuft cells inhibit injury under conditions of chronic pancreatitis through IL-25 synthesis and secretion. The immune landscape changes during injury when there is a lack of TCs or IL-25. Further studies are required to determine the mechanism(s) by which immune cells respond to IL-25 and how this suppresses inflammation and injury.

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LB-08

ELUCIDATING ROLES FOR LEUKEMIA INHIBITORY FACTOR IN INNATE IMMUNITY

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Abstract Content: Leukemia inhibitory factor (LIF) is a member of the interleukin-6 (IL-6) cytokine family. LIF binds to gp130, along with its unique receptor subunit LIFR. This interaction results in recruitment of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT3. LIF is a pleiotropic cytokine, broadly expressed across tissues with many well-established roles; nonetheless, the role for LIF in immunity and inflammation remains elusive. Our group and others have observed that LIF is upregulated in the circulation of mice during inflammatory challenge, such as exposure to Toll-like receptor (TLR) agonists or infection with enteropathogenic bacteria. Additionally, our group identified multiple immune cell subsets that express LIFR, such as mononuclear phagocytes and dendritic cells (DCs), suggesting these populations respond to LIF to mediate immune responses. To determine the function of LIF signaling in the myeloid/DC compartment, we generated CD11c-cre^{+} *Lifr*$^{f/f}$ (LIFR KO) mice. Our studies show that LIFR KO mice have reduced amounts of circulating inflammatory factors following intraperitoneal injection of lipopolysaccharide (LPS) at a sub-lethal dose compared to wild type controls. This includes lower amounts of IL-1β, IL-18, interferon-gamma (IFN-γ), IL-15 and IL-22, suggesting a pro-inflammatory role for LIF signaling in the myeloid/DC compartment. Moreover, IL-1β intracellular staining of myeloid populations derived from blood, bone marrow (BM), or colon after LPS challenge indicate LIFR KO mice have a lower capacity for de novo IL-1β production in CD11b^{+} CD64^{+} and CD11b^{+} Ly6C^{+} CD64^{+} myeloid subsets. Since IL-1β and IFN-γ can induce proliferation of hematopoietic stem and progenitor cells (HSPCs), we profiled BM progenitors in adult LPS-challenged mice (aged 8-12 weeks). These studies

revealed that LPS-mediated expansion of Lin^- Sca^+ $cKit^+$ (LSKs), short-term HCS (ST-HSCs), and myeloid-biased multipotent progenitors was inhibited in adult LIFR KO mice compared control animals, suggesting LIF signaling in $CD11c^+$ cells directly or indirectly mediates HSPC proliferation in response to LPS challenge. Due to the well-established connection between inflammation and aging, we challenged aged LIFR KO mice (42 weeks old) with LPS. These assays revealed that ST-HSCs expanded in response to LPS in aged LIFR KO mice, but not in wild type controls, suggesting LIF signaling in the myeloid/DC compartment may influence HSPC aging. In conclusion, our data show LIF signaling in $CD11c^+$ cells enhances expression of IL-1β and other pro-inflammatory cytokines upon inflammatory challenge. Additionally, our results suggest this pathway regulates the ability of BM HSPCs to respond to inflammatory challenge and may influence HSPC aging.

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LB-09

PROBING CYTOKINE INDUCEMENT OF CELL SURFACE EXPRESSION OF E-SELECTIN IN PRIMARY HUMAN ENDOTHELIAL CELLS USING CRISPR-CAS9-MEDIATED PROTEIN TAGGING WITH NANOBIT

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Abstract Content: E-Selectin is a Ca^{2+}-dependent C-type lectin de novo synthesized and expressed on the surface of endothelial cells in response to inflammatory cytokines where it interacts with leukocytes to deliver them to sites of inflammation (Read et al., 1997). E-Selectin has been targeted in drug discovery, but a lack of knowledge on the extent and dynamics of its expression in live cells has contributed to poor efficacy in clinical trials.
We have developed a sensitive method to detect real-time cell surface expression of E-Selectin in live Human Umbilical Vein Endothelial Cells (HUVECs) using CRISPR-Cas9-mediated protein tagging in conjunction with NanoLuciferase Binary Technology (NanoBiT). NanoBiT utilizes complementary fragments of the novel luciferase NanoLuc. Here, CRISPR-Cas9 gene editing was used to append the 1.1KDa high affinity fragment of NanoLuc (termed HiBiT) onto the N terminus of endogenously expressed E-Selectin in HUVECs. The complementary membrane impermeable larger fragment (LgBiT; 18KDa) was then added as a purified protein. These fragments re-complement producing luminescence, indicative of cell surface expression of E-selectin.
HUVECs or immortalized TERT2 HUVECs were electroporated with 'HiBiT CRISPR mix' and grown (16hrs; 37°C, 5% CO_2). Cells were stimulated with TNF alpha, IL-1a, IL-1b, lipopolysaccharide (LPS), histamine or vascular endothelial growth factor 165a (VEGF165a) in Medium 200 supplemented with 2.2% large endothelial vessel supplement (LVES) for 6hr (37°C, 5% CO_2). Complementary LgBiT (1:400, 20min, 37°C) and the NanoLuc substrate furimazine (1:400) were then added and luminescence read using a PHERAstar FSX platereader. For kinetic experiments, cells were stimulated with the maximal concentration of mediator and luminescence read over 15hr (37°C) using a slow release NanoLuc substrate (Endurazine).
TNF alpha showed comparable potency at inducing E-Selectin surface expression in mixed population wildtype HUVECs, TERT2 HUVECs and a clonal TERT2 HUVEC cell population (EC_{50} values range: 0.0569 - 0.104nM; n=5). The clonal TERT2 HUVECs were homozygous for HiBiT insertion and showed increased reproducibility, sensitivity and assay windows. TNF alpha was the most potent stimulator for E-selectin expression compared to LPS (EC_{50} ± S.E.M, 2.80 ± 0.703nM n=5), IL-1a, (EC_{50} ± S.E.M, 32.84 ± 5.734nM; n=5) or IL-1b (EC_{50} S.E.M, 30.903 ± 6.225nM; n=5). Moreover, TNF alpha induced prolonged maximal surface expression over the course of 15hr, whilst other mediators displayed a reduction by 50% after 6 – 8 hours. A novel stimulator of E-selectin was also discovered; VEGF165a, that like Histamine, increased E-selectin expression above vehicle ($p < 0.0001$) and potentiated the TNF alpha induced response ($p = 0.0044$). The reproducibility of the clonal HiBiT E-Selectin TERT2 HUVECs combined with the high dynamic range of NanoBiT therefore makes this an exquisitely sensitive method to measure E-Selectin induced expression in real time in living endothelial cells.
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03. Cytokines and macrophages/myeloid cells

P50

ROLE OF GM-CSF-INDUCED CCL17 IN INFLAMMATION

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Abstract Content: Introduction: Rheumatoid arthritis is a chronic inflammatory autoimmune disease, which leads to poor quality of life due to the debilitating effect of inflammation. Glucocorticoids (GCs) are potent anti-inflammatory and immunosuppressive agents broadly used in anti-inflammatory therapy, albeit with adverse side effects assocated with long-term usage. The negative consequences of GC therapy provide an impetus for research into gaining insights into the molecular mechanisms of GC action on immune cells. We have previously reported that an inflammatory cytokine, GM-CSF drives CCL17 production via interferon regulatory factor 4 (IRF4)-dependent pathway in human monocytes and mouse macrophages. In this study, we investigated the molecular regulation of CCL17 production by GCs.
Methods: Gene and protein expression were measured by quantitative PCR and Western blotting, respectively. Secreted protein in culture medium was by ELISA. Genes were silenced using siRNA technology. Enrichment of histones and transcription factors at the gene loci was determined by chromatin immunoprecipitation.
Results: We report here that GM-CSF-induced CCL17 expression is inhibited by GCs in human monocytes and mouse macrophages. We provide evidence for the first time that GCs suppress GM-CSF-induced IRF4 expression via regulating the expression and activity of JMJD3, which demethylases trimethylated-H3K27. Moreover, GCs ameliorated GM-CSF-induced inflammatory arthris and pain development in animal models, correlating with marked decrease in the levels of CCL17 in arthritic joints. Significantly, spontaneously secreted CCL17 by synovial fluid mononuclear cells from RA patients was inhibited by GC tretment.
Conclusion: We provide molecular evidence for the anti-inflammatory actions of GCs in rheumatoid arthritis patient samples. The delineated pathway potentially provides new therapeutic options for the treatment of inflammatory diseases and their associated pain.

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P51

IL-27 REGULATES MONOPOIESIS AND MONOCYTE FUNCTION DURING ACUTE TOXOPLASMA GONDII INFECTION

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Abstract Content: IL-27 is an immuno-modulatory cytokine composed of p28 and EBi3 subunits. During infection with the protozoan parasite *Toxoplasma gondii*, mice that lack IL-27 show dramatic alterations in their responses to infection, culminating in severe, infection-driven immunopathology. While this pathology is driven by aberrant $CD4^+$ T cell responses, the events that proceed, and contribute to, this response are unclear. During *T. gondii* infection, IL-27 was produced in the bone marrow of infected mice and corresponded to waves of monocyte development in the bone marrow and seeding in the periphery. Hematopoietic stem-cells showed the highest expression of the IL-27 receptor in the bone marrow, and as early as three days post-infection substantial changes in monocyte development were apparent in IL-27 deficient mice. While granulopoiesis remained unaltered in these mice, monopoiesis was enhanced, corresponding to an enhanced monocyte response in the periphery. These monocytes showed significant enhancement in their TNFα and iNOS responses, which were mediated, at least in part, by $CD4^+$ T cells. These data suggest a novel role for IL-27 in biasing cell fate and restraining the induction of a monocytic response during infection.

Furthermore, IL-27 may have a previously unappreciated role in restraining HSCs from contributing to aberrant and pathological immune responses by balancing the ratio of innate to adaptive immune cells.

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P52

DIFFERENTIAL GENE REGULATION THROUGH TYPE I IFN DEPENDENT PATHWAYS IN PRIMARY MOUSE MYELOID CELLS

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Abstract Content: Monocytes and neutrophils reside in the bone marrow and can be found in the circulation at the steady state. Upon immune challenge, they are promptly recruited to sites of injury, infection and inflammation where they play a key role in protective immune responses and pathogen clearance, largely by their production of proinflammatory cytokines. However, if such responses are not properly regulated this may result in pathology and chronic inflammation, which in part can be controlled by the cytokine IL-10. Although both monocytes and neutrophils can respond to pathogen derived products, such as LPS, a component of gram-negative bacteria, each have different roles during infection. My research aims to investigate how responses to LPS and type I IFN are regulated in primary monocytes and neutrophils using *in vitro* and *in vivo* models of inflammation.

Herein, I show that *Il10* gene regulation in response to LPS is partially controlled by type I IFN in primary murine bone marrow monocytes and neutrophils, recapitulating published findings from our lab performed on *in vitro* differentiated bone marrow-derived macrophages. Furthermore, I show that primary bone marrow monocytes and neutrophils show differential transcriptional and epigenetic responses *in vitro* to LPS, which signals through the receptor TLR4. Monocytes and neutrophils showed different requirements to the autocrine signals resulting from type I IFN, which signals through the type I IFN receptor (IFNAR). Neutrophils showed a potent transcriptomic signature in response to LPS, which was partially reduced upon deletion of *Ifnar1*. On the contrary, monocytes showed a modest response to LPS, which was mostly dependent on the presence of type I IFN signalling. Additionally, complementary ATAC-sequencing data on these *in vitro* stimulated primary cells, revealed minimal chromatin remodelling upon LPS treatment in neutrophils only, but a considerable change in the accessibility of chromatin upon deletion of *Ifnar1* in primary monocytes and to a minimal degree in neutrophils.

To verify the physiological relevance of these findings, I assessed the dependency for type I IFN in shaping responses of monocytes and neutrophils to LPS *in vivo* in the peritoneal cavity in an LPS immune challenge model. In this *in vivo* model, I show that monocyte and neutrophil recruitment to the site of inflammation is partially decreased in the absence of IFNAR signalling. Furthermore, I am generating single cell RNA-sequencing and flow cytometry datasets in order to assess the transcriptional and cellular activation changes induced by TLR4 and type I IFN in monocytes and neutrophils *in vivo*. Integration of the *in vitro* and *in vivo* "omic" datasets produced by my research will be key to define the gene regulatory networks underlying TLR4 and type I IFN responses in myeloid cells, forming the basis of their response during *in vivo* immune challenges.

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P53

LOSS OF EPIGENETIC READER SP140 DRIVES CROHN'S DISEASE DUE TO UNCONTROLLED MACROPHAGE TOPOISOMERASES

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Abstract Content: How mis-regulated chromatin directly impacts human immune disorders is poorly understood. Speckled Protein 140 (SP140) is an immune-restricted PHD and bromodomain-containing epigenetic 'reader' and SP140 loss-of-function mutations associate with Crohn's disease (CD), multiple sclerosis (MS) and chronic lymphocytic leukemia (CLL). However, the relevance of these mutations and mechanisms underlying SP140-driven pathogenicity remain unexplored. Using a global proteomic strategy, we identified SP140 as a repressor of topoisomerases (TOP) that maintains heterochromatin and macrophage fate. In humans and mice, SP140 loss resulted in unleashed TOP activity, de-repression of developmentally silenced genes and ultimately defective microbe-inducible macrophage transcriptional programs and bacterial killing that drive intestinal pathology. Pharmacological inhibition of TOP1/2 rescued these defects. Furthermore, exacerbated colitis was restored with TOP1/2 inhibitors in $Sp140^{-/-}$ mice, but not wild-type mice, *in vivo.* Collectively, we identify SP140 as a TOP repressor and reveal repurposing of TOP inhibition to reverse immune diseases driven by SP140 loss.

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P54

CHARACTERIZING THE METABOLIC ROLE OF GSK3B IN IFNG-DEPENDENT MACROPHAGE ACTIVATION

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Abstract Content: IFNγ induces transcriptional and metabolic changes required for pathogen restriction. Specifically, IFNγ restricts pathogenesis by inducing the expression of antigen presentation machinery like MHCII. Previous work from our lab in bone marrow derived macrophages (BMDMs) suggests that the multifunctional kinase, GSK3β is a key regulator of IFNγ-dependent MHCII. Not only was GSK3β required to induce transcriptional activation of MHCII, but RNAseq analysis suggests broad changes to the IFNγ response in the absence of GSK3β. Recent work in our group suggests that changes in metabolism are essential for macrophages to respond to IFNγ. While GSK3β is known to directly modulate metabolism, its role in controlling metabolic changes following IFNγ activation remains unknown.Given the role of GSK3β in metabolic pathways and now in IFNγ responses, we hypothesize that the loss of GSK3β inhibits important metabolic shifts required for macrophages to effectively respond to IFNγ. Our lab recently developed an alveolar-like macrophage model (FLAMs) that we are using to test the role of GSK3β broadly in IFNγ-driven macrophage metabolism and activation. We are now using Seahorse assays to broadly test how the inhibition of GSK3β changes the rate of glycolysis and oxidative phosphorylation following IFNγ stimulation in both BMDMs and FLAMS. We will then directly target metabolic networks in the presence and absence of GSK3β to determine how these impact IFNγ responses including the expression of MHCII. Our findings will help define the requirements of successful host macrophage activation and host immunity.

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P55

TISSUE-SPECIFICITY OF IL-9R-EXPRESSING TUMOR-ASSOCIATED MACROPHAGES

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Abstract Content: The immunosuppressive function of myeloid cells that support tumor progression is controlled by secreted factors found in the tumor microenvironment. Interleukin 9 (IL-9) is a pleiotropic cytokine that signals through the IL-9 receptor (IL-9R) and can function as a positive or negative regulator in tumor immunity. Our recent work demonstrates that IL-9 signaling promotes tumor progression in a B16F10 mouse model of lung metastasis by expanding a CD11c+ interstitial macrophage population and inducing Arginase 1 (ARG1) activity. However, whether this IL-9R+ macrophage population is found in other cancer types remains unknown. Here, we use orthotopic models of breast cancer, colorectal cancer, melanoma, and a spontaneous model of colorectal cancer to identify IL-9R expressing myeloid cells that contribute to tumorigenesis. Using a 4T1 triple-negative breast cancer (TNBC) model, we identified a population of IL-9R expressing CD11b+ LY6C+ monocytes that are recruited to the tumor microenvironment. Furthermore, we found an IL-9R/ARG1+ macrophage population that is phenotypically similar to the pro-tumorigenic IL-9R/ARG1+ pulmonary macrophage population. In contrast, the spontaneous and orthotopic models of colorectal cancer and melanoma lack the IL-9R+ monocyte and macrophage populations. Thus, our work defines the tissue and tumor tropism of the IL-9R+ monocyte/macrophage populations and provides the basis for additional functional studies.

Disclosure of Interest: None Declared
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P57

SKIN SENSORY NEURONS DOWNREGULATE MYELOID-DERIVED IL-33 FOR IL-17-DEPENDENT IMMUNITY AGAINST SCHISTOSOMA MANSONI.

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Abstract Content: Interleukin 33 (IL-33) is considered an alarmin cytokine produced by damaged structural cells at barrier sites including the skin to elicit immune responses against injurious substances. However, it is becoming increasingly clear that IL-33 is expressed by diverse cellular sources that mediate divergent biological functions, obscuring our understanding of how IL-33 production is regulated. This work demonstrates that IL-33 is expressed by both hematopoietic and non-hematopoietic cell lineages at baseline, most notably in cutaneous dendritic cell (DC) and macrophage subsets. Our data show that selective deletion of IL-33 in CD11c-expressing cells resulted in a basal increase of dermal IL-17/IL-23 and γδ T cell responses accompanied by increased keratinization and epidermal thickening. Single-cell RNAseq analysis revealed that myeloid-specific IL-33 deficiency increased cell intrinsic expression of IL-17 inducing cytokines (e.g.IL-12p40, IL-18 and IL-1β,) in DC and macrophage subsets under steady-state conditions. This phenotype was consistent with enhanced IL-17 or IL-23-dependent resistance to percutaneous infection with the helminth *Schistosoma mansoni,* in mice with myeloid-specific IL-33 deficiency, as compared to their littermate controls. Unexpectedly, regulation of myeloid-specific IL-33 was dependent upon skin sensory neurons, inasmuch as neuron activation using optogenetics selectively reduced IL-33 protein content in skin cDC2 and tissue macrophage subsets, accompanied by increased IL-17-expressing γδ T cell responses and resistance to *S. mansoni* infection. Moreover, stimulation of bone marrow derived macrophages with supernatants derived from activated neurons caused a reduction in IL-33 protein content and increased pro-inflammatory cytokine secretion. Overall, these data support a hypothesis that sensory neurons can curtail IL-33 expression in myeloid cells to unleash their cytokine secretion that promotes the expansion of IL-17 producing γδ T cells for host protective immunity against skin-penetrating parasites.

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P59

PIM1 REGULATES M1 MACROPHAGE POLARIZATION

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Abstract Content: The proviral integration site for Moloney murine leukemia virus 1 (Pim1) protein is a highly conserved serine/threonine kinase. Numerous studies demonstrated that Pim1 is associated with diverse types of cancer via regulating diverse cellular pathways such as cell survival, growth, proliferation and apoptosis. However, the role of Pim1 in innate immune response is largely unknown.
Here, we demonstrated that Pim1 can regulate M1 macrophage polarization. We generated Pim1-/- mice using the CRISPR/Cas9 system and all mice were viable, fertile and normal in teeth. Although Pim1-/- mice exhibited reduction in body size compared with WT littermates, the development of innate immune cells were comparable. By contrast, we found that Pim1 deletion promotes bone marrow-derived macrophages to polarizate into M1 macrophages. The expression levels of M1 macrophage marker NOS2 and TNF-a were markedly increased with Pim1-/- macrophages. This study provides a new insight that Pim1 may be a new immunomodulatory target for macrophages.

Disclosure of Interest: None Declared
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P60

INFLUENZA-INDUCED HOST DEFENSE AGAINST BACTERIAL INFECTIONS BY MONOCYTE-DERIVED ALVEOLAR MACROPHAGES AND NEUTROPHILS

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Abstract Content: At the steady state in humans and mice, macrophages residing in the alveolar space (AMs) are derived from fetal progenitors and maintained by self-renewing in situ, hence are called tissue resident AMs (ResAMs). Using a murine model, we have previously shown that once the AM niche was perturbed following severe influenza infection, it could be repopulated with monocyte-derived AMs (MoAMs). We demonstrated that MoAMs were retained in the lung long after influenza infection was resolved, without the additional contribution of bone marrow-derived cells. At 1 month post flu, we observed increased Streptococcus pneumoniae (Strep) survival, and MoAMs were crucial for this phenotype. This could partially be explained by elevated cytokine production, including IL-6, by MoAMs.
In addition to being high producers of cytokines, MoAM gene expression was enriched in pathways associated with the immune response when compared to ResAMs at 1 month post flu. Gene set enrichment analysis of RNAseq also identified Il6 as a top gene in the immune response pathway in stimulated MoAMs. Further analysis of RNAseq data identified scavenger receptors, including Marco, as top downregulated genes in MoAMs, compared to their resident counterparts. Indeed, we found that ResAMs had high expression of MARCO, and could further upregulate it after stimulus, whereas MoAMs failed to express MARCO as their surface receptor. As MARCO is known to play an important role in mediating phagocytosis, we studied Strep uptake by AMs in 1 month post flu mice, and found that MoAMs had reduced phagocytic capacity. Nevertheless, flu-experienced mice had elevated defense against Strep infection, so we investigated the involvement of other immune cells. We found increased levels of neutrophils in bronchoalveolar lavage after Strep stimulus in post-flu mice, but also in other models where MoAMs were present. We are currently investigating whether increased neutrophil recruitment is dependent on Mo-AMs or other lung cells that received an imprint during primary infection. Also, we are testing which chemoattractants are involved in increased neutrophil recruitment to fight bacterial infections.
The importance of this research is highlighted by the current COVID-19 pandemic. As healthy individuals previously were thought to maintain ResAMs throughout their lifetime, it is reasonable to speculate that SARS-CoV-2 infection, especially in severe cases, could lead to depletion of ResAM population and subsequent repopulation with monocyte-derived cells (MoAMs), as observed in our murine model using influenza A virus. Therefore, it is crucial to assess MoAMs in regard to their immunoreactivity, as well as their direct and indirect roles in fighting subsequent infections for prolonged time.

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P61

LONG NONCODING RNA LUCAT1 REGULATES INFLAMMATORY GENE EXPRESSION IN HUMAN MACROPHAGES

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Abstract Content: Immune cells including macrophages rely on a dynamic immune gene expression program to confer immunity against pathogens. Emerging evidence indicates that long noncoding RNAs (lncRNA) represent a novel regulatory layer in the immune gene expression program. LncRNAs are expressed in all immune cells in context-specific manner, although their biological function remains largely unexplored. Here we describe the expression, function and the mechanism of action of a TLR4-regulated lncRNA, LUCAT1 (Lung Cancer Associated Transcript 1), as a novel immunoregulatory lncRNA in human macrophage. LUCAT1 is differentially expressed in a variety of tumor cells; however, its physiological function in immune cells remain largely unclear. Our results indicate that LUCAT1 is expressed in resting human neutrophils, monocytes, and macrophages. Using RACE, Sanger sequencing and subcellular fractionation, we demonstrate that *LUCAT1* gene uses an alternative polyadenylation sequence to produce two major isoforms with distinct cellular localization - a 890 bp long cytosolic transcript and a predominant, 2.5 kb long nuclear RNA. LUCAT1 expression is highly induced via ERK-MAPK-AP1 axis in TLR4-activated macrophages. Transient perturbation of LUCAT1 expression, coupled with bulk RNA-seq analysis, reveal that LUCAT1 regulates the expression of a subset of TLR4-induced genes including cytokines and chemokines (*CSF3, IL24, CXCL5* and *CXCL6*), matrix metalloproteinases (*MMP1* and *MMP3*) and others. Further, we demonstrate that the nuclear LUCAT1 and not the cytosolic transcript is the functional modulator of immune gene expression. The nuclear LUCAT1 mediates these functions by promoting the transcription of its target genes by associating with chromatin. Ongoing studies are focused on identifying LUCAT1-interacting nuclear proteins, and understanding how LUCAT1 is recruited to specific genomic loci in macrophages. Our study highlights the emerging paradigm that lncRNAs are important components of the gene regulatory circuits of the immune system.

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P62

TYPE I IFN INDUCTION AND RESPONSE DURING MTB INFECTION OF HUMAN MONOCYTE DERIVED MACROPHAGES (HMDMS): FOCUS ON TYPE I IFN'S ROLE IN MTB INDUCED HMDM CELL DEATH

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Abstract Content: Macrophages are both critical for the control of Mycobacterium tuberculosis (Mtb) infection and a niche in which Mtb can grow. Non-apoptotic cell death of macrophages has been implicated in promoting Mtb pathogenesis by inhibiting efferocytosis of infected cells and allowing for bacterial spread. Type I interferons (IFNs) have been found to mediate non-apoptotic, Mtb-induced cell death in both murine cell line and primary macrophages. Although Mtb infection induces both murine macrophages and human macrophage cell lines to secrete Type I IFNs, it is unclear whether primary human macrophages upregulate Type I IFNs during Mtb infection. It is also unknown whether Type I IFNs play a role in Mtb-induced cell death in these cells. Here we show that although human monocyte-derived macrophages (hMDMs) are capable of secreting Type I IFNs in response to stimulator of IFN genes (STING) activation and express IFN stimulated genes (ISGs) in response to Mtb infection, Type I IFN proteins are not detectable in the supernatant of Mtb infected hMDMs. Additionally, we show that Mtb infected hMDMs undergo cell death in an ostensibly Type I IFN independent manner, but that addition of Type I IFNs during Mtb infection induces greater hMDM cell death. These results indicate that Type I IFN arising from other cells may play a role in human

macrophage cell death in vivo and therefore in Mtb pathogenesis. Furthermore, these results highlight a potential deviance in the innate immune response to Mtb between mice and humans and between transformed human cell lines and primary human macrophages.

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P63

AGE SPECIFIC DIFFERENCES IN MURINE ALVEOLAR ENDOTHELIAL CELLS TO PATHOLOGICAL CONDITIONS

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Abstract Content: Neonatal pulmonary viral infections can have diverse outcomes ranging from bronchiolitis and pneumonia in respiratory syncytial virus (RSV) infection to more subdued response in SARS-CoV-2 infection. In this study, we compared the murine lung alveolar endothelial cell (MLEC) responses of neonatal and adult mice to toll-like receptor agonists representing viral stimuli as well as the ability of neonatal serum to modulate endothelial cell responses. In this study, we investigated the responses of murine alveolar endothelial cells (MLEC) to neonate mice serum in presence of Poly I:C or ssRNA40 activated macrophages, which can mimic SARS-Cov2 induced innate cell activation. We found that when neonatal and adult MLECs were cocultured with macrophages activated with poly I:C and ssRNA40, significantly decreased TNFα and elevated IL-6 were detected in macrophages. Macrophage co-culture with adult MLECs downregulated ACE2 and kdr (VEGFR2) regardless of the stimulation conditions. In neonatal MLECs, stimulation with Poly I:C or ssRNA downregulated ACE2 expression when macrophages were not present. In the presence of macrophages, the expression of ACE2 in neonatal MLECs increased with Poly I:C stimulation but decreased with ssRNA40 stimulation. Addition of neonatal mouse serum to MLECs increases the reactive oxygen species but downregulates multiple inflammatory signal pathways. Concurrently, significantly decreased expression of VE-cadherin and Shp2 were detected in neonatal mice exposed MLECs. In addition, neonatal serum compromised MLEC barrier integrity whereas adult mouse serum did not. Morphologically, neonatal mouse serum, but not adult mouse serum triggered the formation of lipid droplets in MLECs by upregulating the expression of scavenger receptors SR-BI and CD36. Taken together, neonatal and adult MLECs differentially regulate cytokine-secretion from activated macrophages, and neonatal serum breaks the tight junction formation between MLECs by down regulating Shp2 and VE-Cadherin expression and induces the formation of lipid droplets in MLECs through receptors SR-BI and CD36. The differences in neonatal and adult MLEC responses to pathologic conditions may reflect the age specific pulmonary manifestations to viral infections.

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CROSSTALK BETWEEN BMP2-PRODUCING MACROPHAGES AND CSF-1-SECRETING TISSUE CELLS REGULATES MACROPHAGE SURVIVAL

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Abstract Content:
Introduction: The intestine is a complex organ comprised of diverse sets of tissue compartments working together to carry out critical functions including nutrient absorption and immunological tolerance against orally ingested antigens. Macrophages are key regulators within the intestine that survey commensal microbes, tissue niches, and other immune cells to promote optimal tissue function and immunity. Characterization of the gut macrophage population has revealed substantial heterogeneity, particularly when comparing cells from different microanatomic locations of the intestinal tract. Specifically, at least three distinct subsets have been identified based on the surface

expression of CCR2 and Tim4, marking monocyte-derived and tissue resident macrophages, respectively. This suggests that heterogeneity may be driven by niche specific regional specialization and maintenance of macrophages. Indeed, macrophages residing in the muscularis layer of the intestine have been shown to form a codependent relationship with enteric neurons, wherein macrophage derived bone morphogenetic protein (BMP)2 enabled enteric neurons to modulate intestinal motility, while the neurons provided the macrophages with colony stimulating factor (CSF)1. We have recently identified that Tim4+ macrophages outside of the muscularis layer also produce BMP2. However, little is known about what effects BMP2 has on surrounding tissue cells and what mechanisms regulates macrophages within the microanatomic niches of the gut.
Methods: To determine the role of macrophage-derived BMP2 on the tissue cells and the subsequent feedback on the intestinal macrophage populations, we developed a novel conditional knockout mouse model that allows for inducible macrophage-specific labelling with simultaneous abrogation of macrophage-derived BMP2. Immunofluorescence, RNAscope, qPCR, and flow cytometry analyses of these mice was carried out to characterize changes in the intestinal tissue following BMP2 deletion.
Results: We report a critical role for BMP2 in sustaining intestinal macrophage numbers by regulating the CSF1 production of surrounding tissue cells. Strikingly, BMP2-deficiency had little effect on CSF1-independent macrophages, suggesting a possible macrophage-BMP2-tissue cells-CSF1 axis that regulates intestinal macrophage homeostasis and diversity. This would suggest that the BMP2-CSF1 crosstalk between macrophages and neurons in the muscularis could extend to additional macrophage-tissue compartments and may be a system wide feedback loop that regulates macrophage heterogeneity and tissue function.
Conclusions: Taken together, this project reveals an important and previously unknown role for a distinct niche of tissue resident macrophages in cytokine signaling that not only affects their survival through crosstalk but may also control tissue cell survival and function.

Disclosure of Interest: None Declared
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P65

HIGH-THROUGHPUT IDENTIFICATION OF NOVEL INHIBITOR(S) TARGETING NLRP3 INFLAMMASOME ACTIVATION

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Abstract Content: The Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome plays a key role in the innate immune response by activating caspase-1, resulting in the generation and secretion of mature IL-1β and IL-18. Dysregulation of this pathway has been associated with several autoimmune and inflammatory disorders. Cytidine/uridine monophosphate kinase 2 (CMPK2), a key enzyme of the salvage pathway of mitochondrial DNA (mtDNA) synthesis, provides new mtDNA that, when oxidized, activates the NLRP3 inflammasome (Shimada et al., 2012, Zhong et al., 2018). Ablation of CMPK2 in mouse myeloid cells prevents endotoxin induced acute respiratory distress syndrome (Xian et al., 2021). Using CRISPR-mediated knockdown of CMPK2 in primary human macrophages, we further validate the role of CMPK2 in NLRP3 inflammasome activation and strengthen the rationale for targeting the pathway in human diseases. To identify CMPK2 inhibitors, we performed high throughput screening of small molecule libraries using an enzymatic kinase assay. Our efforts led to the identification of several tractable chemical series which were further evaluated in cell-based assays. Our early hits demonstrate modulation of the NLPR3 inflammasome pathway across several orthogonal assays. Ongoing biology and chemistry efforts are aimed at further characterizing and improving the physiochemical properties of these series of compounds as potential treatments for autoimmune and inflammatory diseases.
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P66

AN UNLIKELY PAIRING: A ROLE FOR IL-17 IN SARS-COV2 ORF8-INDUCED MACROPHAGE ACTIVATION?

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Abstract Content: COVID-19, caused by the novel SARS-CoV-2 virus, has contributed to over 6 million deaths worldwide. The SARS-Cov-2 accessory protein, open reading frame 8 (ORF8), plays a key role in eliciting proinflammatory responses during infection and mediates immune evasion through down-regulation of MHC-I. Recently, two papers suggested that ORF8 activates the IL-17R signaling pathway, promoting expression of proinflammatory factors that contribute to a cytokine storm. These reports provided data that secreted ORF8 may bind to human IL-17RA and IL-17RC and trigger signaling in mouse and human bone-marrow derived macrophages (BMDMs) and THP-1 cells. These results were very surprising because the vast majority of literature over 20 years has indicated that IL-17 does not signal on most hematopoietic cells due in part to low IL-17RC expression. Rather, IL-17 is a key player in mediating immunity in non-hematopoietic cells, such as epithelial and mesenchymal cells. Accordingly, we were compelled to re-visit the role of IL-17 and ORF8 in macrophages and monocytes. Here, we demonstrate that ORF8 but not IL-17 can promote gene expression of inflammatory factors in mouse BMDMs and human monocytes. Studies in IL-17RA- and Act1-deficient BMDMs will be presented, and we speculate that cell contamination and TLR-inducing contaminants may underlie these conflicting findings. We propose instead that ORF8-induced gene expression occurs via an IL-17R independent mechanism. Our study confirms that IL-17 does not function significantly in hematopoietic cells and aims to resolve distinct mechanisms of IL-17 and ORF8 induction in macrophages and monocytes.

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P67

CXCL10 CONTRIBUTES TO IMMUNE PRIMING BY TLR2-DEPENDENT RECOGNITION OF EXTRACELLULAR FILAMENTOUS ACTIN RESULTING IN IMPROVED CLEARANCE OF STAPHYLOCOCCUS AUREUS.

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Abstract Content: Background: Toll like receptor 2 (TLR2) is a promiscuous pattern recognition receptor known to be triggered by a range of pathogen-associated as well as non-pathogen-associated molecular patterns, such as repeating arrangements of subunits within proteins, lipoproteins, and glycoproteins. Previously, we found that TLR2 can distinguish repeating protein subunit pattern (RPSP) exhibited by virus exteriors and by host cellular proteins. As such, we found that viral RPSP was recognized via TLR2/6 heterodimer and resulted in induction of classical anti-

viral responses. Conversely, RPSP exhibited by cellular proteins like filamentous actin (F-actin) or ferritin, was recognized via TLR2/1 heterodimer. Further, we found that TLR2-dependent recognition of F-actin, but not its globular monomeric form resulted in improved clearance of subsequent pulmonary infection with *Staphylococcus aureus (S. aureus)*. This protective phenotype was intriguing because prior to *S. aureus* challenge, intratracheal F-actin inoculation did not trigger neutrophil recruitment or result in any cellular or cytokine inflammation.

Results: We found that unlike saline, F-actin inoculation primed wild type (WT) mice to produce CXCL10 after *S. aureus* challenge only. Being one of the markers of trained immunity, CXCL10 is known to regulate function of monocytes and macrophages. Using both clodronate liposomes-based depletion and adoptive transfer approaches we found that alveolar macrophages are both required and sufficient for improved *S. aureus* clearance following F-actin inoculation. Because F-actin inoculation of TLR2-deficient mice did not result in improved *S. aureus* clearance or CXCL10 production, we next sought to determine whether CXCL10 contributes to this TLR2-driven protective phenotype. Treatment of naïve WT mice with murine recombinant CXCL10 resulted in similar improvement in *S. aureus* clearance, whereas antibody blocking of CXCL10 cognate receptor CXCR3 reduced the protective effect of F-actin. While at this time the precise cellular source of CXCL10 produced in response to pulmonary F-actin inoculation remains unknown, studies in WT:TLR2$^{-/-}$ bone marrow chimeric mice revealed that TLR2 expression only on cells of non-hematopoietic origin was required for F-actin induced improved *S. aureus* clearance. This suggests that while macrophages are likely targeted by CXCL10 for improved responsiveness to bacterial pathogens, other cell types, such as respiratory epithelium, may be producing CXCL10 after TLR2-based F-actin recognition.

Conclusions: As a cytoskeletal protein F-actin is expressed intracellularly by healthy cells, and its extracellular presence likely serves as a danger signal alerting innate immune cells of either infection or injury. Importantly, our evidence suggests that TLR2-based mode of F-actin recognition on the respiratory mucosal surface does not induce classical DAMP-like inflammatory response. Instead, our data suggests that it primes lung resident alveolar macrophages for enhanced responsiveness to a subsequent *S. aureus* infection. Understanding the mechanisms involved in TLR2 immune priming and subsequent protective effects could help to inform rational design of therapies or treatments to mitigate immune-responses to infection or damage.

Disclosure of Interest: None Declared
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P68

APOPTOSIS-ASSOCIATED SPECK-LIKE PROTEIN CONTAINING A CARD (ASC)-MEDIATED RELEASE OF MATRIX METALLOPROTEINASE 10 (MMP10) STIMULATES A CHANGE IN MICROGLIA PHENOTYPE

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Abstract Content: Inflammation contributes to amyloid-β and tau pathology in Alzheimer's disease. Microglia facilitate this altered immune response that includes microgliosis, upregulation of inflammasome proteins, and elevation of matrix-metalloproteinases (MMPs). Studies of cerebrospinal fluid and blood in dementia patients show upregulation of two potential biomarkers of inflammation at the cellular level, MMP10 and apoptosis-associated speck like protein (ASC). However, little is known about their relationship in the context of brain inflammation. Therefore, we stimulated primary mouse microglia cultures with ASC and MMP10 to elucidate their role. We found that assembled ASC speck aggregates stimulated the release of MMP3 and MMP10 when measured with ELISA and mesoscale discovery, respectively. We then investigated the impact of this MMP10 release further in primary microglia and determined that MMP10-stimulated microglia displayed morphofunctional changes indicative of a proinflammatory phenotype. To quantify cell morphology, immunocytochemistry was conducted with the microglia specific marker, IBA1. Morphological measurements indicated that MMP10-exposed microglia displayed a change in appearance consistent

with a shift from a rod-like steady state to a more ameboid shape. The proinflammatory phenotype was confirmed, as MMP10-treated microglia released additional MMP10 along with the inflammatory cytokine, tumor-necrosis factor-α (TNFα). A broad spectrum MMP inhibitor, GM6001, prevented morphology changes and TNFα release. With this, we conclude that MMP10 and ASC act on microglial cells to propagate inflammation. In the future, we will investigate the molecular mechanisms responsible for ASC-mediated release of MMP10 in microglia. Furthermore, we will evaluate the potential targets of MMP10 present on microglia that lead to TNFα release. Potential targets of MMP10 include β-integrin, protease-activated receptor (PAR1), and TNFα Receptor 1.

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P69

FC-MDNA413 IS A NOVEL LONG-ACTING IL-4/IL-13 SUPER-ANTAGONIST THAT SUPPRESSES M2A TAM SKEWING AND IN VIVO TUMOR GROWTH INCLUDING SYNERGY WITH AN IL-2 SUPER-AGONIST

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Abstract Content: Introduction:
The IL-4/IL-13 pathway promotes an anti-inflammatory (Th2) tumor microenvironment (TME) by stimulating M2a skewing of tumor associated macrophage (TAM) and myeloid derived suppressor cells (MDSCs), resulting in suppression of the Th1 response and promotion of tumor growth. Therefore, inhibition of IL-4/IL-13 signaling has the potential to inhibit tumor growth by shifting the TME towards a pro-inflammatory condition. We engineered and characterized a long-acting Fc fusion IL-13 dominant-negative antagonist (Fc-MDNA413) to target the IL-13Ra1 component of type II IL-4 receptor (IL-4Ra/IL-13a1) expressed on TAMs and MDSCs to inhibit their differentiation and expansion for immuno-oncological (IO) applications.

Experimental Procedure:
Studies conducted include binding analyses by Biacore/SPR, signaling analyses in IL-4/IL-13 cell reporters, in vitro M1/M2 macrophage polarization and in vivo efficacy in syngeneic tumor models.

Summary of Data:
In comparison to Fc-IL13 control, Fc-MDNA413 exhibits higher affinity for IL-13Ra1 (~10-fold increase) and lower binding to the non-functional IL-13Ra2 decoy receptor (~30-fold decrease), indicating increased selectivity towards the functional IL-4Ra/IL-13Ra1 receptor complex. In a cell-based p-STAT6 reporter assay, Fc-MDNA413 showed dose-dependent inhibition of IL-4 and IL-13 induced signaling. Fc-MDNA413 antagonism was further confirmed in a macrophage polarization assay with human PBMCs where inhibition of IL-4 and IL-13 induced M2 polarization was observed in a dose-dependent manner, consistent with an inhibitory effect on IL-4/IL-13 signaling. In mice, Fc-MDNA413 has a longer half-life than scaffold-free MDNA413 (or rh IL-13) but exposure was reduced more rapidly than anticipated of Fc fusion construct, suggestive of rapid target engagement following administration. Treatment of B16F10 melanoma tumor bearing mice with Fc-MDNA413 resulted in tumor growth inhibition, supporting the potential of inhibiting IL-4/IL13 signaling and ensuing Th2 response for IO therapy. The effect was however relatively modest, consistent with a weak adaptive (Th1) response towards non-immunogenic B16F10 tumors. Accordingly, combination treatment with Fc-MDNA413 to suppressive Th2 response and a long-acting IL-2 super-agonist (MDNA19) to boost Th1 response resulted in synergistic tumor growth inhibition. These data demonstrate the potential of Fc-MDNA413, particularly in combination with an IL-2 super-agonist, to therapeutically target immunologically ‘cold’ cancer.

Conclusion:
Fc-MDNA413 is a long-acting IL-4/IL-13 super-antagonist demonstrating in vivo therapeutic activity as monotherapy and in synergy with IL-2 agonist. Ongoing studies are investigating the effect of monotherapy and combination therapy on tumor infiltrating immune cells to understand the underlying mechanism of therapeutic response.

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P70

COMPARATIVE ANALYSIS OF CYTOKINE RELEASE BY MONOCYTE-DERIVED HUMAN M1 AND M2 MACROPHAGES AND SC-DERIVED MACROPHAGES

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Abstract Content: Analysis of human macrophages is generally hampered by the necessity to differentiate them from peripheral blood monocytes and the inability to expand them *ex vivo*. We have analyzed to which extent the non-cancerous SC monocyte cell line could be used as an *in vitro* macrophage model. Macrophages differentiated from peripheral monocytes using standard CSF1 and CSF2 protocols for M2 and M1 precursors, respectively, were compared to THP-1-derived macrophages treated with PMA, and to SC-derived macrophages differentiated either by CSF1, CSF2, or PMA according to different protocols. The optimal condition for generation of SC-macrophages was treatment with PMA for 3 days, followed by 5-days culture without PMA, and 24-hours polarization with LPS/IFN-γ or IL-4/IL-13. Similar to THP-1, SC cells do not express the monocyte marker CD14 and differentiation to macrophages results neither in CD68 nor in CD14 expression, both of which were expressed by monocyte-derived macrophages. Similar to THP-1-macrophages, a proportion of SC-macrophages can be polarized to the M1-like subtype that is characterized by higher expression of CD38, CD86, CD80, TNF-α, and IL-1ra, whereas treatment with IL4/IL13 did not lead to expression of the M2-associated receptors. Still, SC-M1 express much lower levels of the M1-associated markers compared to monocyte-derived M1, and produce neither IL-1β, nor IL-10, or CCL22, the cytokines released in high amounts by MΦ-M1 and partially also by THP-1-M1. Similarly, SC-M2- and THP-1-M2-macrophages exhibited deficient phagocytosis when compared to monocyte-derived macrophages. Hence, the data demonstrate that SC-derived macrophages differ from monocyte-derived macrophages in respect of their morphology and expression of important macrophage receptors and cytokines. Yet, polarized SC-M1-like cells may with restrictions serve as a model for M1 macrophages, though this model does not provide significant advantages over already well-described THP-1-M1-like cells.

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P71

M-CSF/IL-34-DIFFERENTIATED BONE MARROW CELLS DIFFERENTIATE INTO MICROGLIA-LIKE CELLS

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Abstract Content: The increase in diseases is becoming more serious with the advent of an aging society. Alzheimer's disease (AD) is one of the most common causes of dementia in old age. There is still no effective treatment because of a limited information of mechanism of pathogenesis. The onset mechanism of AD is complicated, and several theories have been put forward. Accumulation of amyloid β42 (Aβ) is thought to cause neuronal cell death and neurodegeneration. Microglia, the immune cells present in the brain, are thought to play an important role in the neuroinflammation of AD. In this study, we investigated the establishment of preparing microglia-like cells from bone marrow cells. Bone marrow cells were differentiated 7 days with M-CSF/IL-34 (M/34) or GM-CSF for microglia-like cells or macrophages respectively. By confocal microscopy, branching out like microglia in M/34-differentiated (M/34-D) cells was observed. Flow cytometry analysis demonstrated that M/34-D cells expressed CD11b-high, CD11c-low, CD14-int, F4/80-high, TLR4-int. IBA1 is commonly used as a marker for microglia. Mean fluorescence intensity level of IBA1 in M/34-D cells is 1.5 times or more higher than that of differentiated by GM-CSF cells (GM-D cells). Western blotting demonstrated that the level of IBA1 in M/34-D cells significantly increased. After treatment with red fluorescent particulate matter (PM) or green fluorescent Aβ for 3 h with or without a prior 1 h treatment of dynamin inhibitors, cells were harvested and analyzed by flow cytometry. M/34-D cells endocytosed more 0.1 μm PM compared to 1 μm PM.

Inhibitors for the PH domain of dynamin, OcTMAB and MiTMAB decreased endocytosis level of PM. These results suggest that M/34-D cells has a function resembling microglia.

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P72

FEN1-GENERATED OXIDIZED DNA FRAGMENTS ESCAPE MITOCHONDRIA VIA MPTP- AND VDAC-DEPENDENT CHANNELS TO LICENSE NLRP3 INFLAMMASOME AND STING ACTIVATION

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Abstract Content: Mitochondrial DNA (mtDNA) escaping stressed mitochondria provokes inflammation via the cGAS-STING pathway and when oxidized (Ox-mtDNA) it binds cytosolic NLRP3, triggering inflammasome (Inf) activation. However, it is unknown how and in which form Ox-mtDNA exits stressed mitochondria in non-apoptotic macrophages. We show that diverse NLRP3Inf activators rapidly stimulate calcium uptake via the mitochondrial calcium uniporter (MCU) to open mitochondrial permeability transition pores (mPTP) that trigger VDAC oligomerization independently of mtDNA or reactive oxygen species that convert newly synthesized mtDNA to Ox-myDNA. Within mitochondria, Ox-mtDNA is repaired by DNA glycosylase OGG1 or cleaved by endonuclease FEN1 to 500-650 bp fragments that are released via mPTP- and VDAC-dependent channels to initiate NLRP3Inf activation. This pathway also leads to cGAS-STING activation and generation of pro-inflammatory extracellular mtDNA. Its improved understanding will facilitate development of new treatments for chronic inflammatory diseases, exemplified by FEN1 inhibitors that suppress IL-1b production and mtDNA release in mice.

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P73

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

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Abstract Content: Macrophages are highly plastic cells of innate immune system with diverse functions in host pathogen defense and regulation of tissue homeostasis. Despite their relative abundance in the tumor microenvironment (TME), the exact functions of tumor-associated macrophages (TAMs) and how they are regulated in TME remain largely unknown. Macrophages are also key players in various metabolic processes including lipid metabolism and its dysregulation can result in perturbed macrophage functions and pathologies. Since TME has been characterized as a “lipid-rich” environment by various recent studies, we are interested in understanding how lipids in TME regulate TAMs' metabolism and their pro- or anti-tumor functions.
In this research, we firstly characterized the lipid metabolic phenotype of TAMs in several mouse tumor models. We found that tolerogenic subset of TAM (marked by high expression of F4/80 and PD-L1) is highly lipid-laden and has greater ability to import lipids comparing to other subsets. We also found F4/80^{hi} TAMs highly express scavenger receptor CD36 which mediates uptake of oxidized low density lipoprotein (oxLDL) from TME. Using germ line knockout (*Cd36*$^{-/-}$) and myeloid-specific knockout (*Cd36*$^{flox/flox}$ *x Csf1r-Cre*) mouse models, we observed that uptake of oxLDL by TAMs was significantly blocked, along with decreased expression of immunosuppressive molecules PD-L1 and CD206, and elevated secretion of inflammatory cytokines (TNF, IL-12, IL-1b) and type I interferons by CD36-deficient TAMs. Altogether, this reprogrammed TAM functional state mediated by CD36 knockout leads to significantly slower tumor growth. We further conducted single-cell RNA sequencing on CD36-deficient and wild-type TAMs and found inflammatory response and type-I interferon response pathways were upregulated in CD36-deficient TAMs. We also

showed that the upregulation of type-I IFN response in CD36-deficient macrophages is cell-intrinsic using *in vitro* bone-marrow derived macrophage model; and type-I IFN production is critical for TAM-mediated tumor control.
In summary, our data suggests that scavenger receptor CD36 plays critical roles in functional reprogramming of tumor-infiltrating macrophages through inhibiting inflammatory cytokine production; and blocking of CD36 may have beneficial effects for cancer immunotherapy.

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P74

CHRONIC PARASITE INFECTION RESHAPES SYSTEMIC VIRAL INFECTION BY ALTERING RETINOIC ACID METABOLISM IN TISSUE RESIDENT MACROPHAGES.

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Abstract Content: Chronic infections have the potential to dramatically alter tissue resident populations of cells, such as macrophages, and thus change the course of subsequent infections. In the peritoneal cavity, large peritoneal macrophages (LPMs) are the most abundant macrophage population in an uninfected mouse and contribute to homeostasis of the peritoneal cavity by phagocytosing apoptotic cells. During inflammation of the peritoneal cavity, LPMs often “disappear”, localizing to the omentum or the periphery of the peritoneal cavity. In contrast, chronic intestinal parasite infection expands LPMs. In order to examine the dynamics of the tissue resident macrophage population during chronic infections, we used a two-pathogen coinfection model. We first infected with an intestinal helminth, *Heligmosomoides polygyrus* (HP), which is known to induce a strong Th2 immune response and promotes LPM expansion. A week later when the helminth infection is established, we infected with murine gammaherpesvirus-68 (MHV68) via intraperitoneal injection. MHV68 is the mouse model for the human herpesviruses, Epstein-Barr virus (EBV) and Kaposi’s sarcoma-associated herpesvirus (KSHV), which cause lymphoproliferative disorders in immunocompromised people. Gammaherpesviruses chronically infect macrophages, B cells, and dendritic cells, all cell types that are found in the peritoneal cavity. We found that the helminth infection induced expansion of the LPMs and maintained this expansion in the coinfected mice, during both early and late timepoints of the viral infection, in contrast to mice that are only infected with MHV68. Further, we found that HP-induced LPM expansion is independent of STAT6, which differs with literature on helminth-induced large cavity macrophage expansion. We also found that there are more infected LPMs in the peritoneal cavity of coinfected mice during acute replication of the virus. Because MHV68 is a virus that establishes latent infection, we examined whether parasite coinfection altered MHV68 latent infection and reactivation from latency. We found that infection and latency were increased in LPMs of coinfected mice. In addition to increased infection, coinfected mice had increased reactivation of the virus from latency. Further, the increased infection and reactivation were independent of STAT6, but are dependent on vitamin A. Vitamin A is metabolized to retinoic acid, which is required by LPMs. The LPMs from helminth infected mice had increased capacity for retinoic acid production and altered expression of retinoic acid metabolism genes. Taken together, our data suggest that intestinal helminth infection changes systemic viral infection by altering retinoic acid metabolism in peritoneal tissue resident macrophages.

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P75

RNASE L SUPPRESSES THE EXPRESSION OF CYTOKINES IN MACROPHAGES INDUCED BY S-PROTEIN

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Abstract Content: Introduction

Coronavirus disease 2019 (Covid-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has created a public health crisis and devastating economy across the globe. The significant increase and hyperactive nature of inflammatory myeloid cells are generally considered as a key hallmark of the disease pathogenesis. The production and release of proinflammatory cytokines from hyperactivated myeloid cells such as macrophages mainly contribute to the development of cytokine release syndrome (CRS), i.e. a cytokine storm, leading to severe COVID-19. Control of the cytokine storm using immunomodulators, cytokine antagonists and down regulation of cytokine expression in myeloid cells is essential for improving the survival rate of patients with severe COVID-19. Current medical intervention consists primarily of disease prevention via vaccination, or early disease treatment with antivirals or neutralizing antibodies. However, taming the cytokine storm is crucial for treating late stage COVID-19. Such treatment is urgently needed. In this study, we revealed the role of RNase L, an interferon (IFN) inducible enzyme, in suppressing the expression of proinflammatory cytokines in macrophages induced by the spike protein (S-protein) of SARS-CoV-2.
Methods
RNase L knockdown and wild type mouse macrophages (Raw 264.7) were treated with S-protein for various times. The expression of anti- and pro-inflammatory cytokines was measured by Western blot analysis, RT-PCR and ELISA. The activation of the signaling pathways induced by S-protein was determined by analyzing the phosphorylation status of the components in the pathways. Bone marrow derived macrophages (BMDMs) from RNase L wild type and knockout mice were used to further demonstrate the results.
Results
Deficiency of RNase L enhanced the expression of a variety of cytokines and chemokines in macrophages upon the stimulation with S-protein. Further investigation of the molecular mechanism revealed that RNase L suppressed the activation of the TLR4 signaling pathway induced by S-protein. In addition, deficiency of RNase L attenuated the phagocytic functions of macrophages after activation by S-protein.
Conclusion
The studies can shed mechanistic insight into the role of RNase L in regulating macrophage functions and cytokine production under S-protein stimulation and targeting RNase L may be a novel therapeutic strategy for severe COVID-19.

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04. Cytokines and dendritic cells

P76

IL-6 MEDIATED SUPPRESSION OF VACCINE RESPONSES IN NEONATAL MICE

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Abstract Content: The inability of neonates to develop CD4+CXCR5+PD-1+ T follicular helper (Tfh) cells contributes to their weak vaccine responses. In previous studies, we measured diminished IgG responses when IL-6 was co-injected with a pneumococcal conjugate vaccine (PCV) in neonatal mice. This is in sharp contrast to adults, where IL-6 improves vaccine responses. Our studies also indicated that IL-6 mediated suppression of Tfh response is likely due the expansion of the inhibitory follicular regulatory T helper (Tfr) cells in neonates. In sharp contrast to neonates, IL-6 increases the frequency of Tfh cells in adult mice. In this study, we expanded the early studies to investigate the changes in IL-2 response in immunized neonates because recent reports suggest that in adults IL-6 promotes the expansion of Tfh cells by protecting them from IL-2 mediated suppression through the ablation of IL-2Rβ expression on Tfh cells. Indeed, we found decreased IL-2Rβ expression on Tfh cells in IL-6 co-injected adult mice. In sharp contrast, co-injection of neonatal mice with PCV and IL-6 resulted in a significant increase in Tfh cell IL-2Rβ expression. Reflecting the differences in IL-2Rβ expression on adult vs neonatal Tfh cells, analysis of IL-2R signaling indicated that the frequency of phospho-STAT5+ Tfh cells increases in neonates and decreases in adults after IL-6 co-injection with PCV. We also observed that CpG containing PCV increased antibody responses against PCV in neonatal mice, which was accompanied by an increase in IL-21 producing Tfh frequency and a decrease in Tfr cell population. Moreover, CpG co-injection also led to a sharp decrease in neonatal IL-6Rα as well as IL-2Rα and IL-2Rβ

on Tfh cells. These findings further underscore age specific differences in IL-6 mediated vaccine responses and highlight the need to consider age related immunobiological differences in designing vaccines.

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P77

PRODUCTION OF PROINFLAMMATORY CYTOKINES BY TISSUE RESIDENT MEMORY T CELLS IS REGULATED BY THE INTEGRATED STRESS RESPONSE PATHWAY

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Abstract Content: Background:
CD4⁺ T cells produce cytokines and play a central role in immunity. Tissue-resident memory T (Trm) cells remain in organs after infection and contribute to efficient host defense by immediate production of cytokines. Recently, it was demonstrated that Trm cells also promote autoimmunity. Therefore, tight regulation of cytokine production by Trm cells is of great importance to achieve efficient host defense without excessive inflammation. However, the underlying mechanisms of cytokine production by Trm cells are not well understood.

Method:
Human and mouse T cells including renal Trm cells were analyzed by single cell RNA sequencing (scRNAseq), polysome profiling, RT-PCR, flow-cytometry, immunocytochemistry, and mRNA FISH. Mouse models for *Staphylococcus aureus* infection and crescentic glomerulonephritis were used to induce and study Trm cells *in vivo*.

Results:
scRNAseq analysis of T cells from human healthy kidney, colon, and lung revealed that Trm cells express high mRNA levels of proinflammatory cytokines such as *IL17A*, *INFG*, and *CSF2*. However, combination of flow-cytometry, tissue signature analysis, and polysome profiling showed that Trm cells do not translate cytokine mRNA into protein without re-stimulation. Mechanistically, we demonstrated that the phosphorylation of eIF2a, a key feature of the integrated stress response (ISR), results in recruitment of cytokine mRNA into stress granules, which are organelles crucial for regulating mRNA translation during ISR, thereby inhibiting mRNA translation in resting Trm cells. Re-stimulation of Trm cells through T cell receptor resulted in eIF2a dephosphorylation, leading to rapid translation of cytokine mRNA into protein. Moreover, we found that blocking eIF2a phosphatase by Raphin1 efficiently suppresses eIF2a dephosphorylation and cytokine production from Trm cells. *In vivo* administration of Raphin1 ameliorated immune-mediated kidney injury in mice.

Conclusion:
Tissue-resident memory CD4⁺ T cells express high level cytokine mRNA. Under homeostatic conditions, the cytokine mRNA is stored in stress granules and not translated into protein. These Trm cells rapidly produce cytokine protein upon re-stimulation. Our study identifies a novel mechanism of how the integrated stress response regulates rapid cytokine production of poised Trm cells in health and disease.

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P78

VISUALIZING IMMUNE CELLS MARKERS AND CYTOKINES TO CHARACTERIZE TUMOR INFILTRATING IMMUNE CELLS USING THE SPATIAL RNASCOPE™ HIPLEX V2 IN SITU HYBRIDIZATION ASSAY

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Abstract Content: The tumor microenvironment (TME) is highly complex, comprised of tumor cells, immune cells, stromal cells, and extracellular matrix. Interrogating spatial interactions between various cell types and detecting their activation states in the TME is crucial for implementing successful immunotherapies against cancer. This study demonstrates a highly sensitive and specific multiplexed technique, the RNAscope HiPlex v2 in situ hybridization (ISH) assay for detecting target gene expression and assess immune regulation in human lung, breast, cervical and ovarian FFPE tumor tissues.

We have expanded our current RNAscope HiPlex assay capability of iteratively multiplexing up to 12 targets in fixed and fresh frozen samples to include formalin fixed paraffin embedded (FFPE) tissues. The novel FFPE reagent effectively reduces background autofluorescence, improving the signal to noise ratio. We have leveraged this technology to investigate spatial expression of 12 oncology and immuno-oncology target genes, including tumor markers, immune checkpoint markers, immunosuppression markers, immune cell markers and secreted chemokine RNA expression profile within the TME. The targets were simultaneously registered using HiPlex image registration software v2 that enables background subtraction.

We visualized T cell infiltration and identified T cell subsets within tumors using *CD3* and *CD8* expression and activated T cells by *IFNG* expression in cervical and lung tumors. Cells with *CD3*+ *CD8*+ Cytotoxic Tcell phenotype and the *CD3*+ *CD8*+ *IFNG*+ *PD1*+ Activated Tcell phenotype were quantified using HALO image analysis. We further identified subsets of pro- and anti-inflammatory macrophages by *CD68* and *CD163* expression as well effector cells which secrete chemokines and cytokine such as *CXCL10* and *CCL22*. Quantitative analysis indicated higher degree of macrophage and tumor associated macrophage (TAM) infiltration in the lung tumor compared to the cervical tumor. Average copy number per cell of secreted cytokines *CXCL10* and *CCL22* in cervical and lung cancer were also evaluated in these tumors which showed higher copy numbers in cervical tumor as compared to the lung tumor. We also detected the hypoxia markers *HIF1A* and *VEGF* to elucidate the immunosuppressive state of tumor cells. Preliminary analysis and quantification of the *HIF1A* expression using HALO® image analysis software showed higher copy numbers in the lung tumor as compared to the other tumors, demonstrating the sensitivity of the assay through differential expression.

Using a highly sensitive multiplexed RNAscope HiPlex v2 ISH assay, we have demonstrated the capability of this technique to spatially resolve 12 targets in four different tumor types. The FFPE reagent efficiently quenched background autofluorescence in the tissues and identified immune cell signatures within the TME. This technology is highly beneficial for investigating complex and spatial tumor-stroma interactions and the assay can also provide valuable understanding of the biological crosstalk among various cell types in complex and heterogeneous tissues.

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P79

HISTONE DEACETYLASES CONTROL IL-15-MEDIATED PATHOGENIC REPROGRAMMING OF CD8+ INTRAEPITHELIAL LYMPHOCYTES

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Abstract Content: IL-15 is an inflammatory cytokine that is upregulated in a variety of inflammatory T cell disorders, including Celiac Disease (CeD). In CeD, expression of IL-15 by distressed intestinal epithelial cells results in the pathogenic reprogramming of cytotoxic $CD8^+$ intraepithelial lymphocytes (IE-CTLs), promoting tissue destruction. Strikingly, IL-15 induces major transcriptional changes in IE-CTLs in the absence of significant changes in chromatin accessibility, especially at early timepoints that are associated with potent effector function in IE-CTLs. We found that histone deacetylases (HDACs) are required for more than 40% of the early transcriptional changes that we see in IE-CTLs following IL-15 stimulation. However, an increase in nuclear HDAC activity is only observed 24h post-IL-15 stimulation. Using selective HDAC inhibitors and small interfering RNA, we identified class II HDACs, which have minimal catalytic activity, as the main HDACs driving transcriptional changes 3h post IL-15 stimulation. Taken

together, these results suggest that HDACs are not mediating early transcriptional changes in IE-CTLs through epigenetic changes (dependent upon HDAC catalytic activity), but, instead, are controlling accessibility of transcription factors. Furthermore, this work identifies class II HDACs as key regulators of transcriptional programs in terminally differentiated CTLs. Identifying which HDACs are controlling the IL-15-mediated reprogramming of IE-CTLs could lead to the implementation of novel therapeutic options that use specific HDAC inhibitors to treat IL-15-mediated inflammatory T cell disorders.

Disclosure of Interest: None Declared
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P80

RUNX3 AND TGFB COLLABORATE TO DRIVE A TISSUE RESIDENCY PROGRAM THAT IS ABSENT IN CD4+ T CELLS

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Abstract Content: Tissue-resident memory T (T_{RM}) cells provide rapid and superior control of localised infections. The transcription factor Runx3 was recently identified as a master regulator of $CD8^+$ T cell tissue residency. However, Runx3 also drives $CD8^+$ T cell lineage commitment and is repressed in $CD4^+$ T cells, raising the possibility that this transcription factor defines a form of tissue residency unique to the $CD8^+$ T cell compartment. Here, we show that as a direct consequence of Runx3-deficiency, $CD4^+$ T_{RM} cells lack the TGFb-responsive transcriptional network that underpins epithelial $CD8^+$ T_{RM} cell residency. While $CD4^+$ T_{RM} cell formation was found to involve the action of Runx1, this along with modest Runx3 expression was insufficient to engage the TGFb-driven residency mechanism. In contrast, ectopic expression of Runx3 in $CD4^+$ tissue T cells incited this TGFb-transcriptional program to promote prolonged survival, decreased tissue egress and a microanatomical redistribution towards epithelial layers culminating in enhanced effector functionality. Thus, our results reveal a fundamental discordance in the form of tissue residency adopted by $CD8^+$ and $CD4^+$ T_{RM} cell subsets attributable to diametric Runx3 activity.

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P82

STRUCTURAL AND FUNCTIONAL INSIGHTS INTO THE ROLE OF THEMIS1 IN T CELL RECEPTOR SIGNALLING

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Abstract Content: Themis1, a recently discovered T cell-specific protein, is a key regulator of thymocyte development. Maturation from double positive (DP) thymocytes to effector T cells is blocked in Themis1-deficient mice, resulting in a marked reduction in single positive (SP) thymocytes and peripheral T cells. Themis1 also plays a role in mature T cell signalling and has been implicated in integrating TCR and cytokine signals.

Themis1, and related protein Themis2, contain tandem copies of a novel structural domain, dubbed the cysteine-containing all beta in Themis (CABIT) domain, crucial for Themis activity and signalling. CABIT domain-containing proteins have been identified in a wide variety of species, pointing to Themis1 as the archetypal member of a new metazoan protein family. Themis1 does not exhibit catalytic activity, and is thought to act as a molecular scaffold in TCR signalling. Themis1 binds to the cytosolic adaptor protein Grb2, which facilitates recruitment of Themis1 to the TCR after activation.

In addition to its crucial role in T cell development, accumulating evidence also links Themis1 to inflammatory diseases, such as rheumatoid arthritis, yet the structural and mechanistic basis of these pleiotropic functions remain poorly understood. Interestingly, Themis1 and Themis2 are functionally interchangeable, suggesting that conserved domains in Themis family proteins, particularly the CABIT domain, may be important for their biological activity, particularly in signal transduction downstream of TCR activation. Themis1 has been shown to act as a positive regulator of TCR signalling, with reduced IL-2 production and *in vitro* effector functions in Themis1-deficient $CD4^+$ T cells. Moreover, Themis1 is required for IL-2 and IL-15-induced $CD8^+$ T cell proliferation via the activation of JAK-STAT and mTOR signalling.

We have combined an integrative structural biology approach with cellular and *in vivo* experiments to elucidate the structure-function landscape of Themis1 and to investigate the unexplored roles of Themis1 in inflammatory signalling and disease models of rheumatoid arthritis. Here, we present the biochemical reconstitution of Themis1-Grb2 complexes and progress on the structure determination of the Themis1-Grb2 complex by X-ray crystallography and cryoEM. Furthermore, we characterised the binding kinetics and affinity dictating the Themis1-Grb2 interaction and report camelid single-domain VHH antibody fragments with binding specificity for Themis1. Characterisation of Themis structural domains, particularly the CABIT domain, and elucidation of how they serve to recruit and modulate binding partners such as Grb2 will provide timely insights into the function of CABIT domain-containing proteins as signalling scaffolds in T cell biology and inflammatory pathways.

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P83

MIXED IMMUNE POPULATIONS CORRUPT IL-21 MEDIATED CD8 T CELL TUMOR IMMUNITY

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Abstract Content: IL-21 enhances the antitumor activity of adoptively transferred $CD8^+$ T cells, while IL-2 impairs T cell immunity by inducing terminal differentiation. The effects of IL-21 on T cells have primarily been studied with T cells in isolation, yet it can act on many different immune cells. Therefore, we asked if this cytokine directly augments T cells or rather if it acts upon other immune cells in the culture that indirectly influence T cell therapy. To test this question, splenocytes from pmel-1 transgenic mice were used, where all $CD8^+$ T cells express a transgenic TCR specific for tumor-antigen $gp100_{25\text{-}33}$ overexpressed on melanoma. We activated a mixed immune population of pmel-1 splenocytes or activated enriched naïve pmel-1 $CD8^+$ T cells and expanded them in presence of IL-21 for four days. The *in vitro* memory phenotype of these cultures was then assayed. Additionally, we infused pmel-1 T cells expanded from a mixed immune population or from enriched $CD8^+$ T cells into mice bearing B16KVP melanoma tumors and compared their capacity to engraft, persist, and regress tumor *in vivo*. Interestingly, we discovered that IL-21-treated T cells generated from bulk splenocytes are phenotypically distinct from IL-21-treated isolated T cells. IL-21 cells generated from bulk splenocytes were marked by increased expression of the effector molecules granzyme B and CD44 and decreased expression of the stemness associated transcription factors, TCF-1 and LEF-1. *In vivo,* IL-21-treated T cells expanded from a mixed immune population engrafted and persisted poorly, thereby mediating suboptimal melanoma regression. Conversely, when T cells were first isolated from the spleen prior to their expansion and infusion into the animal, IL-21 dramatically bolstered T cell's engraftment and antitumor activity. This corruption of IL-21 mediated $CD8^+$ T cell stemness due to a mixed immune population was not improved by varying the dose of IL-21, implying this is not an issue of bioavailability. Nor was it improved by individually depleting B cells, $CD4^+$ T cells, macrophages, or neutrophils from the mixed immune population. Interestingly, we found that this IL-21 induced stemness phenotype in isolated T cell was corrupted by transferring the supernatant of a culture of IL-21 treated bulk splenocytes, 24 hours after peptide activation, onto a new culture of IL-21 treated isolated T cells, at peptide activation. This finding implies that a soluble factor produced in response to IL-21 only in mixed immune populations corrupts IL-21 mediated stemness and antitumor activity on isolate T cells. Overall, our data demonstrates that IL-21 may improve

ACT therapy best when used directly on antitumor CD8$^+$ T cells. Ongoing studies will define the mechanism behind this difference as these findings are important for defining the best conditions in which to use IL-21 for adoptive T cell transfer therapy.

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HCMV EXPLOITS STING SIGNALING AND COUNTERACTS ISG AND IFN-B, BUT NOT IFN-Λ1 INDUCTION TO FACILITATE VIRAL GENE EXPRESSION IN MONOCYTE-DERIVED DENDRITIC CELLS

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Abstract Content: Human cytomegalovirus (HCMV) is a widespread human pathogen with a seroprevalence of 40–99% among the global population. Although infection of healthy individuals normally is asymptomatic, the virus establishes latency and can cause life-threatening disease in immunocompromised hosts. During viral infections, monocytes are recruited to sites of inflammation where they typically differentiate into macrophages and/or dendritic cells (DC) to further coordinate anti-viral immunity. Upon infection of monocyte-derived DC (moDC) with HCMV at MOI 3, only 2-30% of the cells are productively infected. We hypothesize that the balance between the expression of anti-viral and pro-viral factors determines the infectivity of moDC. To address this, we studied HCMV infection of moDC on the single-cell level using single-cell RNA sequencing (scRNAseq).
We found that the majority of moDC got infected, whereas only a fraction of the cells initiated immediate early (IE) viral gene expression. We identified three distinct subsets of moDC, which support virus infection to different extents. Upon HCMV entry, cGAS/STING sensing induces IFN-β responses, and HCMV exploits NF-κB- dependent STING activation to exacerbate IE viral gene expression. We could show that upon the progression of the viral life cycle, IFN-β induction is inhibited. This inhibition did not apply to IFN-λ1, highlighting differential induction mechanisms for IFN-β and IFN-λ1. Analysis of nascent RNA indicated that upon HCMV exposure, ISGs were initially induced also in productively infected cells. However, at 8 hpi HCMV-encoded viral modulators efficiently shut off ISG expression.
Thus, this work points to several subpopulations of moDCs and correlations between virus and host gene expression. We reveal a dynamic interplay of seemingly contrasting functions with STING playing a role in driving virus gene expression, as well as the support of productive infection that governs the outcome of HCMV infection in moDCs.

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P86

AXL INHIBITION ENHANCES TYPE 1 INTERFERON (IFN) RESPONSE AND POTENTIATES CHEMO-IMMUNOTHERAPY

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Abstract Content: Chemotherapy elicits anti-tumor immune responses by inducing immunogenic tumor cell death, targeting suppressive immune cells and activating Type 1 interferon (IFN) responses (1, 2, 3). Chemotherapeutic agents lead to cGAS - STING cytosolic DNA sensing pathway activation that results in Type 1 IFN release (4). IFN receptor (IFNAR1, IFNAR2) signaling mimics viral immune responses, is associated with clinical benefit (5, 6) and is exploited by chemo-immunotherapies (7, 8). The receptor tyrosine kinase AXL is associated with immune evasion

and immunotherapy resistance (8). AXL serves as a critical regulatory checkpoint for TLR-induced IFN responses in dendritic cells, macrophages and natural killer cells (9, 10). IFN receptor signaling induces AXL expression (11) and AXL activation on dendritic cells is targeted by viruses (e.g. Zika) to abrogate IFN responses and inhibit anti-viral immunity. AXL serves as an IFN-response checkpoint by blocking IFNAR1 and IFNAR2 signaling. We hypothesized that tumor cells exploit AXL signaling to abrogate Type 1 IFN responses and inhibit antitumor immunity. We evaluated whether AXL inhibition promotes Type 1 IFN signalling and enhances immune checkpoint inhibitor efficacy.
AXL kinase inhibition (bemcentinib) in combination with chemotherapy (doxorubicin) increased IFN response gene expression in mammary carcinoma and melanoma cell lines. In vivo, bemcentinib treatment potentiated the efficacy of immune checkpoint inhibitor treatment in combination with intratumoral doxorubicin injection (i.t) in the syngeneic myeloid derived suppressor cell (MDSC)- rich refractory mammary adenocarcinoma 4T1 model. In addition, bemcentinib treatment in conjunction with i.t. doxorubicin enhanced Type 1 IFN response, reduced cancer stemness and epithelial to mesenchymal (EMT) gene expression in this model. Furthermore, this combination treatment regimen sensitized the immune checkpoint inhibitor refractory *Braf*-mutant melanoma (YUMM1.7) by enhancing the type 1 IFN response resulting in significantly improved median overall survival. In conclusion, this study provides evidence that bemcentinib potentiates chemo-immunotherapy by enhancing tumor Type 1 IFN response and dampening EMT.
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P87

RNASCOPE MULTIPLEX ASSAY ENABLES SPATIAL INTERROGATION OF CHEMOKINE SIGNALING THAT FACILITATES CONVENTIONAL TYPE 1 DENDRITIC CELL-MEDIATED CYTOTOXIC T CELL RECRUITMENT IN CERVICAL CANCER

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Abstract Content: The role of T cells in mediating anti-tumor immunity has been harnessed to develop some of the most successfully immunotherapies in recent years. Although direct presentation of tumor antigens by tumor cells plays an important role in effector function of cytotoxic T cells, cross-presentation by professional antigen presenting cells is vital for priming naive CD8 T cells and develop a sustainable cytotoxic response. NK cells within the tumor recruit specific population of DC cells called conventional type 1 DCs (cDC1s) into the TME (tumor microenvironment) by secreting chemokines such as CCL5 and XCL1. These cells are characterized by expression of specific markers and their detection can be challenging due to their low number.
By using specific RNAscope *in situ* hybridization probes for *XCR1*, *THBD* and *CLEC9A*, we identified cDC1 cells using the RNAscope Multiplex Fluorescence assay within 4 cervical cancer tumors. These tumors were then assessed for the presence of NK cells by using NK cell specific marker probes such as CD56 and NCR1. Furthermore, cytotoxic T lymphocytes specific markers CD8 and IFNG were used to establish a correlation between the presence of cDC1 cells and T cell infiltration within the cervical cancer tumors.
There was a strong correlation observed between the presence of NK cells, cDC1 cells and CTLs (cytotoxic T lymphocytes) within ¾ cervical cancer samples. One of the 4 samples demonstrated relatively lower levels of NK cells which correlated to lower cDC1 cells and in turn lower CTLs infiltration. The NK cells showed expression of *XCL1* and *CCL5* indicating that the $XCR1^{+}/CCR5^{+}$ cDC1 cells could have been potentially recruited by these NK cells secreting these specific chemokines. High cDC1 and NK cell regions also showed significantly higher level of CTL recruitment indicated by $CD8^{+}/IFNG^{+}$ cells that can initiate anti-tumor immune response within the cervical cancer tumors.
In conclusion, by using specific RNAscope probe combinations, we were able to identify and visualize NK cells secreting activating chemokines, T cells secreting cytokines and cDC1 cells, a subset of DC cells that excel at presenting tumor antigens to T cells. Using this tool, it is possible to spatially interrogate the TME and detect specialized immune cells when assessing the effect of new immunotherapies.

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P88

REVEALING THE FACTORS THAT POSITION MEMORY T CELLS WITHIN SECONDARY LYMPHOID ORGANS

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Abstract Content: The maintenance of immune memory is essential to conferring superior protection against infection. Current vaccine strategies promote $CD8^+$ T cell responses with varying success, and as such there is a need to better understand the mechanisms that drive and maintain T cell memory fate and longevity. The factors that regulate central memory T cell (T_{CM}) location within lymph nodes remain elusive. We propose that there is a precise positioning of T_{CM} within a lymph node memory niche and that this provides enhanced recall function and durability. Here, we used light-sheet fluorescence microscopy to image draining lymph nodes following the resolution of viral infection, to determine T_{CM} positioning in 3D. We have demonstrated that T_{CM} cells are distributed peripherally, relative to naïve cells (T_N), with a greater density of T_{CM} occupying the cortical ridge than $T_{N,}$ which primarily reside in the paracortex. Additionally, this positioning was conserved across various infection models. We have employed RNA-sequencing (RNAseq), in combination with high resolution confocal microscopy, to examine cell-cell contacts and determine the key regulators of T_{CM} positioning. This will provide critical insight into the enhanced recall, maintenance and longevity of immune memory.

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P89

HUMAN PLASMACYTOID DENDRITIC CELLS DEMONSTRATE MULTIPLE LEVELS OF DYSREGULATION IN HOSPITALIZED COVID-19 PATIENTS IN THE PRESENCE OR ABSENCE OF DEXAMETHASONE THERAPY

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Abstract Content: Plasmacytoid dendritic cells (pDC) are innate immune cells and the most potent producers of interferon-a (IFN-α) in the body in response to viruses. Hospitalized patients with severe COVID-19 have reduced plasma IFN-α compared to those with mild/moderate cases. In addition, pDC numbers are reduced in patients with severe COVID. These findings suggest a role of decreased plasma IFN-a and decreased numbers of pDC with SARS-CoV-2 pathogenesis. To address this hypothesis, we evaluated hospitalized COVID-19 patients prior to the emergence of delta and omicron variants who were treated with or without dexamethasone (DEX), a corticosteroid that is used to reduce inflammation and the cytokine store in COVID-19. We have shown that DEX is able to deplete pDC in vivo and to suppress pDC function and maturation and enhance apoptotic death in vitro. We isolated PBMC from fifty-seven DEX-treated or untreated, hospitalized COVID-19 patients, 16 post-COVID convalescent subjects, and healthy controls. We categorized patients into younger and older groups, which were less than or greater than 50 years-old, respectively. PBMC were stimulated with either Influenza-A virus (IAV) or HSV-1 and pDC IFN-a production was assessed by flow cytometry. Absolute pDC numbers and percent of pDCs producing IFN-a were dramatically reduced in both the DEX-treated and untreated patients. Thus, dysfunction of pDC in COVID-19 is cell intrinsic and not solely due to DEX therapy. Numbers and function of pDC were partially restored in the convalescent patients. In addition, older subjects without DEX therapy had more highly compromised pDC numbers and function than younger

subjects. IRF-7 expression, which is the transcription factor required for initiation of IFN-a production in pDC, was reduced in COVID-19 patients compared to healthy controls. We also observed that TNF-α production was reduced in pDC from COVID-19 patients, which, along with low IFN-α production, suggests that pDC are functionally exhausted during COVID-19 progression or may have matured into antigen-presenting cells, or both. Analysis of freshly-isolated pDC from COVID patients demonstrated that co-stimulatory markers CD80, CD86 and CD40, and the migratory marker CCR7, were upregulated in patients compared to healthy controls, indicating that pDC maturation is contributing to pDC IFN-adeficiency. Additionally, pDC from COVID-19 patients had an increase in senescence-associated β-galactosidase activity, which is a measure of immune cell senescence, suggesting a role for increased pDC senescence in their dysfunction. Increased Ki67 expression in pDC from COVID patients vs. healthy controls indicated increased turnover of peripheral pDC. Using disease severity scoring, higher disease severity correlated with decreased absolute pDC numbers. Overall, these multi-faceted compromises in the numerical, phenotypic and function of pDC likely contribute to SARS-CoV-2 pathogenesis.

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P90

PHENOTYPIC, MOLECULAR AND FUNCTIONAL CHARACTERISATION OF IN VITRO INDUCED HUMAN IL-17+ CD8+ T-CELLS

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Abstract Content:
IL-17A+CD8+ T-cells (Tc17 cells) are present in inflamed tissue in several immune-mediated inflammatory diseases including psoriasis and spondyloarthritis. Much of our understanding of these cells derives from murine studies, whilst human Tc17 cells remain less well-characterised. The aim of this study was to phenotypically, molecularly and functionally characterise human IL-17A+CD8+ T-cells.
Using flow cytometry, we detected low ex vivo frequencies of IL-17A+ and IL-17F+ CD8+ T-cells in healthy human donor peripheral blood (median 0.09%, n=48 and 0.02%, n=20, respectively). These frequencies were significantly increased when peripheral blood mononuclear cells were stimulated with anti-CD3/CD28 mAbs in the presence of IL-1β+IL-23 vs stimulated cultures without polarising cytokines (IL-17A, n=25: 1.05% vs 0.33% and IL-17F, n=16: 0.77% vs 0.11%, respectively, p<0.0001). A similar increase in IL-17A+CD8+ T-cells was observed when purified CD8+ T-cells were stimulated in the presence vs absence of IL-1β+IL-23 (1.44% vs 0.28%, n=16, p=0.0005), and this was not further enhanced by the addition of IL-6, IL-2 or anti-IFNγ mAb.
Phenotypically, a high proportion of in vitro induced IL-17A+CD8+ T-cells co-expressed the typical type-17 surface markers CCR6 and CD161 compared to IL-17A- counterparts (CCR6: 88% vs 11% and CD161: 93% vs 19%, respectively). In vitro induced IL-17A+CD8+ T-cells additionally displayed a type-17 related transcriptional signature defined by high expression of *IL17A, IL23R* and *RORC* compared to IL-17A- CD8+ T-cells.
In vitro induced IL-17A+ and IL-17F+ CD8+ T-cells contained both conventional and Vα7.2+ / MR1-5-OP-RU-tetramer+ mucosal-associated invariant T (MAIT) cells, with a predominance of MAIT cells (IL-17A: 58% vs 34% and IL-17F: 59% vs 30% MAIT vs conventional, respectively). SPICE analysis revealed that both Vα7.2- and Vα7.2+ IL-17A+CD8+ T-cell subsets mostly comprised of polyfunctional cells (IL-17A+IFNγ+TNFα+ or IL-17A+IFNγ+TNFα+GM-CSF+). Both conventional and MAIT IL-17A+CD8+ T-cells co-secreted IL-17AA/AF, IFNγ and TNFα with more consistent production of IL-17FF/AF, GM-CSF, IL-22 and IL-10 by Vα7.2+ CD8+ T-cells; neither subset secreted IL-21. In vitro induced IL-17A- CD8+ T-cells did not produce type-17 associated cytokines but secreted IFNγ, TNFα and GM-CSF.
Importantly, sorted in vitro induced IL-17A+CD8+ T-cells (using a cytokine secretion assay) were biologically functional, as evidenced by their ability to induce proinflammatory IL-6 and IL-8 production by synovial fibroblasts from psoriatic arthritis (PsA) patients. This response was reduced in the presence of either secukinumab (anti-IL-17A) or adalimumab (anti-TNFα) and particularly so with combined blockade (p=0.0153).

In conclusion, human IL-17A+CD8+ T-cells are induced in vitro when T-cells are stimulated in the presence of IL-1β+IL-23. These cells display a polyfunctional type-17 profile and comprise conventional as well as unconventional T-cells. Our data further indicate proinflammatory crosstalk between IL-17A+CD8+ T-cells and synovial fibroblasts, suggesting a functional contribution of Tc17 cells to joint inflammation in PsA.

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P91

INTERPLAY OF TYPE I AND II IFN FOR THE GENERATION OF CD8+ STEM-LIKE MEMORY T CELLS

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Abstract Content: The timing and location of the interferon (IFN) response to viral infection is tightly coordinated. High expression or type-I IFN (IFN-I) therapy enhances viral clearance, whereas deficiency in the IFN-I receptor, IFNAR, promotes the formation of $CD8^+$ T cell stem like memory cells (T_{SCM}). Here, we demonstrate that blocking IFNAR during LCMV infection results in potent T_{SCM} formation that is associated with increased CXCR3 ligands, desensitization of CXCR3 and retention in the lymph node paracortex. Blocking IFNAR increased IFNg and the recruitment of monocyte-derived inflammatory DCs (moDCs) in an IFNg-dependent fashion. In the absence of both IFNAR and IFNg CXCR3 chemokine expression was lost. In this setting T_{SCM} fate remained enhanced and occurred along-side T cell exhaustion and persistent viral load. Leveraging the interplay of IFN-I and IFN-II cytokines, we demonstrate that potent T_{SCM} memory can be promoted following mRNA-LNP vaccination to establish superior protection from infection.

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IL-38 SHAPES REGULATORY T CELL ACTIVATION

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Abstract Content: **Introduction:**
Interleukin-38 (IL-38) is a newly discovered IL-1 family member that shares homology with both IL-1Ra and IL-36Ra, and is therefore proposed as a negative regulator of inflammation. IL-38 is known to be released from apoptotic cells to limit inflammatory macrophage activation and downstream T lymphocyte IL-17 production by blocking X-linked IL-1 receptor accessory protein-like 1 (IL1RAPL1) signalling. IL-38 polymorphisms are associated with increased susceptibility for auto-inflammatory diseases such as psoriasis, spondyloarthritis, rheumatoid arthritis, and psoriatic arthritis, but the mechanisms by which IL-38 regulates inflammation *in vivo* are still ill-defined.
Methods:
The zymosan-induced peritonitis mouse model was used to study both the initiation and resolution of inflammation. WT and IL-38 KO mice were injected with 10 mg/kg body weight of zymosan A for 1, 3 and 6 days. The content of the peritoneal cavity was studied using flow cytometry, Cytometric Bead Array, targeted proteomics, and transcriptome analysis. To modulate Treg homeostasis and activation, WT and IL-38 KO mice were injected with a CD39 inhibitor or vehicle during peritonitis. Statistical comparisons between two groups were performed using Mann Whitney or unpaired two-tailed Student's t test. One- or two-way analysis of variance (ANOVA) followed by appropriate post-tests was used for multiple comparisons.
Results:

Complete absence of IL-38 in IL-38 KO mice lead to delayed resolution of inflammation in the zymosan-induced peritonitis mouse model, compared to WT mice. This was marked by a persistent neutrophilia and a lower production of pro-resolving mediators during the resolution phase, such as TGF-β produced by macrophages following efferocytosis of apoptotic cells. Reduced TGF-β production from macrophages reduced TGF-β levels in the peritoneal lavage and coincided with reduced levels of regulatory T cells (Tregs), which are known to promote the resolution of inflammation. Unexpectedly, the TGF-β production capacity of macrophages did not influence the induction of Tregs from naïve T cells, but affected Treg activity markers, particularly features of the adenosine production machinery. Finally, blocking adenosine production in WT mice mimicked the delayed resolution of inflammation and persistent neutrophilia that were observed in IL-38 KO mice.

Conclusion:
These data indicate a potential key function of IL-38 in the regulation of Treg biology, which is relevant for autoimmune disease. Moreover, they provide evidence that the pro-resolving function of IL-38 may be indirectly linked to the activation and function of Tregs in the peritoneal cavity by regulating the TGF-β production in macrophages and the production of adenosine. However, further studies are required to identify the mechanism by which IL-38 regulates TGF-β production and its role in Treg activation.

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A SEMI-SUPERVISED REGRESSION MODEL TO PREDICT BULK INTERFERON-GAMMA (IFNG) KINETICS FROM IFNG-PRODUCING CD8+ T CELL CLUSTERS CO-CULTURED WITH TUMOR CELLS

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Abstract Content: Interferon-gamma (IFNg) is a cytokine that plays an important role in the apoptotic killing of tumor cells by T cells. IFNg is produced by activated CD8+ T cells after they bind to cognate peptides on the surface of tumor cells. Tumor cells consume IFNg and upregulate surface markers such as H-2Kb and PDL1. These surface markers activate new naïve T cells that produce more IFNg. Eventually, activated T cells become exhausted and stop producing IFNg. Despite the temporal nature of these processes, researchers have not studied how IFNg levels change over time; instead, they often only measure IFNg at single time points. This lack of data about the temporal dynamics of IFNg limits our understanding of how IFNg signals in the tumor-immune microenvironment. To that end, we measured bulk IFNg levels in tumor-T cell co-cultures using a robotic platform that collected and stored supernatant every six hours for 120 hours. Using these samples, we constructed detailed time kinetics of IFNg and fit the curve to a tanh function. From this function, we numerically computed three biologically relevant parameters: t_{start}, the time that IFNg levels first rose above baseline; slope, the constant exponential increase of IFNg levels; and $t_{shutdown}$, the time at which IFNg levels sharply plateau. By varying experimental conditions such as the number of T cells, the number of tumor cells, and the number of IFNg receptors, we were able to modify the t_{start}, $t_{shutdown}$, and slope respectively.
Using the same robotic platform used to collect supernatant, we collected single-cell samples at each timepoint and ran these samples on a forty-channel spectral flow cytometer. We then fed the multiparametric single-cell data into a regression algorithm that clustered the cells and assigned weights to each cluster representing the average amount of IFNg produced by a cell in that cluster. Using this model, we recapitulated the bulk IFNg kinetics from the frequencies of IFNg-producing clusters without directly staining for intracellular-IFNg. Furthermore, by studying the relationships between clusters via pseudo-time trajectory analysis, we built a compartment model to describe the transition of T cells from naïve to activated to IFNg-producing to exhausted cells. By varying experimental conditions such as the numbers of tumor and T cells, we determined that new naïve T cells activate and begin producing IFNg quicker when they are surrounded by other IFNg-producing cells; this indicates positive feedback that explains the exponential slope of the tanh function. We also determined that tumor cells secrete a metabolic factor into the microenvironment that prevents the activation of new T cells but does not pre-exhaust already activated T cells; this explains the earlier $t_{shutdown}$ in co-cultures with more tumor cells.

Our regression model serves as a broadly applicable method to predict bulk cytokine kinetics from single-cell phenotypic data and vice versa. We aim to use this model to predict IFNg levels in more complex *in vivo* and patient-derived co-cultures of tumor and immune cells as well as to predict the kinetics of other cytokines. As such, our model serves as a useful tool to better understand the relationship between heterogeneous cellular phenotypes and bulk cytokine production/consumption.

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SELECTIVE IL-27 PRODUCTION BY INTESTINAL REGULATORY T CELLS PERMITS GUT-SPECIFIC REGULATION OF TH17 IMMUNITY

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Abstract Content: Regulatory T (Treg) cells are instrumental in establishing immunological tolerance. However, the precise effector mechanisms by which Treg cells control a specific type of immune response in a given tissue remains unresolved. Here, by simultaneously studying Treg cells from different tissue origins under systemic autoimmunity, we found that IL-27 is specifically produced by intestinal Treg cells to regulate Th17 immunity. Selectively increased intestinal Th17 responses in mice with Treg cell-specific IL-27 ablation led to exacerbated intestinal inflammation and colitis-associated cancer, but also helped protect against enteric bacterial infection. Furthermore, single-cell transcriptomic analysis of gastrointestinal immune systems in patients with ulcerative colitis (UC) suggested that such a Treg-IL-27 regulatory axis could also exist in humans. Collectively, our study uncovers a previously unexplored Treg cell suppression mechanism crucial for controlling a specific type of immune response in a particular tissue, and provides further mechanistic insights into tissue-specific Treg cell-mediated immune regulation.

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ARYL HYDROCARBON RECEPTOR PROMOTES INDUCIBLE NITRIC OXIDE PROTEIN BY REGULATING EUKARYOTIC ELONGATION FACTOR-2 KINASE UBIQUITINATION

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Abstract Content: Aryl hydrocarbon receptor (AhR) is known as a ligand-activated transcription factor, and also as an ubiquitin E3 ligase. It is implicated in the regulation of anti-inflammatory and pro-inflammatory immune responses. Mesenchymal stem cells (MSCs) possess immunomodulatory activities, including suppression of T-cell activation. Inducible nitric oxide synthase (iNOS) is a mediator responsible for the immunomodulatory functions of MSCs. MSCs were investigated for the treatment of inflammatory diseases such as graft-vs-host disease (GvHD). Here, we observed less efficacy of *Ahr*-deficient MSCs on GvHD compared to the wild type (WT) -MSCs. We found that the *Ahr*-/- MSCs less inhibited T cell activation. In addition, *Ahr* -/- MSCs express a lesser amount of iNOS than WT-MSCs upon IFN-γ, TNF-α, and IL-1β stimulation. Interestingly, increased the expression of eukaryotic elongation factor 2 kinase (eEF2k), a calcium/calmodulin- dependent kinase, which inhibits the elongation stage of protein synthesis by phosphorylating eEF2 was found in the *Ahr*-/- MSCs. Furthermore, decreased ubiquitination of eEF2K in *Ahr* -/- MSCs was observed. When the *Ahr*-/- MSCs were treated with eEF2K inhibitor, expression of iNOS was increased. Therefore, we think that AhR ubiquitinated the eEF2K which may regulate the expression of iNOS. These results are supposed to be useful evidence for understanding how AhR regulates iNOS proteins. Our results are valuable in explaining the immunomodulatory mechanism of MSCs. (NRF2022R1A2C1010354)

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SOLUBLE IL-2RA/CD25 IS GENERATED BY THE METALLOPROTEASES ADAM10 AND ADAM17

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Abstract Content: Interleukin-2 (IL-2) is one of the main regulators of immune responses through modulating a variety of immune cell functions, including $CD4^+$ T-cell differentiation, cytotoxic activity of NK cells, and, most importantly, the homeostasis of regulatory T-cells. Signal transduction of IL-2 is mediated via a receptor complex consisting of IL-2Rα (CD25), IL-2Rβ and IL-2Rγ. Of those receptors, IL-2Rβ and IL-2Rγ are intracellularly associated with kinases and consequently required for signal transduction whereas the IL-2Rα is not directly involved. Instead, IL-2Rα expression regulates the affinity of the receptor complex and thus the whole cell for IL-2. High IL-2Rα expression is found primarily on regulatory T-cells and involved in self-tolerance, but also transiently on other T-cell types upon activation of the T-cell receptor. In addition to the membrane-bound form, there is also a soluble (s)IL-2Rα that is reported to be mainly produced by activated T-cells, most likely through proteolysis. Of note, blood sIL-2R levels are elevated in a variety of diseases and discussed as a clinical marker. However, the exact mechanism(s) that lead to sIL-2Rα generation remain elusive.
Here, we analyzed the contributions of different proteases to constitutive and induced production of sIL-2Rα. First, we employed different small molecules that inhibit specific classes of proteases and identified the related metalloproteases ADAM10 and ADAM17 as the most likely sheddases of the IL-2Rα. We confirmed these findings using protease-deficient cells, which identified ADAM10 as the protease responsible for constitutive IL-2Rα shedding. We next analyzed sIL-2Rα production upon T-cell activation using a T-cell line as well as primary human T-cells. Here, ADAM17 rather than ADAM10 was mainly responsible for induced IL-2Rα shedding. In a next step, we analyzed whether the sIL-2Rα affects IL-2 signaling in T-cells. We stimulated primary human T-cells with IL-2 and increasing concentrations of sIL-2Rα and found that the soluble receptor acts as an antagonist. Finally, we examined constitutive sIL-2Rα in various protease deficient mice to assess whether the *in vivo* situation complements our *in vitro* data. Indeed, in mice lacking ADAM10 in $CD4^+$ T-cells, we found a decrease in sIL-2Rα with a concomitant increase in membrane-bound receptor, confirming that ADAM10 is also responsible for constitutive shedding of the IL-2Rα *in vivo*.
In summary, we identify ADAM10 and ADAM17 as sheddases of the IL-2Rα and could show that constitutive and induced generation of sIL-2Rα are executed by different proteases, which opens new opportunities to modulate sIL-2Rα levels and thus IL-2 function.

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P100

DISCOVERY OF A MOLECULAR CLOCK THAT CONTROLS CD8+ T CELL FUNCTION AND EXHAUSTION

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Abstract Content: 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 distinguish acute and chronic signals to program the exhausted or effector transcriptional states remains poorly understood. We found that members of the protein kinase

C (PKC) family function together as a “molecular clock,” sensing acute or chronic agonism to drive distinct transcriptional programs. Continuous stimulation of PKC is sufficient to induce many features of T cell exhaustion, including a loss of production of the cytokines IFNγ and TNF, and an increase in the expression of GzmB. PKC agonism triggers other hallmarks of terminal exhaustion, including altered expression of genes in the AP-1 transcription factor family and upregulation of the exhaustion regulator TOX. Mechanistically, $CD8^+$ T cells express several different PKC proteins, and chronic agonism of PKC leads to degradation of multiple family members and selective maintenance of only one PKC protein, PKC-η. This “PKC switch” alters the downstream signaling cascade to support the transcriptional reprogramming of T cells into a terminally exhausted state. Remarkably, chronic agonism of PKC even in the absence of antigen is sufficient to induce elements of the terminal exhaustion gene expression program, illustrating how the different forms of PKC function together as a signal transduction nexus during T cell exhaustion differentiation. 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.

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P101

TH9-DERIVED IL-9 PROMOTES CCR2-DEPENDENT MAST CELL ACCUMULATION IN THE ALLERGIC LUNG

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Abstract Content: Allergic asthma is a chronic lung disease characterized by airway hyperresponsiveness and cellular infiltration that is exacerbated by IgE-dependent mast cell activation. Our work in mouse models of allergic airway inflammation identified interleukin-9 (IL-9)-producing T helper 9 (T_H9) cells as important effector cells in promoting mast cell accumulation in the lungs. However, the precise mechanism of IL-9-mediated mast cell expansion remains unclear. Here, we demonstrate that intranasal recombinant IL-9 expands, while blockade of IL-9 reduces, mast cell progenitor numbers in the lungs and the bone marrow. These findings suggests that there are systemic effects of local IL-9 production in the allergic lung. Using adoptive transfer models and newly generated mice with an inactivation of the IL-9 gene restricted to T cells generated by CD4-Cre/loxP-mediated targeting of the IL-9 gene, we show that T_H9 cells promote mast cell progenitor and mature mast cell recruitment from the bone marrow to the lungs in a chemokine receptor 2-dependent manner. Our research further demonstrates that T cell-derived IL-9 is critical for mast cell-mediated airway hyperresponsiveness. Together, these findings define a contribution of T cell-derived IL-9 in promoting mast cell expansion and function in allergic airway inflammation. Therefore, IL-9 may be a promising therapeutic target for targeting mast cell-specific pathologies.

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P102

LIPID NANOPARTICLE INDUCED IL-27 PROMOTES VACCINE ELICITED MEMORY CD8+ T CELLS

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Abstract Content: Lipid nanoparticle (LNP)-mRNA vaccines have proven to be a flexible platform that promotes robust protective antibody and T cell responses. Despite widespread use, there remain significant knowledge gaps in how LNP-mRNA vaccines induce protective immunity. The LNP-mRNA induced IL-6 is critical for the development of T follicular helper cells and the antibody response, but how it and related family members impact the induction of $CD8^+$ T cell responses following immunization remain unexplored. IL-27 is a member of the IL-6 family and immunization with LNP-mRNAs induces inflammatory monocytes and cDC1s to produce IL-27. Immunization of mice deficient in IL-27 revealed that IL-27 is necessary for maximal generation of antigen-specific $CD8^+$ T cells. $CD8^+$ T

cell-specific loss of the IL-27 receptor demonstrated that optimal expansion following LNP-mRNA doses requires $CD8^+$ T cell-intrinsic IL-27 signaling for the generation of circulating memory $CD8^+$ T cells. Immunization with LNPs formulated with mRNA encoding IL-27 plus antigen rescues the $CD8^+$ T cell response in IL-27-deficient mice and augments the $CD8^+$ T cell response in wildtype mice. Thus, IL-27 promotes the induction of protective $CD8^+$ T cell populations following LNP-mRNA vaccination and optimizing the induction of this cytokine can improve the generation of protective $CD8^+$ T cell populations.

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P103

MIDKINE AND PLEIOTROPHIN PRODUCTION BY HUMAN MACROPHAGES AND DENDRITIC CELLS SUGGESTS NOVEL IMPLICATIONS FOR THESE CELLS

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Abstract Content: Midkine (MK) and pleiotrophin (PTN) are implicated in different processes including inflammation, the development of different cancers and tissue repair. The production of MK and PTN by primary DCs and the production of MK by primary human macrophages was never described.
We demonstrate that the TLR stimulation of human monocytes, macrophages, pDCs and MDDCs induced the production of MK and PTN by these cells. Primary mDCs produced PTN but not MK. MK production was detected in $CD11c^+$ cells, $CLEC4C^+$ cells and $CD68^+$ cells in biopsies of human tonsils showing reactive lymphoid follicular hyperplasia. Although the percentage of MK producing cells was similar in these cell populations the number of $CD11c^+$ cells producing MK was the highest and that of $CD68^+$ cells was the lowest ($p<0.05$). Selective inhibition of NF-κB and PDE4 activities, decreased the TLR-induced production of MK ($p < 0.05$). The inhibition of MK production by MDDCs and macrophages using anti-MK siRNA decreased the capacity of their supernatants to stimulate the proliferation of endothelial cells ($p < 0.05$).
This is the first study demonstrating that MK and PTN are produced by primary human DCs and that MK is produced by primary human macrophages upon TLR triggering, and that macrophages and MDDCs can stimulate endothelial cell proliferation through MK production. Our results suggest that cells that are $CD11c^+$ are the primary source of MK in the tonsils and that NF-κB and PDE-4 play a role in the production of MK. These novel immunological phenomena have potentially important therapeutic implications.

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P104

STAT5-REGULATED AUTOCRINE IL-6 SIGNALING DICTATES IL-10-DEPENDENT REGULATORY FUNCTIONS OF NEONATAL B10 CELLS

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Abstract Content: Neonates and infants have suboptimal immune responses to immunization compared to adults, resulting in higher susceptibility to microbial infection. B lymphocytes have a central role in humoral immunity to immunization and infection, and antigen recognition by B cell receptors (BCRs) is essential for the activation and differentiation of B lymphocytes. Neonatal B cells, however, do not respond to BCR activation as robustly as adult cells do. Our hypothesis is that unique features of neonatal BCR signaling may be contributing to suboptimal humoral responses. Thus, the aim of this project is to decipher the neonatal BCR signaling pathways and identify those that may be responsible for suboptimal B cell functions.
Splenic B cells purified from neonatal and adult mice were stimulated ex vivo with anti-mouse IgM F(ab')2 to crosslink BCRs. RNA sequencing analysis revealed that gene sets related to signal transducer and activator of transcription

(STAT) 3 and STAT5 signaling pathways were enriched in neonatal B cells following BCR cross-linking. We found that STAT3 and STAT5 were highly phosphorylated in neonatal B cells compared to adult B cells in response to BCR activation. Using small molecule inhibitors and small interfering RNA, we determined that BCR-mediated STAT5 phosphorylation induces rapid IL-6 production, which in turn activates STAT3 in an autocrine or paracrine manner. Moreover, IL-6-induced STAT3 activation led to the production of anti-inflammatory cytokine, IL-10. Further underscoring the role of IL-6 in the induction of IL-10, B cells from IL-6 knock-out mouse did not secrete IL-10 in response to BCR activation. IL-10 is known to act as a negative regulator of inflammatory cytokine production by macrophages. To assess whether the autocrine IL-6 is essential for IL-10-dependent immunosuppression, we incubated peritoneal macrophages with conditioned medium from adult B cells, wild-type neonatal B cells, or IL-6-deficient neonatal B cells. We found that conditioned medium from wild-type neonatal B cells suppressed TNF-α production by macrophages in an IL-10-dependent manner, whereas conditioned medium from IL-6-deficient neonatal B cells or adult B cells did not.
Our studies unveiled the essential role for STAT5-induced autocrine IL-6 signaling in IL-10-mediated immunosuppressive functions of neonatal B cells.

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P105

TET2 IS A CELL INTRINSIC REGULATOR FOR INTESTINAL TH2 IMMUNITY

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Abstract Content: The intestinal immune system facilitates nutrient absorption in the presence of diverse commensal microbiota while establishing a protective barrier to prevent infection. Prototypically studied microbes induce specific immune programs and these models provide insight into how the immune system is regulated in this unique environment. *Tritrichomonas* species are protozoan pathosymbionts that are common in many animal facilities. These protozoa typically induce a type-2 immune program in the small intestine characterized by interleukin-25 (IL-25) signaling and secretory cell hyperplasia that is primarily mediated through the action of GATA3+ innate lymphocytes (ILC2s). However, unlike immunity to helminths, for which the type 2 immune program is evolved, this immune response is self-limiting; a state of tolerance or anergy is developed whereby the protozoa continue to occupy the lumen without significant continued immune activation. We previously identified small intestinal barrier dysfunction correlated with increased IL-25 signaling in mice deficient for the methylcytosine dioxygenase Tet2. In these mice, *Tritrichomonas* colonization induced an adaptive Th2 response which chronically propagated this IL-25 circuit. Naïve lymphocytes typically require paracrine signaling from various innate populations for efficient Th2 differentation in helminth infections. Tet2-deficient naïve cells however are able to sufficiently drive autocrine Th2 polarization even in the absence of helmith induced innate activation. In a model of peanut allergy, the loss of Tet2 and associated type 2 immune activation predisposed mice to anaphylaxis. Collectively, our findings provide evidence for a cell-intrinsic checkpoint that prevents allergic immunopathology in the microbe-rich intestinal environment.

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P106

DYNAMIC CHROMATIN ACCESSIBILITY LICENSES STAT5- AND STAT6-DEPENDENT INNATE-LIKE FUNCTION OF TH9 CELLS TO PROMOTE ALLERGIC INFLAMMATION AND DEFINE A NOVEL CLINICOPATHOLOGIC ENDOTYPE

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Abstract Content: Allergic diseases are a major global health issue, causing significant morbidity and mortality. T helper 9 (Th9) cells drive allergic inflammation through interleukin-9 (IL-9). Yet Th9-driven allergic inflammation is incompletely characterized, in part because Th9 cells are a uniquely unstable subset. The mechanisms and functions of Th9 lineage instability are unknown, although T cell receptor (TCR) activation is hypothesized to play a key role. Conversely to this paradigm, we found that resting Th9 cells did not require TCR restimulation for IL-9 production, which was instead promoted by STAT5- and STAT6-dependent paracrine cytokines. This mechanism was restricted to recently activated cells because withdrawal of TCR stimulation reduced accessibility of STAT-dependent *IL9* enhancers, promoting lineage instability. Th9 cells induced allergic lung inflammation *in vivo* via TCR-independent, STAT-dependent mechanisms. In patients with allergic disease, Th9 expansion was associated with STAT activation and responsiveness to JAK inhibitors. These findings suggest that Th9 instability is a negative checkpoint on TCR-independent inflammation that breaks down in allergic disease, and that jakinibs should be considered as treatments for Th9-associated diseases.

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P107

NEURONS CAN INHIBIT THE ACTIVATION OF T CELLS VIA REGULATING THE TGF-ß PATHWAYS

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Abstract Content: The brain is considered to be an immune-privilege organ, in which adaptive immunity and inflammation are highly controlled. After brain tissue damage occurs, e.g. stroke, various immune cells are infiltrated and brain cells such as neurons died, and T cells play an important role in brain inflammation. We established a neuron and T-cell co-culture system *in vitro*, to study how neurons can regulate T cell activation. Under stimulation of T cells by anti-CD3/CD28 antibodies, neurons isolated from the brain can inhibit expression of IFN-g and CD62L compared to T cells alone. In addition, neurons induced CD73 expression, known as a marker of regulatory T cells. Interestingly, all these proteins were fully restored in the presence of SB431542, an inhibitor for TGF-ß pathways including ALK 4, 5, and 7 receptors. Therefore, we think that neurons may inhibit T cell activation by regulating TGF-ß pathways. Probably, the TGF-ß pathways might be involved in the immune-privilege mechanisms suppressing T cell activation. The detailed molecular mechanisms are currently under investigation. (NRF2022R1A2C1010354)

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P109

HETERODIMERIC IL-15 (HETIL-15) MONOTHERAPY INDUCES TUMOR INFILTRATION OF A NOVEL DENDRITIC CELL POPULATION ASSOCIATED WITH REGRESSION OF ORTHOTOPIC BREAST AND PANCREATIC CANCER IN MOUSE MODELS

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Abstract Content: Introduction: We have produced the native heterodimeric form of IL-15 (hetIL-15), which has advanced in clinical trials due to its anticancer activities in many mouse cancer models. The objective of this study was to identify the effect of hetIL-15 on the tumor-infiltrating lymphoid and myeloid cell populations in different mouse models of breast and pancreatic cancer. We identified and characterized a new population of infiltrating dendritic cells (DCs) present mainly after hetIL-15 treatment.
Study design and methods: In this study we analyzed the therapeutic efficacy of hetIL-15 administration in three different mouse cancer models: the EO771 and 4T1 breast cancer models, and a KPC derived pancreatic cancer model. hetIL-15 effects on tumor-infiltrating immune cells were evaluated by flow cytometry (FC) and immunohistochemistry (IHC). The novel DCs were further studied using single-cell RNAsequencing (scRNAseq) and RNA-ISH.
Results: hetIL-15 treatment resulted in complete tumor regression in 40% of the treated mice of the EO771 cancer model. Additionally, hetIL-15 monotherapy caused a significant tumor delay in 4T1 and KPC derived cancer models. FC analysis revealed a significant accumulation of both $CD8^+$T and NK cells in the hetIL-15 treated tumors. The tumor-infiltrating $CD8^+$T and NK cells were characterized by higher content of the cytotoxic marker Granzyme B and increased proliferation. These results were also verified using IHC. Furthermore, when we analyzed the myeloid tumor-infiltrating cells, FC analysis showed an increase of conventional type 1 dendritic cells (cDC1s), only in EO771 breast tumors whereas 4T1 breast and KPC derived pancreatic tumors displayed an accumulation of cDC2s. Importantly, FC revealed an additional novel distinct DC population characterized by $CD103^{int}CD11b^+$immunophenotype, which was mostly evident in hetIL-15 treated tumors in all three models. Phenotypic profiling of this novel DC population identified expression of several cDC1 specific markers. Both cDC1 and the novel DC populations were inversely correlated with the tumor size in EO771 cancer model. In addition, scRNAseq data demonstrated that $CD103^{int}CD11b^+$DCs form a distinct cluster with a transcriptional profile most closely associated with monocyte-derived DCs and may have functional role intratumorally due to the expression of genes associated with antigen presentation. Using RNAscope, we monitored these novel DCs into the treated tumors, due to the unique high co-expression of *Mgl2*, *CD24a* and *Ccl17*.
Conclusions: hetIL-15 affects both T cells and cDCs in syngeneic murine models of breast and pancreatic cancer. We report that the treatment with hetIL-15 increases a novel distinct $CD103^{int}CD11b^+$DC population in all three models, which enhances the immune response against cancer. These findings suggest hetIL-15 as a promising therapeutic agent in the treatment of triple negative breast and pancreatic cancer enhancing both the cytotoxic arm of the immune system but also affecting positively the interplay with the myeloid cells

Disclosure of Interest: None Declared
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LB-10

IDENTIFICATION OF DC-T CELL INTERACTIONS DRIVING IMMUNE RESPONSES TO FOOD

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Abstract Content: The intestinal immune system is charged with maintaining tolerance to harmless stimuli from food and commensal bacteria, while providing protective immunity against pathogens. Dendritic cells (DCs) are key players in orchestrating immune responses by presenting luminal antigens and inducing functional differentiation of $CD4^+$ T cells into regulatory (Treg) or pro-inflammatory (Th) subsets. Here, we investigated the cellular mechanisms underlying the decision between tolerance or immunity to dietary antigens using an intestine-adapted LIPSTIC (Labeling Immune Partnerships by SorTagging Intercellular Contacts) technology. By combining LIPSTIC with single-cell transcriptomics, we found that a small fraction of total DCs in the duodenum-draining lymph node present cognate dietary antigen to $CD4^+$ T cells, including migratory classical dendritic cells (cDC1 and cDC2), but not resident DCs. While cDC2 induce T cell activation to dietary antigens, cDC1 display a tolerogenic program and are responsible for Treg differentiation. Infection with the helminth *Strongyloides venezuelensis* during oral antigen exposure, known to break oral tolerance, abrogated tolerogenic cDC1 presentation of dietary antigens and Treg induction concomitantly expanding cDC2 subsets inducing type-2 immunity. In contrast, infection with helminth *Heligmosomoides polygyrus*,

only partially abrogated dietary antigen presentation by the tolerogenic cDC1, allowing for maintenance of oral tolerance generated by cDC1-induced Tregs. Our studies reveal that dietary antigen presentation by a small fraction of tolerogenic cDC1 is sufficient and required for the induction of Treg cells during the development of oral tolerance. Additionally, our studies indicate that break in oral tolerance can be achieved by targeting these cDC1 cells during inflammation induced by co-infection events.

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P110

A NEXT GENERATION BIFUNCTIONAL SUPERKINE FOR IMMUNOTHERAPY (BISKIT) ENCOMPASSING THE COMBINED THERAPEUTIC POTENCY OF IL-2 SUPER-AGONIST AND ANTI-PD1

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Abstract Content: Introduction:
Combination treatment with engineered IL-2 variants and the immune-checkpoint inhibitor anti-PD1 demonstrated potent therapeutic efficacy by activating CD8 T and NK cells while preventing these effector immune cells from becoming functionally exhausted. Co-administration of IL-2 and anti-PD1 can potentially face challenges related to overlapping toxicities when both therapeutics need to be delivered at sufficiently high dose to ensure sufficient target engagement on the same cells to achieve synergistic response. To overcome this challenge, we fused our IL-2 super-agonist (MDNA109FEAA) to a therapeutic anti-PD1 antibody to generate a single BiSKIT, designated as anti-PD1-MDNA109FEAA, to facilitate cis (i.e., on the same cell) engagement of IL-2 receptor (IL-2R) and PD1. MDNA109FEAA is a human IL-2 super-agonist exhibiting enhanced affinity for IL-2Rb (CD122) and no binding to IL-2Ra (CD25), designed to preferentially activate effector immune cells over immune suppressive T regulatory cells (Tregs).
Experimental Procedure:
Studies included binding analyses with Biacore/SPR, in vitro pSTAT5 signaling in human PBMCs, in vitro PD1/PDL-1 blockade and efficacy studies in syngeneic tumor models.
Summary of Data:
Anti-human (h)PD1-MDNA109FEAA and surrogate anti-mouse (m)PD1-MDNA109FEAA demonstrated selective binding to either hPD1 or mPD1 and inhibition of PD1/PDL-1 blockade in respective human and mouse in vitro functional assays. Studies of p-STAT5 signaling in human primary peripheral blood monomorphic cells (PBMCs) with both constructs showed enhanced activation of CD8 T cells and NK cells with reduced activity on Tregs when compared to rhIL-2. In mice, anti-mPD1-MDNA109FEAA has a shorter half-life than expected of antibodies such as anti-PD1, consistent with rapid binding to IL-2R expressing immune cells and the observed increase in proliferation. In the CT26 colon tumor and B16F10 melanoma syngeneic models, treatment with anti-mPD1-MDNA109FEAA was significantly more effective at tumor growth inhibition than equal molar dose of anti-mPD1, MDNA19 (i.e., MDNA109FEAA-Fc) or their co-administration. These data demonstrate the in vivo therapeutic superiority of anti-mPD1-MDNA109FEAA over the combined effect of anti-PD1 and MDNA19 when co-administered independently.
Conclusion:
We have engineered anti-PD1-MDNA109FEAA to enable cis engagement with IL-2 receptor and PD1 as an approach to activate immune cells while reducing their exhaustion and demonstrated superior in vivo therapeutic efficacy. These data exemplify the versatility of the superkine platform to empower next generation BiSKITs to leverage the combined therapeutic potential of cytokines with approved therapeutics including but not limited to check-point inhibitors such as anti-PD1.

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P111

POST-TRANSCRIPTIONAL REGULATORY CIRCUITY MODULATING CD69 IN T CELLS

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Abstract Content: Intersecting transcriptome-wide RNA binding protein occupancy with probability estimation of causal single-nucleotide polymorphisms (SNP) associated with immune-mediated diseases identified candidate RNA cis-regulatory elements in 3'untranslated region (3'UTR). Functional testing of these candidate regions confirmed post-transcriptional regulation of important cytokine signaling proteins and cell surface molecules, including CD69, a membrane protein induced by T cell receptor engagement and interferon stimulation. The presence of autoimmune disease associated genetic variants, as well as numerous predicted RNA binding protein sites within the 3'UTR, suggests that *CD69* may contain post-transcriptional regulatory circuity that affects its protein expression as well as T cell function. To understand the mechanism by which *CD69* is post-transcriptionally regulated, we dissected the 3'UTR of the gene using CRISPR and identified a protein occupied region, conserved between mouse and human, that promotes transcript degradation and modulates protein expression in mouse and human T cells. We deleted this region, as well as the whole 3'UTR, in mice to determine the effect of post-transcriptional regulation on T cell function. Mice that lacked *Cd69* 3'UTR showed impaired thymic egress and accumulation of mature-like single positive thymocytes, a phenotype that was not observed in mice with deletion of the specific regulatory region (Δβ) at baseline. We adoptively co-transferred WT and Δβ naïve OTII into recipient mice and induced allergic inflammation using house-dust mite and ovalbumin, and found that lung Δβ OTII expressed higher levels of CD69 after challenge compared to WT. Together, our data demonstrates the crucial role post-transcriptional circuitry plays in modulating CD69 expression and T cell migration, and identified a specific region that contributes to this regulation. Future studies will evaluate how post-transcriptional regulation of CD69 contributes to tissue residency of memory T cells.

Disclosure of Interest: None Declared
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05. Cytokines and metabolism

P112

SCHISTOSOME INDUCED INNATE METABOLIC TRAINING: THE ROLE OF BIOLOGICAL SEX

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Abstract Content: Despite strong evidence that helminth infections are protective against the development of metabolic disease, a major gap exists in understanding the mechanism(s) underlying this protection. We have recently demonstrated that macrophages derived from the bone marrow (BMDM) of *S. mansoni* infected male ApoE$^{-/-}$ mice have dramatically increased mitochondrial respiration and mitochondrial mass compared to those from uninfected mice. This is accompanied by increased glucose and palmitate shuttling into TCA cycle intermediates and accumulation of free fatty acids, with decreased accumulation of cellular cholesterol esters. We have demonstrated that this metabolic reprogramming is not due solely to IL-4, and that IFNgamma signaling is involved in this phenotype. The systemic effect of metabolic modulation by schistosome infection is a function of biological sex, where schistosome infection protects ApoE$^{-/-}$ male mice from obesity and glucose intolerance, but not female mice. The sex-dependent effects of infection extend to myeloid cells specifically, where the metabolic transcriptional profile, basal metabolism, and carbon substrate flexibility of BMDM from infected females is significantly distinct from BMDM from infected males. These data indicate that females are resistant to infection induced metabolic reprogramming. Importantly, we have demonstrated that the resistance to infection induced modulation in females is linked to female sex hormones and can reversed. This work provides evidence that *S. mansoni* induces innate training that reprograms the metabolism of the myeloid compartment in a sex-dependent manner.

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P113

THE CONTRIBUTION OF ADIPOSE TISSUE INFLAMMATION TO PLASMA CYTOKINE LEVELS IN PERSONS WITH HIV

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Abstract Content:
Background: Persons with HIV (PWH) have heightened innate and adaptive immune activation, which persists despite suppression of plasma viremia on antiretroviral therapy (ART) and contributes to an increased risk of cardiometabolic diseases. HIV infection establishes an adipose tissue viral reservoir and increases adipose inflammation. We assessed the contribution of the adipose tissue immune environment to circulating cytokine levels in PWH with long-term viral suppression.
Methods: 123 PWH with >12 months of well-controlled viremia (plasma HIV-1 RNA <50 copies/mL) on ART had fasting blood collection and abdominal subcutaneous adipose tissue (SAT) biopsy. Plasma cytokines (interleukin [IL]-1β, -4, -5, -6, -8, -10, and -12p70, interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], serum amyloid A [SAA], vascular cell adhesion molecule-1 [VCAM-1] and intracellular cell adhesion molecule-1 [ICAM-1]) were measured by Mesoscale multiplex assay, and high-sensitivity CRP (hs-CRP) by nephelometry. SAT gene expression was measured on total RNA using a NanoString panel of 255 immune-related genes. Associations between cytokine levels and gene expression were assessed using multivariable linear regression adjusted for body mass index (BMI) and other covariates. Canonical correlation analysis (CCA) was used to identify combinations of genes and cytokines with the maximum correlation.
Results: Participants had a median age of 47 years, BMI 32 kg/m^2, CD4+ count 849 cells/μL, and 7.0 years of ART exposure; 78% were male and 40% Black. After adjustment for multiple comparisons, only hsCRP and SAA were significantly associated with expression of >2 individual SAT genes (*IRF3*, *CCL24*, *MAX,* and *CCL24, IRF3, MRC1,* respectively) in linear regression models (FDR<0.05). In the CCA, 22 genes with FDR <0.2 were included to avoid overfitting. The first canonical dimension had a significant correlation of 0.74 (p=0.001) between the sets of variables (cytokines and genes). For the cytokines, the first canonical dimension was most strongly influenced by lower levels of IL-4 (-0.66) and IL-10 (-0.56), and higher SAA (0.35), TNF-α (0.31), ICAM-1 (0.29), and hsCRP (0.26). For the gene variables, this dimension was most strongly influenced by higher expression of *DEFA1* (0.72; neutrophil granule defensin), *CXCR1* (0.62; IL-8 receptor), *HLA-DRA* (0.58; MHC2 antigen presentation), *CCL21* (0.39; T cell chemotaxis), and lower expression of *ALOX12* (-0.4; production of lipid mediators), and *TLR1* (-0.31; pathogen recognition).
Conclusions: Lower plasma IL-4 and IL-10, potentially reflecting less Th2 polarization and regulatory function, and higher TNF-α, hsCRP and ICAM-1, markers of inflammation and vascular endothelial activation, in PWH on long term ART were associated with several SAT immune response genes. Interventions to reduce adipose tissue inflammation may represent an important avenue to decrease chronic systemic inflammation and the high burden of cardiometabolic disease among PWH.

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P114

METFORMIN AMELIORATES OLANZAPINE-INDUCED OBESITY AND GLUCOSE INTOLERANCE VIA REGULATING HYPOTHALAMIC INFLAMMATION AND MICROGLIAL ACTIVATION IN FEMALE MICE

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Abstract Content: Olanzapine (OLZ) is an atypical antipsychotic medication that is used to treat schizophrenia, but its use is associated with the development of obesity and metabolic disorders. A series of pharmacological trials to overcome OLZ-induced metabolic side effects were evaluated, but current evidence has been limited due to the lack of knowledge about how OLZ induces obesity and metabolic disorders. Metformin (MET), a commonly used type 2 diabetes medicine, has been prescribed to treat OLZ-induced weight gain and metabolic disorders. Here we found that 5-days administration of OLZ induces hypothalamic microglial activation and inflammation, and systemic glucose intolerance in advance of obesity onset. Notably, both hypothalamic inflammation and systemic glucose intolerance were far more evident in female mice than in male mice. Coadministration of MET with OLZ remarkably prevented OLZ-induced hypothalamic leptin resistance and systemic glucose intolerance by attenuating microglial activation, pro-inflammatory cytokine expression. Our findings not only reveal a critical causative mechanism of OLZ on the development of obesity and metabolic disorders but also explain how MET modulates pathogenesis of OLZ-induced metabolic disturbances by alleviating hypothalamic inflammation.

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P115

GATA4 CONTROLS REGIONALIZATION OF PEYER'S PATCH DEVELOPMENT AND INDUCTION OF MUCOSAL IMMUNE RESPONSES

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Abstract Content: The gastrointestinal tract encounters a wide range of both dietary and microbial antigens requiring regional metabolic and immune functions to support host physiology. Despite these observations, the mechanisms driving regionalization of intestinal immunity and its role in disease are unknown. Here we reveal that the intestinal epithelial transcription factor GATA4 regulated M cell function, maturation of Peyer's patches, and the mucosal immune response between the proximal and distal small intestine. In the absence of epithelial GATA4, the jejunum lost its identity and upregulated ileal specific genes. This phenomenon drastically altered the mucosal immune landscape leading to a reduction in Peyer's patch immune responses, and a deficiency of IgA and intraepithelial lymphocytes in the proximal intestinal tissue. This immune deficiency was a result, in part, through GATA4 controlled retinol metabolism, a critical feature of the proximal intestine. A key consequence of GATA4 regulated loss of intestinal regionalization was an inability of the host to mount antigen specific IgA responses to oral immunization in the Peyer's patches. These data uncover an underappreciated role of the proximal intestine in maintaining both inductive and tissue effector responses through GATA4 dependent metabolic pathways.

Disclosure of Interest: None Declared
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P118

CHEMERIN TO RESISTIN RATIOS IN SERUM AND SYNOVIAL FLUID IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS OF THE KNEE JOINT- A POTENTIAL ROLE BEYOND METABOLISM

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Abstract Content: Adipocytokines regulate not only the lipid metabolism but also take part in some obesity-induced inflammatory diseases. For this reason,they are studied in the light of potential biomarkers for various diseases including rheumatoid arthritis (RA) and knee joint osteoarthritis (KOA). Previous studies of serum and synovial concentrations of chemerin and resistin resulted in ambiguous findings. Therefore, we conducted the current study aiming a better understanding of their possible interaction in these pathologies.

Aim: To examine the chemerin to resistin ratio in serum and synovial fluid as well as the serum to synovial ratio of both chemerin and resistin in patients with RA and KOA. To examine the possible association of these ratios with established inflammatory markers and disease activity score in these patients.
Patients and methods: In total, 54 participants with RA and 28 age-, sex- and BMI-matched patients with KOA were enrolled(synovial fluid was collected from 10 patients with RA and 4 patients with KOA). Circulating and synovial resistin and chemerin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) and the calculated ratios were compared between groups using Mann-Whitney U analysis. Correlation analysis was then performed to describe the assiotion between them and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum level of uric acid (UA), Disease Activity Score-28 for Rheumatoid Arthritis with ESR (DAS28-ESR).
Results:
Results: In the group of 10 RA patients, higher resistin level in synovial fluid was observed (19.54± 15.51 ng/mL), compared to KOA patients (5.29 ± 9.07ng/mL), P=0.034.The serum chemerin to resistin ratio was higher is patients with KOA (37 ± 14) than in the RA group (24±12), P = 0.006. The other ratio that showed significantly higher level was serum to synovial resistine in patients wit KOA (7.8 ± 6.1), compared with the same ratio in RA patients (3.1 ± 3.5), P = 0.034. The synovial chemein to synovial resistin ratio negatively correlated only with serim uric acid (r= -0.733, P = 0.016).
Conclusion: The serum chemerin to resistin ratio might serve as a possible marker for differential diagnosis in patients with RA and KOA. The different ratio between these two adipokines indicates thei potential interactions between these adipokines in patients with RA and KOA. Further studies are needed to determine the causal relationship for this difference and evaluate its importance for the routine medical practice.

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LB-11

GLYCOLYTIC IFN-GAMMA-PRODUCING GAMMA DELTA T CELLS WITH ANTITUMOR FUNCTION ARE REPLACED BY OX-PHOS PROTUMOR IL-17-PRODUCING GAMMA DELTA T CELLS DURING COLON CANCER.

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Abstract Content: gd T cells are the major immune cells in the epithelial layer of the intestine at steady state, but also constitute a major lymphocyte population infiltrating the tumor during colorectal cancers (CRC). However, gd T cell contribution during early phase of CRC development or late phase of cancer progression remains unclear. Analysis of human CRC samples showed that gd T cells located in non-tumor areas exhibit glycolytic metabolism and cytotoxic markers while tumor-infiltrating gd T cells express a pro-tumorigenic profile and an Ox-Phos metabolic state. Murine CRC models confirm these initial observations. Moreover, gd T cell profiles were associated with distinct TCR-V gd gene-usage in both humans and mice. Longitudinal intersectional genetics and antibody-dependent strategies targeting murine gd T cells enriched in the epithelium at steady state led to increase tumor development, without affect tumor size. On the other hand, targeting gd T cell subsets that accumulate during CRC resulted in reduced tumor growth, without affecting tumor number. Our results show that tissue resident IFN-g-producing gd T cells with glycolytic metabolism can suppress tumor formation while tumor-infiltrating IL-17-producing gd T cells with Ox-Phos metabolism can promote tumor growth. Understanding the signals that drive gd T cells anti- and pro-tumor functions can help to control CRC development and progression.

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LB-12
T-BET+ B CELLS ACCUMULATE IN ADIPOSE TISSUE AND EXACERBATE METABOLIC DISORDER DURING OBESITY

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Abstract Content: Obesity is accompanied by inflammation in adipose tissue, impaired glucose tolerance, and changes in adipose leukocyte populations. In adipose tissue from overweight and obese humans and mice, we found increased frequencies of T-bet+ B cells compared to lean control subjects. This increase depends on invariant NKT cells and correlates with weight gain during obesity. Transfer of B cells enriched for T-bet+ cells exacerbates metabolic disorder in obesity, while ablation of Tbx21 specifically in B cells reduces serum IgG2c levels, inflammatory cytokines, and inflammatory macrophages in adipose tissue, ameliorating metabolic symptoms. Furthermore, transfer of serum or purified IgG from HFD mice restores metabolic disease in T-bet+ B cell-deficient mice, confirming T-bet+ B cell-derived IgG as a key mediator of inflammation during obesity. Together, these findings reveal an important pathological role for T-bet+ B cells that should inform future immunotherapy design in type 2 diabetes and other inflammatory conditions.

Disclosure of Interest: None Declared
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06. Cytokine storm/inflammation

P119
INTERFERONS INDUCE CANCER CELL DEATH IN COMBINATION WITH IAP ANTAGONISTS

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Abstract Content: The Inhibitors of Apoptosis (IAPs) are oncogenes that enable cancer cells to evade numerous death signals, including signals originating from the immune system. A number of IAP antagonists have been developed to degrade two IAPs, cIAP1 and cIAP2, which are proteins important to immunity and cancer cell survival. The eradication of cancer cells by IAP antagonists require an immunological death ligand, such as Tumor necrosis factor alpha (TNFα). In addition, IAP antagonists modulate inflammation and immunity by regulating the activity of the alternative Nuclear Factor Kappa B (NF-κB) signaling pathway, a pathway that is critical for the control of immune cell activity. We are exploring the combination of IAP antagonists with Interferon (IFN) for their potency in various animal tumor models. Our data shows that the combination is effective at generating long-term durable cures in mouse models of cancer. Mechanistically, IFN and IAP antagonists induce complementary cytokine expression profiles that converge to induce cancer cell death, and this synergy is dependent on macrophages. Our findings from these pre-clinical studies will guide the use of IAP antagonists and IFNs for the treatment of cancer.

Disclosure of Interest: None Declared
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P120
INCREASED CYTOKINE STORM AND ALTERED SIGNALING IN A 'HUMANIZED' MURINE CD28 MODEL

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Abstract Content: Animal models do not always reflect the human condition, which complicates translation of laboratory findings to the clinic. One such example was the phase one clinical trial of a CD28 super agonist antibody (CD28SA). In mice, CD28SA treatment expanded regulatory T cells (Tregs) and reduced autoimmune disease.

However, when healthy human volunteers received CD28SA, they experienced life-threatening cytokine storm and multiorgan failure requiring intensive care hospitalization. CD28 is constitutively expressed on T cells and provides the canonical co-stimulatory “signal 2” needed for T cell activation. The proline residues in the CD28 PYAP signaling domain are required for downstream signaling and this motif is conserved across species. However, the human PYAP domain is adjacent to an additional proline, while murine PYAP is followed by an inactive alanine residue. Previous in vitro studies have suggested this single amino acid variation modulates downstream signaling leading to interspecies differences in T cell activation. We have developed a ‘humanized’ CD28 mouse with a CD28 A to P substitution at residue 210 adjacent to the C-terminal proline rich domain ($CD28^{A210P}$). In vitro, $CD28^{A210P}$ T cells had increased Akt phosphorylation, JunB nuclear localization, and produced more IL-2 compared to WT T cells when stimulated with anti-CD3 and CD28. When driven to differentiate into Th17 cells, $CD28^{A210P}$ T cells were more susceptible to CD28-mediated suppression of Th17 development, that was only partially corrected by IL-2 neutralization, corresponding to our findings in human Th17 cell development. In addition, we assessed the *in vivo* response of the “humanized” CD28 mice to the CD28 super agonist antibody that induced a regulatory response in wild type mice and a cytokine storm in human patients. After injection with CD28 super agonist, $CD28^{A210P}$ mice had significantly increased weight loss, elevated and sustained pro-inflammatory cytokine production, and less Treg expansion compared to WT mice. Overall, this study suggests interspecies variation in CD28 signaling due to differences outside of the well-studied signaling domains can alter outcomes of CD28 ligation and promote divergent outcomes when targeted *in vivo.*

Disclosure of Interest: None Declared
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P121

TRANSCRIPTIONAL ANALYSIS OF CD14+ MONOCYTES DURING MACROPHAGE ACTIVATION SYNDROME HIGHLIGHTS ROLE FOR INTERFERONS AND RNA SENSING IN MONOCYTES

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Abstract Content: Macrophage activation syndrome (MAS), a form of secondary hemophagocytic lymphohistiocytosis (HLH), is a potentially fatal complication of rheumatic diseases. Most commonly associated with systemic juvenile idiopathic arthritis (sJIA), MAS affects at least 10% of sJIA patients with up to 50% exhibiting signs of subclinical inflammation. MAS is a dysfunctional hyperinflammatory response characterized by abnormal activation of lymphocytes and phagocytes, leading to an overproduction of inflammatory cytokines and damage to host tissues. Myeloid cells such as circulating monocytes play a crucial role in the pathogenesis of MAS, but have not been well-studied in this disease. Circulating monocytes are particularly responsive to their surrounding environment and are known to exhibit phenotypic and functional changes during inflammation. We analyzed classical CD14+ monocytes from children with active MAS compared to individuals with sJIA without MAS and age/sex/race matched healthy children. We found significant upregulation of CD16 surface expression during active MAS, which rapidly reversed post-treatment with systemic steroids. We performed transcriptional analysis using RNA sequencing of these monocytes to define differentially expressed genes in MAS monocytes. Our analyses show broad transcriptional changes in CD14+ monocytes from children with active MAS, including upregulation of RNase 2 (involved in processing RNAs for the innate immune sensor TLR8) and SLAMF7 (associated with monocyte/macrophage hyperinflammation in response to interferon gamma). In parallel, we performed single cell RNA sequencing on myeloid cells from two subjects with active MAS, which revealed a strong interferon signature in MAS monocytes and upregulation of alarmins, including S100A8, S100A9, and S100A12. These data confirm an important role for cytokines, such as interferons, in driving gene expression in monocytes during MAS and suggest potential targets for future therapies. Together, our data show that CD14+ monocytes have a unique transcriptional signature in MAS suggesting interferon signaling and enhanced RNA sensing in active disease.

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P122

DETERMINING THE ROLE FOR LONG NONCODING RNA DURING INFLAMMATION

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Abstract Content: Advances in deep sequencing technologies have revealed that the majority of the human genome is actively transcribed into RNA. Our lab is focused on characterizing the largest group of RNA produced from the genome named long noncoding RNA (lncRNAs) and their associated protein binding partners. To date only 3% of lncRNAs have been functionally validated. Using both long and short read sequencing technologies we have generated an Isoform-level transcriptome atlas of macrophage activation characterizing all inflammatory inducible genes. We have taken both targeted as well as high throughput approaches to identify lncRNAs that function to control inflammation. Using CRISPR technology we have performed systematic unbiased screens to identify functionally relevant lncRNAs involved in viability and inflammatory functions within macrophages. We have begun to functionally characterize both positive and negative regulatory lncRNAs and determine their mechanisms of action. Focusing on conserved lncRNAs we have generated a number of mouse models to showcase the importance of these genes in controlling host responses in vivo. The overall goal of the work in our lab is to identify critical regulators of inflammation with the aim of identifying new targets for therapeutic intervention for inflammatory conditions.

Disclosure of Interest: S. Carpenter Consultant for: NextRNA
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P123

DEFINING THE MOLECULAR BASIS FOR SYSTEMIC INFLAMMATION IN RESPONSE TO SARS-COV-2

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Abstract Content: SARS-CoV-2 infection results in systemic inflammation despite being a respiratory pathogen. To understand this dynamic, we characterized the organ-specific responses to SARS-CoV-2 in the golden hamster. We find that while infectious virus is mainly restricted to the airways and stochastically to a subset of tissues, a strong inflammatory response is consistently observed in the lung, olfactory bulb, kidney, spleen, liver, pancreas, heart, lung, intestines, and whole brain.

In order to characterize the nature of the material initiating the systemic inflammatory response, we studied the blood of infected animals. We found that while no infectious virus could be detected, viral RNA was identified only very early after infection. Additionally, we were able to indirectly detect low levels of circulating interferon in the serum of intranasally infected animals, and the transcriptional profile of whole blood revealed a strong type I interferon (IFN-I) signature. Importantly, we found that the presence of circulating interferon is contingent on active virus replication in the lung, since when by-passing replication in the lung by direct intravenous injection, we no longer detect interferon in circulation.

Next, we wanted to define if the inflammation of peripheral tissues had an impact on disease severity. We hypothesized that inflammation could be priming distal organs and preventing infection of distal tissues. To test this, we dampened organ priming with corticosteroids and found increased dissemination of infectious SARS-CoV-2. Finally, by directly administering virus intravenously, we generated a condition in which we entirely by-passed priming of the organs and lung immune control, and found all organs were susceptible to infection as shown by recovery of infectious virus from each.

All together, these data show that SARS-CoV-2 replication induces early RNAemia and interferon production which is dependent on active viral replication in the lung, leading to priming of peripheral organs. This systemic response limits virus access to distal tissues shortly after infection, since in the absence of priming and high viremia, a multi-organ infection by SARS-CoV-2 can occur.

Disclosure of Interest: None Declared
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P124

A NEW NON-TRANSCRIPTIONAL FUNCTION OF IRF3 INHIBITS VIRAL INFLAMMATION

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Abstract Content: Interferon (IFN) regulatory factor 3 (IRF3) is a critical transcription factor responsible for the induction of IFNs and antiviral genes in virus-infected cells. IRF3 functions require its activation by phosphorylation in the cytoplasm resulting in its translocation to the nucleus to transcribe its target genes. We revealed that IRF3 can be activated by linear polyubiquitination to translocate to the mitochondria resulting in apoptotic cell death. The pro-apoptotic activity of IRF3 is independent of its transcriptional functions. Both transcriptional and pro-apoptotic functions of IRF3 are required for its optimal antiviral activities. Here we present a new non-transcriptional activity of IRF3, the repression of IRF3-mediated NF-kB activity (RIKA), which suppresses the inflammatory gene expression in virus-infected cells and mice. IRF3 inhibits the nuclear translocation of the NF-kB-p65 transcription factor, thereby preventing the inflammatory gene induction in response to virus infection. We created a mutant IRF3, which is defective in transcriptional and pro-apoptotic activities but functional in RIKA. Our results reveal a new functional branch of IRF3 that suppresses virus-induced inflammation, often detrimental to the host. The new function of IRF3, RIKA, may play a key role in preventing the harmful inflammatory response by the host.

Disclosure of Interest: None Declared
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P125

INTERLEUKIN-11 PRODUCED BY MYELOID CELLS DRIVES AUTOIMMUNE DISEASE PATHOLOGY

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Abstract Content:
Introduction: Interleukin (IL)-11 is a member of the IL-6 family of cytokines with emerging roles in autoimmune diseases[1]. The chromosomal region containing the IL-11 gene (19q13) has been linked to Multiple Sclerosis (MS), IL-11 is elevated in the serum of MS patients and in the synovial fluid of arthritis patients, and IL-11 receptor (R) expression is part of a gene signature for multiple autoimmune diseases. However, our understanding of the biological relevance of this cytokine remains in its infancy.
Methods: We questioned the cellular source of IL-11 in MS and arthritis. To this end, we utilised CRISPR-Cas9 gene editing to generate a novel IL-11 reporter mouse (*il11*^{RFP}) that allowed for monitoring the cellular source of IL-11 in real-time, without physiological disruption of IL-11 function. We undertook detailed flow cytometry analysis of mice that had undergone experimental autoimmune encephalomyelitis (EAE), a murine model of MS, and antigen-induced arthritis, a pre-clinical model of rheumatoid arthritis. To define the function of each IL-11 expressing cell population that we identified in disease, we generated an *il11*^{flox} mouse by CRISPR, which was crossed with relevant cell specific Cre-recombinase expressing mice.
Results: Our results reveal that while multiple cell types can produce IL-11, it is the IL-11 produced by myeloid cells that drives disease pathology. We show that myeloid derived IL-11 signals to CD4+ T-cells to promote a pathogenic Th17 cytokine signature, demonstrating a new therapeutic opportunity to dampen the inflammation associated with multiple autoimmune pathologies. The therapeutic potential of blocking IL-11 signalling was modelled using *il11r*KO mice, which mimic systemic administration of a neutralising drug.

Conclusion: We have revealed an unappreciated role for IL-11 in autoimmune pathologies. We define the mechanism by which IL-11 promotes a pathogenic inflammatory response. Together, our results suggest that pharmacological manipulation of IL-11 signalling represents a new therapeutic opportunity.

Disclosure of Interest: None Declared
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P126

ABLATING IFN-I RESPONSES IN ASTROCYTES IS PROTECTIVE IN A MOUSE MODEL OF IFN-ALPHA-MEDIATED NEUROTOXICITY

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Abstract Content: In the central nervous system (CNS), maintenance of a healthy, homeostatic environment relies on a tightly regulated type I interferon (IFN-I) response and uncontrolled IFN-I production results in widespread neurotoxicity. Astrocytes are the most abundant glial population in the CNS and are key effectors of innate immune defences including blood-brain barrier (BBB) function. As a primary responder to IFN-I, astrocytes are unsurprisingly implicated in the pathogenesis of a number of neuroinflammatory and neurodegenerative diseases, including Aicardi-Goutières Syndrome (AGS), the prototypical type I interferon-mediated CNS disorder. Transgenic mice that chronically overproduce IFN-alpha in the CNS (GIFN mice) recapitulate many neuropathological features seen in patients with AGS, including inflammatory encephalitis, intracerebral calcifications, leukocyte infiltration, gliosis, neurodegeneration, and exhibit severe motor and behavioural alterations.
To elucidate the role of astrocytes in the pathogenesis of IFN-a-mediated disease, we generated a novel transgenic GIFN mouse line in which astrocytes are unresponsive to IFN-I, using Cre/lox-mediated recombination of the *Ifnar1* gene. Astrocyte-specific deletion of the IFN-I receptor (IFNAR) improved survival and delayed onset of disease. A reduction in disease severity was observed, accompanied by improved balance and motor coordination. In the CNS, this was associated with protection from neuropathology including severe neurodegeneration, calcifications, aneurysms, gliosis, and improved BBB function. Our findings suggest that cell-specific ablation of IFN-I signalling in astrocytes is protective in a neuroinflammatory environment and highlights their contribution to pathology. Understanding these mechanisms is a key step in the development of targeted, cell-specific therapies and may have potential relevance to other IFN-alpha-mediated CNS disorders.

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P127

COMBINATION OF CYTOKINE STORM LEVELS IN AN IN VITRO MODEL OF THE HUMAN BONE MARROW POTENTIATES GENOTOXICITY: IMPLICATIONS FOR MALIGNANT COMPLICATIONS

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Abstract Content: Haematopoietic stem cell transplantation (HSCT) utilises high doses of chemotherapy known as myeloablative therapy to clear the bone marrow (BM) before transplant, which damages the BM stroma, produces a ‘cytokine storm,’ and leads to a chemotherapy-induced bystander effect (CIBE) between the niche and HSC. Patients have significant post-HSCT long-term complications such as therapy-related leukaemia and donor cell leukaemia (DCL) post chemotherapy and HSCT respectively. DCL is a rare, but increasingly observed new form of malignancy where donated cells become malignant within the recipient whilst the donor remains healthy. We hypothesised that the cytokine storm involved in CIBE contributes to leukaemogenesis in the newly donated cells. As DCL mainly

presents as acute myeloid leukaemia, we speculated that cytokines involved in myeloid lineage development would support the development of DCL. This research investigated the capacity of varying levels of cytokine exposure to induce mutagenesis in a BM model.
Following an 80-cytokine array of chemotherapy-exposed BM stromal cells, we selected GM-CSF, G-CSF, TNFα, TGFβ and IL-6 as candidates for genotoxic capacity. The p53 competent lymphoblast cell line TK6 was used as a standard for genotoxicity testing and a model of HSC. Cells were grown to the start of exponential phase and exposed to both 'healthy' (50-1000 pg/ml) and 'storm' levels (1000-4000 pg/ml) of cytokines for 24 hours. Genotoxicity was assessed using the micronucleus (MN) assay according to the Organisation for Economic Co-operation & Development (OECD) guidelines where MN are only 'scored' when cell viability is ≥50%. At all treatments levels, viability was ≥50%. At healthy doses, each cytokine gave increased MN results but only TNF-α and TGF-β did so by more than twice the PBS control (not statistically significant) which is considered to be 'weakly positive'.
At cytokine storm levels, all five cytokines in isolation increased MN to at least 3-fold above the PBS control ($p<0.05$). Cytokine paired combination treatments were then performed to mimic 'storm', utilising lower and higher end range of storm levels. Formation of MN were further raised in all paired combinations compared to the single storm treatment. MN formation in TNF-α, IL-6, GM-CSF and TGF- β combination treatments were significantly different to the PBS control (between $p \leq 0.05$, to $p \leq 0.0001$) whereas G-CSF was only significantly different in combination with GM-CSF ($p \leq 0.05$). These data infer these cytokines stimulate MN formation and substantially increase genotoxic events in combination treatment by potentiating each other; these may influence DCL development but require further exploration to understand how this occurs.

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P128

EXPLORING SYNOVITIS' HETEROGENEITY THROUGH NEXT GENERATION SEQUENCING

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Abstract Content: Rheumatoid Arthritis (RA) is a complex, heterogeneous immune-mediated inflammatory condition which varies in severity and response to therapy. Synovial biopsies show three broad patterns of disease classified as myeloid-rich, lymphoid-rich and fibroblast-rich synovitis. Based on patient responses to biological drugs that target cytokine signalling pathways, we examined how cytokines shape the course of disease progression.
Previously we have reported that interleukin-6 (IL-6) and IL-27 play pivotal roles in the onset and maintenance of synovitis. WT, *Il27ra*$^{-/-}$ and *Il6ra*$^{-/-}$ mice develop differing forms of synovitis in antigen-induced arthritis (AIA). These patterns of disease resemble those seen in human rheumatoid arthritis. To understand the transcriptional and epigenetic mechanisms contributing the development of disease heterogeneity, we applied next-generation sequencing techniques (specifically Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-seq), and Chromatin Immunoprecipitation sequencing (ChIP-seq) for STAT1, STAT3 and P300).
AIA was established in WT, Il27ra$^{-/-}$ and Il6ra$^{-/-}$ mice. Genomic DNA was extracted from synovial tissues collected on days 3 and 10 post arthritis onset and processed for sequence analysis. To interrogate ATAC-seq, we applied a novel bioinformatic pipeline to consider the frequency and degree of chromatin accessibility in each experimental condition. Dataset were mapped against corresponding ChIP-seq results for STAT1, STAT3 and P300 to evaluate the impact of IL-6 and IL-27 Jak-STAT cytokine signalling. Datasets are currently being mapped to synovial RNA-seq data (previously generated by the laboratory) to investigate the multiple levels of epigenetic control contributing to disease heterogeneity.
Although there are features of both chromatin accessibility and transcription factor binding shared across the genotypes throughout the arthritis model, there are distinct distortions from the WT disease progression. These changes in chromatin accessibility are subtle as a relatively large proportion of genes aligned with open regions contribute to synovitis. These differences offer new and exciting opportunities to establish how disease heterogeneity establishes in response to AIA. The genomic characterisation of STAT1 and STAT3 transcription factor binding by ChIP-seq illustrated how IL-6 and IL-27 signalling deficiencies affect disease activity. Early bioinformatic analysis

using molecular pathway methods to interrogate ChIP-seq and ATAC-seq data has revealed that these alterations relate to genes linked to immune cells activation, alternations in stromal tissue responses and JAK-STAT signalling. These include changes affecting how STAT1 and STAT3 engage with the genomic or become employed under IL-6 or Il-27 signalling deficiency.
In this study, we applied genome-wide sequencing methods to understand the epigenetic mechanisms driving synovitis. This approach has begun to identify regulatory pathways and transcriptional mechanisms affecting synovitis and the development of disease heterogeneity in arthritis. In time, these methods may provide insights into the clinical response to therapies used in treating rheumatoid arthritis.

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P130

NEW BIOLOGICAL DRUG TO IMMUNOMODULATE THE CYTOKINE STORM ASSOCIATED TO INFECTIOUS DISEASES: IN VITRO AND IN VIVO SAFETY AND EFFICACY

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Abstract Content: Cytokine storm is a life-threatening condition caused by uncontrolled pro-inflammatory immune activation. It has been described as a secondary effect of CAR T cell therapy and is at the core of the lethal stage III of COVID-19 and other infectious processes. Individual treatments to resolve the cytokine storm have generally failed, especially in the case of COVID-19, calling for new and more integrative treatments. In this study, we have generated the new supernatant developed from a bicameral culture of mesenchymal stem cells with monocytes differentiated to an M2 phenotype, obtaining a conditioned medium with a clear anti-inflammatory and antifibrotic immunomodulatory profile.
Both in vitro and in vivo tests show that said secretome potently inhibits the release of proinflammatory cytokines from the THP-1 monocytic cell line, controlling and canceling the cytokine storm through a complex immunomodulation process that affects all pathways, especially by decreasing the expression of the NF-kb pathway, and, to a lesser extent, of the NLRP3 inflammasome, but efficiently, avoiding the damage that a cytokine storm is capable of generating in internal organs, mainly characterized by hyperinflammation and fibrosis, all of them without generating side effects, demonstrating a good biosafety and efficacy profile.

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P131

IL-17 PROTECTS AGAINST HYPERACTIVATION AND EXHAUSTION BY REGULATING IFN-GAMMA DURING CHRONIC VIRAL INFECTION

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Abstract Content: The balance between host-protective immune response and immunopathology often determines the fate of the virus-infected host. Recently we discovered that IL-17 levels are increased during the establishment of the chronic phase of LCMV CL13 infection. Unexpectedly, systemic IL-17 blockade resulted in enhanced weight loss during chronic LCMV CL13 infection. This was accompanied by increased activation of Th1 cells, elevated systemic IFN-γ and $CD8^+$ T cell exhaustion along with reduced viral load. These data indicate excessive activation of the antiviral T cell response in absence of IL-17, leading to improved viral control to the detriment of host health. An unbiased RNA-seq analysis from activated ($CD44^{high}$), partially exhausted ($PD\text{-}1^+Tim\text{-}3^-$) and terminally exhausted ($PD\text{-}1^+Tim\text{-}3^+$) $CD8^+$T cells from LCMV-CL13 infected mice showed increased IFN-γ signaling and altered metabolic gene signatures in $CD8^+$ T cells when IL-17 was absent. Since metabolic dysregulation is known to precede T cell exhaustion, we performed metabolic flux analysis on $CD8^+$T cells from LCMV-infected mice, and observed that

exhausted and activated CD8^{+}T cells showed enhanced fatty acid oxidation and reduced glycolytic capacity in IL-17-deficient mice, indicating functional alterations in metabolism. Systemic blockade of IFN-γ signaling in IL-17 deficient mice reversed the increased T cell exhaustion and immunopathology, without altering the enhanced T cell activation and viral control. This suggests an unappreciated interplay between IL-17 and IFN-γ in balancing infection control and immunopathology during establishment of chronic viral infection. While interrogating the targets of the protective IL-17 signaling during chronic LCMV infection, we observed that loss of IL-17 signaling specifically in CD8^{+} T cells (CD8$^{\Delta Il17ra}$) recapitulated some of the effects of the global IL-17 blockade, such as increased T cell activation, exhaustion and elevated systemic IFN-γ. However, viral load and weight loss were not significantly different, suggesting alternative critical targets of IL-17 *in vivo*. We previously showed that IL-17 signaling is required for the survival and proliferation of lymphoid organ stromal cells called fibroblastic reticular cells (FRC) during type-17 immunity in models of multiple sclerosis and colitis. FRC are known to regulate T cell activation, and are targets of LCMV infection. Indeed, FRC-specific ablation of IL-17 receptor (FRC$^{\Delta Il17ra}$) resulted in reduced numbers of FRC during chronic LCMV CL13 infection, indicating that IL-17 plays a role in supporting and repairing the damaged FRC network. Furthermore, FRC$^{\Delta Il17ra}$ mice fully phenocopied the outcome of global IL-17 neutralization, including weight loss, T cell activation and exhaustion, and viral load. In conclusion, we show that IL-17 signaling in CD8^{+}T cells and particularly in FRC is critical for maintaining the balance between immunoregulation and pathology during chronic viral infection. Since enhanced IFN-γ exacerbates exhaustion and immunopathology, our data further suggest that dual targeting of IL-17 and IFN-γ pathways may lead to more effective viral control without exacerbated immunopathology.

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P132

A NOVEL DUAL NLRP1 AND NLRP3 INFLAMMASOME INHIBITOR FOR THE TREATMENT OF INFLAMMATORY DISEASES

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Abstract Content: Inflammasomes induce maturation of the inflammatory cytokines IL-1β and IL-18, whose activity is associated in the pathophysiology of a wide range of infectious and inflammatory diseases including influenza A virus (IAV), SARS CoV2, pulmonary, neurodegenerative, metabolic and cardiovascular diseases, monogenic autoinflammatory syndromes and cancer. As validated therapeutic targets for the treatment of acute and chronic inflammatory diseases, there has been intense interest in developing small molecule inhibitors to target inflammasome activity.
We here describe ADS032 as the first dual NLRP1 and NLRP3 inhibitor that has no effect upon AIM2 or NLRC4 activity. ADS032 is a rapid, reversible and stable inflammasome inhibitor that directly binds to both NLRP1 and NLRP3, reducing secretion and maturation of IL-1β in mouse macrophages, human THP-1 and blood-derived macrophages stimulated with both NLRP1 and NLRP3 agonists. ADS032 suppressed IL-1β secretion from human primary bronchial epithelial cells in response to the NLPR1 agonist poly I:C, and reduced NLRP3-induced ASC-speck formation in ASC-cerulean reporter macrophages, indicative of targeting inflammasome formation and not NF-kB priming.
In vivo, ADS032 reduced IL-1β and TNF levels in the serum of mice systemically challenged with LPS and reduced pulmonary inflammation in an acute model of lung silicosis. Importantly, while we had previously found that ablating NLRP3 functionality with potent inhibitors such as MCC950 had both positive and detrimental disease outcomes during IAV infection; we have found that mice treated with ADS032 protected mice from lethal IAV challenge when administered at any time post-infection, displaying increased survival and reduced pulmonary inflammation.

ADS032 is therefore a potential therapeutic to treat NLRP1- and NLRP3-associated inflammatory diseases and a novel tool to allow examination of the role of NLRP1 in human disease.

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P133

MYELOID AUTOPHAGY GENES PROTECT MICE AGAINST FATAL TNF- AND LPS-INDUCED CYTOKINE STORM SYNDROMES

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Abstract Content: Autophagy regulates inflammation via multiple mechanisms, including lysosomal degradation of specific cellular components. Certain autophagy gene “cassettes” also participate in important immune processes via non-canonical activities. While some autophagy related genes in myeloid cells have previously been implicated in conferring a survival benefit for mice in models of cytokine storm syndromes (CSS), the genetic underpinnings for the activity of autophagy in protecting against CSS remain to be fully defined. We determined that multiple canonical autophagy genes (*Atg5*, *Atg7*, *Fip200*, and *Becn1*) in the myeloid compartment protected against fatal disease in models of CSS (intravenous TNF (ivTNF) and intraperitoneal LPS (ipLPS)), with a notable exception that *Atg14* protected against ivTNF but was dispensable during ipLPS. Importantly, genetic deletion of either IFNγ signaling or *Casp1*/*Casp11* revealed different stimulus-specific dependencies with respect to autophagy gene deficiency in each model. Multiple cytokines were markedly elevated in serum in $Atg5^{\Delta LysM}$ and $Becn1^{\Delta LysM}$ mice after induction of CSS and at baseline. Surprisingly, TNF was dispensable for the increased mortality of myeloid *Atg5*-deficient mice challenged with ipLPS. Further, tissue-specific ablation of *Atg5* in cells expressing CD11c and LysM, but not MRP8, defined a myeloid subset that protected against ivTNF, whereas protection against ipLPS was conferred by *Atg5* in a distinct subset of LysM-expressing cells. In vitro, *Atg5*, *Becn1*, and *Atg14* protected against TNF- and LPS-induced macrophage cell death. However, autophagy gene-deficient cells did not exhibit a cell-intrinsic hyperproduction of cytokines that correlated to in vivo phenotypes, suggesting an as-of-yet defined multicellular interaction occurs in vivo. Together, this study delineates autophagy gene sets and specific cell types required to protect against fatal inflammation due to CSS. Moreover, the results highlight fundamental differences in two commonly used murine models of the disorder.

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P134

THE CORRELATION OF ADM, ANGIOTENSIN II, HIF-1A, MIF, AND THE CYTOKINE RESPONSE (IL1B, IL-6, TNF-A) IN THE HYPOXIC CONDITIONS DURING INFLAMMATION

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Abstract Content: It seems that under hypoxic conditions in a viral inflammation (e.g. Covid-19), there is possible increase of Ang II, HIF-α, MIF, ADM, growth and proinflammatory factors. Our main objective is to study and better understand the relation between the above-mentioned factors and their influence (during inflammation) in the neuroendocrine system, and especially the possible interference of ADM with the HPA and HPG axes. Since endocrinological factors play a major role in inflammation response, we aim to further study the relation between hypoxia, Ang II, HIF1-α, MIF, and ADM, and their involvement on the immune-neuro-endocrine axis, in response to a

pathogen, such as viruses (e.g. SARS-CoV-2). Hypoxia, stress and proinflammatory factors, such as cytokines (interleukin -1a and TNF-α), can increase the expression of ADM. ADM is a peptide composed of 52 amino acids, encoded by the ADM gene that is located in chromosome 11. ADM belongs to the calcitonin family of peptides that includes calcitonin, amylin , ADM II , and αCGRP. These peptides present a similar structure, they signal through common receptors, and they are expressed in several organs and tissues, and although some of their functions may overlap, others differ, and some of them are unique. ADM, having immunoregulatory properties, influences the production of the proinflammatory factors (cytokines & chemokines) in cells, macrophages (the major source), fibroblasts, DC, etc. ADM and Adrenomedullin mRNA is expressed in many tissues and organs e.g. lung, skin, pancreatic islets, surrenal gland, VSMCs, ECs, fat cells, and is considered as a biomarker. According to research, MIF acts as a regulator of innate and adaptive immunity, and an inhibitory regulator in glucocorticoids (GC). ADM probably acts like a new counterregulator for the synthesis and secretion of GC and also can suppress aldosterone release. After the activation of macrophages, it is first observed an increase of IL-β and TNF-αpreceding an increase of ADM and IL-6, according to research. Probably, the contribution of IL-β and TNF-α provokes the expression of ADM gene in activated macrophages. ADM presents anti-inflammatory properties, but in certain cases it exhibits inflammatory properties. Since MIF and IL-1β are considered as significant mediators of inflammatory response, however in some cases, there is a possibility for ADM to operate as a proinflammatory factor in the initiation of this response through the production of MIF and IL-1β, especially in activated macrophages. It is possible that there is a feedback regulating mechanism between ADM and the production of inflammatory cytokines, and a possible self-regulation mechanism involving IL-1β and TNF-α. ADM is also found in the basal cells and alveolar epithelial cells type II, and in the endothelial cells of the lung vessels. We focus on this topic, because in a previous study, we highlighted that after the creation of the complex Sars-Cov 2 Spike with ACE2 (pneumocyte type II) and the destruction of these cells, an increase in Ang II is observed. We would like to study and understand the interactivity of ADM and the (RAAS) system, and the possible interaction of ADM with the ACTH-Cortisol-Glucose Axis, as well as other neuroendocrine axes.

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DETERMINING HNRNP-A2/B1'S ROLE IN INFLAMMATION AND RHEUMATOID ARTHRITIS PATHOLOGY

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Abstract Content: The innate immune system is the first line of defense against pathogens; it functions through various pattern recognition receptors (PRRs) that recognize microbial products or danger signals leading to the activation of signaling pathways to initiate transcription of inflammatory genes. Activation of the innate immune response is essential to resolve infections, however, its dysregulation can result in pathological inflammation, contributing to an array of diseases, such as atherosclerosis, autoimmunity and cancer.
HNRNP-A2/B1, is a biomarker for Rheumatoid Arthritis (RA) due to its abundance in patient's synovial tissue and high level of autoantibodies against it in patients' sera.
Despite its implication in RA, the degree of its involvement in disease pathological inflammation is not yet studied. The aim of our work is to determine if HNRNP-A2B1 plays a direct role in amplifying RA inflammatory circuit. We generated evidence that hnRNP-A2B1 deletion dramatically dysregulates immune gene expression in murine macrophages using a novel conditional KO mouse. Additionally, our studies show that mice in which hnRNP-A2/B1 is depleted in macrophages display an inflammatory response advantage in terms of temperature and cytokine changes under an inflammatory challenge. We also show that silencing this protein in fibroblast-like synoviocytes (FLS) obtained from RA patients dampens ISG expression and inflammatory genes highly associated with RA pathogenesis. We are currently working to determine the molecular mechanism by which hnRNP-A2B1 regulates inflammation within our murine model as well as human healthy macrophages and RA samples. These studies will help boost our understanding of the ways in which an RNA splicing protein is able to regulate gene expression and

splicing leading to inflammatory response modulation and inflammatory circuit amplification in the arthritic joint in vivo.

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CIRCULATING LEVELS OF CXCL-9, CXCL-10 AND IL-6 ARE AUGMENTED IN PATIENTS WITH LONG-TERM PULMONARY DYSFUNCTION 4 AND 12 MONTHS AFTER COVID-19.

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Abstract Content: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the etiological agent of Coronavirus disease 2019 (COVID-19). Patients with severe COVID-19 are characterized by an exacerbated release of cytokines and the development of severe acute respiratory syndrome (SARS), which is the main medical complication after infection. Over time, severe COVID-19 has been associated with sustained lung sequelae after the acute phase, however, the main inflammatory mediators associated with lung dysfunction post COVID-19 remain unknown. The aim of this study was to identify cytokines and chemokines associated with pulmonary dysfunction 4 and 12 months post-COVID-19 in patients with mild, moderate and severe disease. Sixty COVID-19 patients were recruited 4 and 12 months after the acute phase, and 6 cytokines and 4 chemokines were measured from serum samples. Respiratory sequelae of post-COVID-19 patients was analyzed by measuring the structural damage of the lung through computed tomography of the chest (Ct), and pulmonary functional sequelae was measured by spirometry and the diffusing capacity of the lungs for carbon monoxide (DLCO) exam. Our data showed that 14 patients exhibited structural and functional lung sequalae 4 months after COVID-19 infection. After identifying the patients with different degree of pulmonary sequelae, we observed that CXCL-9, CXCL10 and IL-6 were the main inflammatory mediators associated with pulmonary dysfunction at 4 and 12 months post infection. Overall, elevated levels of CXCL-9, CXCL-10, and IL-6 are associated with long-term lung dysfunction after COVID-19.

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TNF-ALPHA PREDICTS LONG COVID SYMPTOMS AND CIRCULATING AUTOANTIBODIES AT 12 MONTHS POST-RECOVERY

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Abstract Content: Background: Recent observational studies have found that up to 88% of convalescent COVID-19 patients report persistent symptoms for many months post-infection, leading to a diagnosis of Post-Acute Sequalae of COVID-19 (PASC). The non-resolution of the T1 hyper response cytokine storm developed during the acute phase of COVID-19 has been conjectured to underlie development of PASC symptoms. We investigated associations between levels of circulating inflammatory cytokines with the development of PASC symptoms and the prevalence of clinically relevant antinuclear autoantibodies which may contribute to PASC etiology.
Methods: Acute markers of inflammation (IL-1β, IL-6, IL-8, TNFα, CRP) and coagulation mediators (D-dimer, E-selectin, ICAM-1, VCAM-1) were assessed in sera of 106 convalescent COVID-19 patients with varying acute phase severities longitudinally at 3, 6, and 12 months post-recovery. Furthermore, for each timepoint, the symptoms fatigue,

cough, and dyspnea were recorded, and a rapid assessment line immunoassay was used to investigate circulating levels of antinuclear autoantibodies.
Results: Strong positive correlations were found between D-dimer and fatigue at 3 months (r=0.33, P=0.002**)**, TNFα and cough at 6 months (r=0.38, P=0.031), and TNFα and fatigue at 12 months (r=0.42, P=0.004). Multiple regression analysis for symptoms demonstrated D-dimer predicted fatigue (β=1.01, P=0.011) and dyspnea (β=0.55, P=0.024) at 3 months, ICAM-1 predicted cough at 3 months (β=1.14, P=0.028), and TNFα (β=4.65, P=0.004) predicted fatigue at 12 months. Regression analysis for general symptomaticity showed that D-dimer (β=1·08, P=0·013) and TNFα (β=2·40, P=0·03) positively predicted symptomaticity at 3 and 12 months respectively. A correlation analysis showed significant positive correlations between inflammatory mediators and various antinuclear autoantibodies. Receiving operator characteristics (ROC) curves were generated for individual and total symptoms using multiple logistic regression models for autoantibodies, in particular, anti-U1-snRNP and anti-SS-B/La. The presence of either of these two autoantibodies at 12 months post-recovery had an 86% certainty (AUC=0.856; 92% specificity, 70% sensitivity) of distinguishing convalescent COVID-19 patients with fatigue versus those without, 82% for dyspnea (AUC=0.815; 97% specificity, 58% sensitivity), and 82% for overall symptomaticity (AUC=0.820; 97% specificity, 58% sensitivity).
Conclusion: Rheumatologically relevant autoantibodies were elevated in convalescent COVID-19 post-recovery. The prevalent autoantibodies at 12 months strongly correlate with TNFα, and all predict common PASC symptoms one year post-infection. An incomplete attenuation of autoimmunity, residual inflammation, and persisting symptoms warrant long-term investigation of autoimmunity in long COVID patients.

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INFLAMMATORY MARKERS ASSOCIATED WITH IN-HOSPITAL MORTALITY IN CHILDREN WITH CEREBRAL MALARIA IN UGANDA.

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Abstract Content: Despite effective antimalarial therapy, cerebral malaria (CM) in children is associated with high mortality. The pathogenesis of CM is driven by both host and parasite factors and associated with multi-organ dysfunction. CM is also a leading cause of acquired neuro-disability in African children and excessive inflammatory responses are implicated in brain injury. However, the contributions of different inflammatory factors to mortality in cerebral malaria is not fully characterized. To better understand the pathogenesis of CM, we evaluated serum levels of 40 different markers of inflammation, angiogenesis and chemotaxis using a Luminex assay. We evaluated biomarkers in 86 Ugandan children, 6 months–4 years of age with cerebral malaria (CM) and 120 community children (CC) as controls. The results showed that 22 out of the 40 different markers were significantly different between children with CM and CC ($p<0.05$). Mortality in children with CM was 35%, to further evaluate whether these markers were associated with disease severity and mortality in the children with CM, we compared levels of these 22 markers in children who died (n=29) vs. those who survived (n=55). Overall, 8 of 22 markers were significantly elevated in children with CM who died compared to the children who survived ($p<0.05$). The median serum concentration (pg/ml) of these 8 markers between the children who died with CM versus those who survived were; inflammatory (IL1-ra: 29 vs 15, IL-6: 1441 vs 241, and PDL1: 326 vs 196), angiogenesis (G-CSF: 123 vs 48 and Flt3 ligand: 202 vs 127) and chemotaxis (CCL11: 182 vs 110, CX3CL1: 3.1 vs 2.0 and CCL20: 1275 vs 198). Following adjustment for age and sex, a $\log_{10}$ increase in levels of the 8 markers were associated with increases in the risk (OR) [95% confidence interval]) of in-hospital mortality; IL-1ra: 7.36 [1.42, 38.16], IL-6: 2.36 [1.24, 4.50], PDL1: 28.70 [3.23, 254.79], G-CSF: 2.23 [1.16, 4.32], Flt3 ligand: 31.52 [3.18, 312.22], CCL11: 130.28 [8.80, 1927.85], CX3CL: 21.92 [2.84, 168.94] and CCL20: 6.89 [2.54, 18.70] after adjustment for multiple comparisons. These results suggest that IL-6, G-CSF, Flt3 ligand, CCL11, CX3CL1 and CCL20 are key mediators of the pro-inflammatory response in the children who die of CM while PDL1 and IL-1ra are involved in inhibitory and anti-inflammatory responses that regulate the immune response. Further analysis will investigate how these markers relate to the inflammatory response in children with CM

and other manifestations of severe malaria. Additional studies are needed to elucidate the immune effector cells associated with the inflammatory response in cerebral malaria.

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CHARACTERIZING COVID-19, CASTLEMAN'S DISEASE AND RHEUMATOID ARTHRITIS BASED ON PATIENTS' SERUM CYTOKINE AND CHEMOKINE PATTERNS BEFORE AND AFTER TOCIRIZMAB TREATMENT USING PARTIAL LEAST SQUARES REGRESSION 2 ANALYSIS

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Abstract Content: Antibody reagents that block IL-6 function are effective in treating COVID-19, idiopathic multi-centric Castleman's disease (iMCD) and rheumatoid arthritis (RA) even though these diseases are thought to be very different pathogenesis. We used Bio-plex Human Cytokine 27-plex panel and Inflammation panel to quantify chemokines/cytokines(59 items in total) in serum of COVID-19(n=13), iMCD(n=19) and RA(n=28) patients before and after an anti-IL-6 receptor antibody, tocilizumab (TCZ) treatment.
COVID-19, iMCD and RA and patients shared some of the same elevated cytokines/chemokines, however they had different patterns for other cytokines/chemokines. PLS2 analysis revealed commonalities and differences in COVID-19, iMCD and RA patients. The widespread overexpression of cytokines/ chemokines is a characteristic of hyperinflammation in patients with severe COVID-19. Our comprehensive analysis demonstrated increased levels of a broad range of biomediators (52 out of 59) in severely to critically ill patients with COVID-19 and the expression profile of cytokines/chemokines in COVID-19 was also much broader than that in RA or iMCD, for which IL-6 blockade therapy has been already approved suggesting activation of a wide spectrum of inflammatory cells. On the other hand, iMCD patients did not reduce many cytokines contained in the 27 plex panel, while the inflammatory cytokines/chemokines contained in the Inflammation panel decreased to some extent, and clinical symptoms improved dramatically. In RA patients, cytokines in the 27 plex and Inflammation panel decreased after 24 weeks. In COVID-19 patients, IL-6 induced cytokine-storm and suppressed the action of IL-6 by the TCZ. TCZ produce better outcomes in severely and critically ill patients with COVID-19 that TCZ monotherapy is beneficial for substantial numbers of patients with severe COVID-19. iMCD patients with systemic lymphadenopathy showed improvement in symptoms and abnormal laboratory values after TCZ therapy. IL-6 production was not inhibited by TCZ, but its functions were blocked. As a result, iMCD patient's inflammatory symptoms decreased. In RA patients, IL-6 induced synovitis was produced by joint inflammation and suppressed the action of IL-6 by the TCZ. As a result, joint inflammation decreased and IL-6 production was also suppressed in both synovial fluid and serum. Finally, IL-6 gradually decreased thereby suppressing inflammation and pannus formation, synovitis and joint destruction by the osteoclastogenic-angiogenesis-suppressing action in rheumatoid synovial tissue. In this way, the mechanism of action of TCZ is very different, but TCZ is effective in each disease. Quantifying cytokines and chemokines and doing PLS2 analysis is useful for classifying and narrowing down the cause of inflammatory diseases.

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P140

QUALITY, LOCATION, & TIMING OF INTERFERON PRODUCTION DICTATES THE IMMUNE RESPONSE.

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Abstract Content: The immune system evolved to protect us against pathogen invasion and to maintain tissue homeostasis. Fundamental for these functions are the interferons (IFNs), a group of cytokines that belongs to three major families: IFN-I, IFN-II, and IFN-III. The amount of interferons produced during an immune response is well known to determine the balance between anti-microbial functions and interferon-mediated diseases, known as interferonopathies. Here, we will discuss how, beside the amount, the type of IFN produced, as well as the location and timing of their production, affect the immune response in the respiratory tract during a viral infection. Also, we will show how the production of IFNs in a specific anatomical location can be exploited to design new adjuvants that increase the efficacy of vaccines against life-threatening viral infections, such as SARS-CoV-2, or Influenza A Virus.

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LB-13

TRACKING INTERLEUKIN-17 PRODUCING CELLS IN THE COLORECTAL TUMOURS OF LIVE MICE

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Abstract Content:
Introduction: Bowel cancer, also known as colorectal cancer (CRC), develops within the lining of the large intestine. It is a highly prevalent cancer in Western societies, with Australia and New Zealand amongst the highest incidence rates world-wide. Chronic inflammation is a well-recognised driver of CRC formation and progression. Interleukin (IL)-17 is a pro-inflammatory cytokine that is highly expressed in the serum and tissue of CRC patients. Whether its function is protective, through orchestration of pathogen clearance, or pathogenic through activation of oncogenic signalling pathways remains debated. Numerous cell types can produce IL-17 within the tumour microenvironment. We sought to track the localisation of IL-17 producing cells in real-time.
Methods: IL-17 reporter mice underwent a model of colitis-associated cancer. We tracked the recruitment and localisation of IL-17 producing cell populations during the onset and progression of colorectal cancer using confocal laser endoscopy and narrow-band imaging-aided endoscopy for *in vivo* imaging of colitis and colon cancer in live mice.
Results: We established methodologies to tracker reporters through confocal laser endoscopy and narrow-band imaging-aided endoscopy of live mice. Our results reveal that IL-17 producing cell populations are disperse within the colonic mucosa; however, they form aggregates within tumours.
Conclusions: The loss of mucin producing cells within colorectal tumours enables the colonisation of pathogens that are known to promote CRC. The localisation and aggregation of IL-17 producing cells within these tumours suggests that they may be recruited to combat pathogens, and inadvertently support tumour growth. Our results highlight potential opportunities to monitor cellular behaviour in live mice during the progression of a cancer, including their response to therapeutics.

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LB-14

PLASMA CYTOKINE LEVELS AS PROGNOSTIC BIOMARKERS AND REPARATIVE MICROSURGERY OUTCOME MEASURES FOR BREAST CANCER-RELATED LYMPHEDEMA

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Abstract Content: Introduction: Breast cancer-related lymphedema (BCRL) is characterized by arm, trunk, or breast swelling, pain, cellulitis susceptibility, and depression. Up to 40% of breast cancer patients who undergo breast cancer surgery and radiation are at risk of developing BCRL, with no known cure. Several studies have shown that early BCRL detection and treatment significantly improves patient quality of life. There is a need for tools to identify and provide early treatment for those breast cancer survivors at highest risk for BCRL, and avoid treating others needlessly. There is also a need to determine objective measures of benefit of BCRL reparative microsurgeries lymphovenous bypass (LVB) and vascularized lymph node transplant (VLNT) that aim to improve outcomes for those BCRL patients for whom first-line, compression therapy fail.
Objective(s)/Purpose: To determine if plasma cytokine/chemokine levels can 1) identify breast cancer patients at highest risk of developing BCRL to enable earlier diagnosis and treatment, and 2) deliver a readout of success or failure of reparative BCRL microsurgeries.
Methods: Plasma samples from two clinical studies were used. In the first study**,** a total of 67 breast cancer patients, all scheduled for mastectomy or lumpectomy with axillary lymph node dissection (ALND) and regional nodal radiation treatment (RT), were longitudinally assessed for clinical BCRL (>5% arm swelling, measured by perometer), with a total of 314 blood specimens collected at four time points (preoperatively, postoperatively, and at 6 and 12 months after RT). Fourteen plasma cytokines/chemokines were measured at each time point, using a MILLIPLEX MAP human cytokine/chemokine magnetic bead panel. Cytokine levels in patients with >5% perometric arm swelling at 12 months post- RT were compared to those with <5% perometric arm swelling. GraphPad/Prism 9 non-parametric Mann-Whitney t-test analysis was used to determine the significance of each cytokine. In the second study, plasma cytokines/chemokines in 10 established BCRL patients, before and 6 months after LVB/VLNT, were measured and analyzed as described for the first study.
Results: IL-15 and MIP-1β were identified to be significantly higher, and G-CSF lower, at baseline in patients who proceeded to >5% perometric arm swelling (clinical BCRL) at 12 months post- RT, compared to those with <5% perometric arm swelling at 12 months post- RT. No plasma cytokine/chemokine levels were found to be significantly different between pre- and post-LVB/VLNT.
Conclusion: IL-15, MIP-1b, and G-CSF could be identifying biomarkers for patients at high risk for developing BCRL, and enable early treatment and improved patient outcomes. Plasma cytokines/chemokines did not differ significantly pre- and post-LVB/VLNT. Thus, surveillance of plasma cytokines/chemokines may be a tool for identifying breast cancer patients at highest risk for developing BCRL, but may not provide useful information about reparative BCRL microsurgeries.

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01. ILC Development and Trajectories

P141

LUNG-RESIDENT ILC3 ARE ESTABLISHED BY T LINEAGE-DERIVED CELLS

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Abstract Content: Although it is becoming increasingly clear that group 3 innate lymphoid cells (ILC3s) play an important function in providing a rapid immune response upon initiation of pulmonary infections, the signals that guide pulmonary ILC3 development remain unclear. To date, it is believed that ILCs develop from progenitors in the bone marrow, but a new evidence of non-productive TCR rearrangements in lung ILC2s strongly suggest that ILCs could also arise neonatally from developing T cell progenitors. Noteworthy, we found in parabiosis experiments that unlike ILC3 counterparts in the gut or other lung ILCs, pulmonary ILC3s are highly heterogenous and include both tissue-resident but also circulatory cells. In line with these observations, the administration of recombinant IL-1b only induced expansion of tissue-resident ILC3s, which suggests that the pool of lung ILC3s is a combination of cells with different developmental origins. We then set out to investigate phenotypical differences in pulmonary ILC3s in Rag1-deficient mice and *Foxn1^{nu}* (nude) mice. Our data reveal the existence of a subset of T lineage-derived lung ILC3s that develop

in the presence of non-functional T cell development. Finally, we confirmed that T-lineage derived ILC3s appear in the neonatal lungs and thus become tissue-resident. Overall, our study reveals a novel player in lung immunity with potential implications to combat pathogenic infections that are associated to pulmonary diseases, as in humans, ILC3s are a major ILC population in the lung.

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P142

MUCOSAL ORGANOIDS PROMOTE THE DEVELOPMENT OF TISSUE-SPECIFIC INNATE LYMPHOID CELLS

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Abstract Content: ILC-restricted precursor (ILCP) populations arise from multipotent common lymphoid precursors (CLP) in the bone marrow, however the final stages of adult ILC-subset maturation likely occur upon exiting this niche. Deciphering what local stimuli drive the differentiation and function of ILC in these tissues remains a pressing question, as ILC frequencies can become dysregulated in disease.
Here, we introduce murine and human co-culture systems of ILCP with mucosal organoids that faithfully capture the maturation of ILC within distal mucosal tissues. This approach promotes significant expansion and functional maturation of all ILC subsets in parallel. Notably, murine germ-free gut and lung organoids cells are sufficient to induce tissue-specific patterns of ILC subset frequencies and phenotypes, even in the absence of organ-specific microbial tropism or additional cells. For example, like their *ex vivo* counterparts, ILC2 are KLRG1^{high} when developed on intestinal organoids and a ST2^{high} when generated on lung organoids. This lung phenotype, is dependent on IL-33, demonstrating the importance of tissue derived factors on the modulation of ILC2 phenotype and function.
Taken together, our work provides unprecedented insight into in situ ILC maturation and function. Moreover, our work introduces a modular organoid platform, which provides exquisite control over both environmental stimuli and host genetics, making it a powerful tool for dissecting immune-epithelial interactions in health and disease.

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P143

THYMUS-DEPENDENT CIRCULATING ILC PRECURSORS

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Abstract Content: ILCs are widely known to arise from bone marrow progenitors or tissue resident progenitors distributed during embryogenesis. Work from our laboratory and others strongly suggest that ILCs at least ILC2s can also be generated in the thymus, not only from multipotent progenitors but also from committed T cell precursors in the thymus. T cell receptor gene rearrangement events have been found in lung ILC2s for wild type (WT) but not nude mice. Our recent data using single cell RNA sequencing (scRNAseq) revealed a substantial fraction of the ILC-enriched population (Lin$^-$Thy1$^+$) in the blood of WT but not nude mice, suggesting their thymic origin and thus dubbed thymus-dependent ILCs (td-ILCs). These cells express general markers of ILCs such as IL7R and TCF1 but not transcription factors specifying each ILC subsets like T-bet, GATA3 or RORγt, which may be interpreted to mean that they are ILC precursors. Indeed, in vitro differentiation assays showed that these cells generated different subsets of ILCs. Interestingly, td-ILCs also expressed transcripts of *Cd3d*, *Cd3e* and *Cd3g*. Flow cytometric analyses detected CD3ε by intracellular staining but not by surface staining, which is consistent with the notion that these cells may arise from committed T cell precursors in the thymus. Using intracellular CD3ε as a marker, we showed that icCD3ε$^+$ cells are present in the lung and small intestine of WT but not nude mice. However, only small fractions of mature ILC2s

were icCD3ε$^+$ while the majority of icCD3ε$^+$ cells are ST2$^-$ and KLRG1$^-$ in the lung and small intestine, respectively. These icCD3ε$^+$ cells may be immature ILC2s and/or other ILCs. In support of this notion, we found an inverse correlation between the levels of GATA3 and those of icCD3ε, suggesting that down-regulation of CD3 occurs during ILC2 differentiation. Consistently, our scRNAseq analyses of small intestine ILCs also revealed a population of thymus-dependent ILC2 with little CD3 expression. Taken together, by profiling ILCs in WT and nude mice, we have uncovered significant fractions of ILCs that are likely derived from the thymus. Understanding the contribution of these thymus-derived ILCs will enrich our knowledge about ILC ontogeny and age-related differences in immunity.

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LB-41
INNATE LYMPHOID CELLS LACKING SURFACE EXPRESSION OF CD90 ARE FUNCTIONAL
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Abstract Content: Huge progress has been made in understanding the biology of innate lymphoid cells (ILC)s by adopting several well-known concepts of T cell biology. As such flow cytometry gating strategies and markers, such as CD90, to identify ILC were discovered. Here we report that most non-NK intestinal ILC have a high expression of CD90 as expected, but surprisingly some have only a low or even no expression of this marker. CD90-negative CD127+ ILC were identified among all ILC subsets in the gut. CD90-negative cLP ILC2 were frequent at steady state. The frequency of CD90-negative CD127+ ILC was dependent on stimulatory cues in vitro and in vivo, and CD90-negative CD127+ ILC played a functional role as a source of IL-13, IFNγ and IL-17A at steady state and upon dextran sulphate sodium-elicited colitis. Hence, this study highlights for the first time that CD90 is not constitutively expressed by functional ILC in the gut.

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02. ILC Tissue Niches
P144
SPATIAL PROFILING OF MURINE ILCS DURING A SYSTEMIC INFLAMMATION USING MULTIPLEXED HISTOLOGY
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Abstract Content: Cells are embedded in a complex network of neighboring cells and matrix components. They are able to react upon internal and external stimuli by regulations on the transcriptional level triggering changes in their cytokine profile, phenotype, and migratory behavior, hence, communicating in this micro-community. Tissue-resident innate lymphoid cells (ILCs) are innate immune cells and potent tissue sensors for cytokines and cellular factors. However, little is known about their localization and spatial behavior in the tissue.
We are interested in the correlation of functional and phenotypical adaptation of ILCs and their microanatomical location during inflammation. By using multi epitope ligand cartography (MELC), a fluorescence-based microscopy technique, we perform multi-parameter analysis with spatial information. Established antibody panels of 50+ markers

– also including ILC's signature transcription factors – in different tissues allows a deep characterization of hematopoietic and stromal populations in tissue context. Our image analysis pipeline enables cell segmentation and extraction of single cell features. Mean fluorescence intensity can be measured on subcellular levels per cell for each marker. With this, we are able to identify ILCs and other rare as well as abundant cells in multiple human and murine organs.
We focus on the detection of ILCs in a mouse model for systemic inflammation aiming to characterize ILC phenotypes and their neighborhood in different immunological-relevant organs. Preliminary results showed RORgt^{high}GATA3^{low} intestinal ILC3s and GATA3^{high} lung ILC2s both located in fibronectin-enriched tissue areas – a first hint for a conserved ILC niche. Furthermore and surprisingly, these ILCs also expressed podoplanin, a marker for lymphatics but not blood endothelial cells. To our knowledge, podoplanin has not been described to be associated with ILC. If this can be supported in our upcoming analysis, this might be a nice example for the great potential of our technology to reveal new markers for immune profiling.

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P145
HETEROGENEITY OF SKIN ILC

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Abstract Content: Skin is one of the largest barrier organs in our bodies and protect the internal environments from external stresses. The skin has heterogeneous populations of ILCs, which provide a variety of immunological functions in cutaneous barrier systems. We have previously found that 3 different anatomical layers of skin: epidermis, dermis and subcutaneous tissues, harbor ILC subsets with unique characteristics. Whereas ST2$^+$ ILC2s were found in subcutaneous tissues, the majority of ILC2s in epidermis and dermis did not express ST2 but expressed ICOS and CCR6. ILCs in epidermis and dermis also showed distinct identities, and epidermal ILCs had both ILC2 and ILC3 transcriptome characters. Further, we have expanded comprehensive survey of skin ILC populations and examined single cell transcriptome profiles of Lin$^-$ Thy1.2$^+$ in skin ILCs of WT and *Rag2*$^{-/-}$ mice in both steady state and MC903-induced atopic inflammation. Comparison between naïve and MC903-treated skin showed significant transcriptome shifts. Differentially expressed genes in clusters that were found in MC903-skin ILCs were associated with the regulation of interferon signaling and leukocyte activation. Furthermore, we found marked alterations in constitutions of ILC subtypes between WT and *Rag2*$^{-/-}$ mice. Distinct clusters which exhibited ILC1 and ILC3 characters emerged in *Rag2*$^{-/-}$ mice. ILC1-like populations which are not previously extensively investigated were also detected in flow cytometry analysis in both WT and *Rag2*$^{-/-}$ mouse skin. The ILC1-like cells were dramatically expanded in *Rag2*$^{-/-}$ mice, particularly in epidermis. Importantly, the marked increase of ILC1-like cells was canceled in *Il15*$^{-/-}$*Rag2*$^{-/-}$ mice, suggesting that IL-15 is an important cytokine that promotes the expansion of skin ILC1s.

Disclosure of Interest: None Declared
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P146
INNATE IMMUNE-MEDIATED CONTROL OF SKIN EPITHELIAL CELL RENEWAL AND STAPHYLOCOCCAL COMMENSALISM

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Abstract Content: The epithelial barrier of the skin is one of the largest border surfaces with the environment and it is exposed to microbes, light, irradiation and physical or mechanical stress. Innate Lymphoid Cells (ILCs) are a recently described subset of leukocytes and its subtypes (ILC1, ILC2 and ILC3) have been linked to important homeostatic and pathological mechanisms in the gut. Recent studies indicate that dermal ILCs are fundamentally

different than the ILCs residing in other tissues. Instead of fitting in the previously described subtypes, they are in a transient-like state that can assume different identities and functions depending on the signals they receive. The mechanisms underlying the lineage and plasticity of dermal ILCs remain to be fully understood.
The pathobiont *Staphylococcus aureus* resides harmlessly on epithelial surfaces but can cause infections under certain conditions. Preliminary data from our group demonstrate that IL-17A and IL-17F produced by ILCs are required to keep *S. aureus* in a commensal state. We hypothesise that staphylococci and, in particular *S. aureus*, may provide essential signals to control the activity of phagocytic cells and ILCs, thereby regulating epithelial and mesenchymal stromal cell function.
We will investigate *S. aureus* virulence factors and their regulatory machinery as a pre-requisite to enhance function of dermal ILCs, the role of ILCs and its derived cytokines on skin epithelial stem cells in the context of colonization/infection with staphylococci and the impact of immune system components on gene regulation of skin commensals and pathobionts.
This project aims to elucidate how skin barrier defense is orchestrated, describing cellular and molecular components of the system. This will open new avenues in our understanding of the crosstalk between the immune system and the transcriptional output of commensal bacterial communities that may in turn shape human health.

Disclosure of Interest: None Declared
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LB-42
GROUP 2 INNATE LYMPHOID CELLS AT THE FETAL MATERNAL INTERFACE
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Abstract Content: Pregnancy is accompanied by unique changes to the maternal immune system to provide protection to both the mother and the fetus. A successful pregnancy relies on the highly coordinated integration of immunomodulatory signals from the fetus as well as the mother at the fetal-maternal interface. Group 2 innate lymphoid cells (ILC2) are key players in type 2 immune responses at mucosal barrier surfaces. They are essential in the defense against helminth infections but also drive atopic disease such as allergic asthma and atopic dermatitis. Importantly, ILC2 have been described in fetal maternal tissue. However, deciphering the physiological relevance and function of ILC2 at the fetal maternal interface is challenging due to lack of established protocols. Using novel isolation methods in combination with multicolor flow cytometry, we functionally characterized mouse decidual and placental ILC2. We observed that the unique microenvironment at the intersection of the mother and fetus strongly defines the phenotype of the resident ILC2 population. We here report an optimized isolation and immunophenotyping protocol for fetal-maternal tissue ILC2 to functionally characterize ILC2 at this key physiological site during pregnancy.

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03. ILC1s and NK cells
P147
THE CD3-ZETA ADAPTOR STRUCTURE DETERMINES FUNCTIONAL DIFFERENCES BETWEEN HUMAN AND MOUSE CD16 FC-GAMMA RECEPTOR SIGNALING IN NATURAL KILLER CELLS
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Abstract Content: The CD16 receptor detects the Fc portion of IgG on antibody-coated cells resulting in NK cell activation. The importance of this receptor on human NK cell biology has long been appreciated; however, how CD16 functions in mouse NK cells remains poorly understood. Therefore, we investigated CD16 function in mouse NK cells. Here, we report drastic differences between human and mouse NK cells whereby human NK cells are potently

activated by engagement of the CD16 receptor whereas the mouse NK cells are poorly activated. We demonstrate that one of the adaptor molecules that CD16 associates with and signals through, CD3ζ, plays a critical role in these functional differences. Using a systematic approach, we demonstrate that key residues in the transmembrane domain of the mouse CD3ζ molecule prevent efficient complex formation with mouse CD16, thereby dampening receptor function. Mutating these residues in mouse CD3ζ to those encoded by human CD3ζ resulted in rescue of CD16 receptor function. In conclusion, we demonstrate critical functional differences in CD16 activation between mouse and human NK cells.

Disclosure of Interest: None Declared
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P148

IFNα14 IS A POTENT ENHANCER OF NATURAL KILLER CELL ACTIVITY AGAINST LEUKAEMIA

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Abstract Content: Over the past 20 years natural killer (NK) cell-mediated immunotherapies have emerged as a safe and effective treatment approach for advanced leukaemia. The importance of an appropriate pre-activation strategy has been highlighted as a major determinant of therapeutic success for adoptive NK cell therapy, with common approaches leveraging proinflammatory cytokines to boost NK cell activity *in vivo*. The type I interferon (IFN) family are well-known for their ability to activate NK cells against malignant cells; though IFNα2 is currently the only subtype approved for the treatment of cancer. Indeed, the capacity for the remaining IFNαsubtypes and IFNβ to enhance NK cell anti-cancer activity remains unexplored. We hypothesised that individual IFN subtypes harbour differing capacities to activate NK cell effector function against leukaemia. As such, we investigated the potential for each of the 12 human IFNα subtypes and IFNβ to enhance the anti-leukaemic activity of healthy donor-derived NK cells. A systematic screen was carried out to identify which IFN subtype(s) harboured the greatest potential to stimulate NK cell degranulation and cytokine production against the leukaemic cell line K562. Interestingly, several IFN subtypes significantly enhanced NK cell activity over IFNα2. Specifically, IFNα14 and IFNβ were identified as superior activators of NK cell effector function *in vitro*. To test the ability of these subtypes to enhance NK cell activity *in vivo*, IFN stimulation was overlaid onto a standard *ex vivo* expansion protocol to generate NK cells for adoptive cell therapy. Strikingly, infusion of NK cells pre-activated with IFNα14, but not IFNβ, significantly prolonged survival in a preclinical model of leukaemia compared to NK cells expanded without IFN. Collectively, these results highlight the diverse immunomodulatory potencies of individual IFN subtypes and support further investigation into the use of IFNα14 in future NK cell therapies.

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P150

CD8A EXPRESSION ON MEMORY-LIKE AND CONVENTIONAL NATURAL KILLER CELLS MARKS A POPULATION WITH IMPAIRED IL-15 INDUCED PROLIFERATION

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Abstract Content: Natural Killer (NK) cells are cytotoxic innate lymphoid cells phenotypically characterized in humans as CD3- CD56+ lymphocytes that play an important role in the surveillance and elimination of virally-infected and malignantly-transformed cells. NK cells exhibit innate memory following IL-12/15/18 stimulation, thereby differentiating into memory-like (ML) NK cells. A phase 1 study using ML NK cells in relapsed/refractory AML patients showed approximately 50% of patients achieving complete remission. Multidimensional immune correlative analysis of donor

NK cells identified a negative association between CD8a expression on donor NK cells and treatment response. However, the role of CD8a on human conventional and ML NK cells remains poorly studied.
In this study, donor NK cells in AML patients with high CD8a expression had diminished proliferation, so we hypothesized that CD8a+ NK cell proliferation is inferior to CD8a- NK cells. Consistent with this, sorted peripheral blood CD8a+ NK cells cultured in IL-15 exhibited diminished proliferation and survival *in vitro* and *in vivo*. One explanation for this proliferative difference is that CD8a marks a terminally differentiated population. However, CD8a is expressed similarly on "immature" $CD56^{bright}$ and "mature" $CD56^{dim}$ subsets, and CD8 did not correlate with CD57, a canonical NK cell maturation marker. Since IL-15 is required for NK cell survival and homeostasis, we hypothesized that CD8a+ NK cells respond less robustly to IL-15 signals. CD8a+ NK cells had lower induction of pERK1/2, pAKT, and pS6 following stimulation with IL-15. Preliminary data also demonstrates that CD8a+ NK cells have impaired metabolic activity when cultured in IL-15. Work is ongoing to further understand the mechanisms for this proliferative defect.
Given that the extracellular domain of CD8a binds human leukocyte antigen (HLA, a ligand for inhibitory KIR receptors), and the cytoplasmic tail associates with the kinase Lck, we also sought to determine if CD8a played an inhibitory role in NK cell activation using a CRISPR-Cas9 electroporation system. In the absence of CD8a, cytotoxic responses (CD107a, IFNg, TNF) following activating receptor ligation were enhanced for several receptors, suggesting an intrinsic role for CD8a in inhibiting NK cell responses.
Together, these results identify a novel association of CD8a with treatment outcome for NK cellular therapy and expand our understanding of basic NK cell biology

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P151
REGULATION OF TBX21 EXPRESSION IN NK AND ILC CELLS

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Abstract Content: Group 1 of innate lymphoid cells (ILCs) include natural killer (NK) cells along with type 1 innate lymphoid cells (ILC1s). Developmental inhibition of NK cells, absence of mature NK cells, and/or impaired NK cytotoxicity, along with impaired function of ILC1s have been associated with acute myeloid leukemia (AML). It has been hypothesized that NK cells have a direct cytotoxic effect on AML cells and that, along with ILCs, have an important role tumor surveillance. Previously, it has been reported that ILC1 and NK cell differentiation is positively regulated by the IKAROS protein. IKAROS is encoded by the IKZF1 gene and functions as a transcription factor and tumor suppressor. Ikzf1 knock-out mice have absent NK cells. IKAROS is a DNA-binding protein that regulates transcription of its target genes. Regulation of gene expression in NK cells and ILC1s by IKAROS is still largely unknown. Here, we report that IKAROS regulates expression of TBX21 (T-BET) gene in NK cells and ILCs. Analysis of global chromatin immunoprecipitation coupled with next-generation sequencing (ChIP-seq) in primary human NK cells showed a strong enrichment of IKAROS at the promoter of the TBX21 gene. IKAROS binding to the TBX21 promoter was confirmed by quantitative chromatin immunoprecipitation (qChIP) in primary cells. The role of IKAROS in regulating TBX21 transcription was tested using gain-of-function and loss-of-function experiments. Luciferase reporter assay showed that Ikaros directly represses transcription from TBX21 promoter. Overexpression of IKAROS via retroviral transduction was associated with increased transcription and overall expression of TBX21. IKAROS knock-down with shRNA results in reduced expression of TBX21. These results suggest that IKAROS directly promotes TBX21 transcription. Since IKAROS function in hematopoietic cells is regulated via direct phosphorylation by Casein Kinase II (CK2), we tested whether CK2 can regulate expression of TBX21. Overexpression of CK2 via retroviral transduction resulted in reduced expression of the TBX21 gene. Increased expression of CK2 was associated with a loss of IKAROS binding to the TBX21 gene promoter. Molecular inhibition of CK2 using shRNA

resulted in increased expression of TBX21. Inhibition of CK2 was associated with increased IKAROS binding at the TBX21 promoter. IKAROS knock-down abolished downregulation of TBX21 expression in B-ALL cells following treatment with CK2 inhibitors. These data demonstrate that CK2 and IKAROS are critical regulators of TBX21 expression. In conclusion, these results indicate that expression of the TBX21 gene is regulated by the CK2-IKAROS signaling axis and provide a novel insight into mechanisms that regulate differentiation of NK cells and ILC1s.

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P152

IL-2 AND TGF-B1 SYNERGIZE TO INDUCE REGULATORY ACTIVITY IN NATURAL KILLER CELLS

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Abstract Content: Harnessing Natural Killer (NK) cells in adoptive cell therapies or promoting NK cell activity are promising approaches in cancer immunotherapy. NK cell function, however, is influenced by cytokines and other microenvironmental factors, which can dampen NK cell anti-tumor responses. We previously identified a regulatory NK-like innate lymphoid cell (ILC) population within tumor infiltrating lymphocytes (TILs) of high grade serous ovarian cancer patients, which was correlated with poorer clinical outcomes. Subsequently, several groups have observed regulatory NK-like cells in the context of breast cancer, sarcoma and non-small cell lung cancer. The regulatory NK-like population in TILs displayed unique and overlapping properties with several ILC family members. In line with NK cell identity, regulatory NK-like cells expressed KIRs, NKp46, NKG2D/CD94 and CD7, but exhibited a unique transcriptional profile. They had minimal expression of IFN-γ, and TNF-α and secreted CCL3, IL-9, and IL-22, associated with ILC1s, ILC2s, and ILC3s, respectively. Whether conventional human NK cells can acquire phenotypes and functions observed in the tumor-associated regulatory NK-like ILC population remains unknown. Here we explored the role of cytokines for promoting regulatory activity in human NK cells. We identified IL-2 and TGF-β1 as being key cytokines that together induced both $CD56^{dim}$ and $CD56^{bright}$ NK cells to gain a regulatory phenotype, with the phenotype being more pronounced in the $CD56^{bright}$ NK cell population. This included a surface marker phenotype consistent with regulatory NK-like cells observed in TILs, low IFN-γ and TNF-α expression and the loss of NK cell cytotoxic functions. While conventional NK cell functions were lost, IL-2 and TGF-β1-treated NK cells acquired the ability to secrete active TGF-β1 themselves, and directly suppressed $CD4^+$ T cells. Of note, culturing naïve $CD4^+$ T cells with IL-2 and TGF-β1-induced 'regulatory' NK cells, resulted in increased proportions of immunosuppressive $CD127^-CD25^+FOXP3^+$ regulatory T cells (Tregs), supporting the potential of regulatory NK cells to promote Tregs. RNA-sequencing revealed these induced regulatory NK-like cells had low expression of cytotoxic genes, maintained expression of canonical NK cell receptor genes, and did not upregulate genes consistent with other ILC family member identities. Instead, increased expression of *IKZF2* (HELIOS), *ZNF683* (HOBIT) and *ITGAE* (CD103) expression was observed. While IL-2 and TGF-β1 polarize NK cells to acquire immunoregulatory functions, this phenotype was not stable, as culturing in different cytokine conditions resulted in the loss of the ability to secrete TGF-β1 and upregulation of IFN-γ and TNF-α. These studies support that IL-2 and TGF-β1 can synergize to induce immunosuppressive function in NK cells, but that this activity can be reversed by altering cytokine milieus.

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P153

A CIS-REGULATORY ELEMENT LIMITS TCF7 EXPRESSION IN LYMPHOCYTES

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Abstract Content: In order to provide protection against a broad spectrum of threats, the immune system must give rise to a diverse range of cell types each with specialized roles in host defense. Transcriptional regulation is key to orchestrating gene expression to establish and maintain distinct cell identities and functional specializations. Transcriptional regulation is achieved by interactions between transcription factors and non-coding cis-regulatory DNA elements such as promoters and enhancers. One of the most important transcription factors in lymphocyte development and function is TCF1 (encoded by the *Tcf7* gene). TCF1 is required for the efficient generation of both innate lymphocytes, such as NK cells, as well as adaptive T cells. TCF1 also regulates the functional abilities of these cell types, such as cytokine production and cytotoxicity. However, the mechanisms controlling TCF1 expression in lymphocytes have remained unclear. By analyzing assay for transposase-accessible chromatin with sequencing (ATAC-seq) data for NK cells undergoing activation we have identified an evolutionarily conserved non-coding sequence within the intronic region of the *Tcf7* gene which, although normally inaccessible, becomes accessible during activation. Chromatin accessibility at this region appears to be inversely correlated with *Tcf7* expression, suggesting it could be acting as a regulatory element. Indeed, deleting this sequence results in an impaired ability for lymphocytes to downregulate *Tcf7* in response to stimulation. To explore what transcription factors could act upon this regulatory element, we disrupted individual transcription factor binding motifs within this conserved region. This revealed that a BLIMP1 binding site was required for the silencing functions of this regulatory element. Thus, this highly conserved cis-regulatory element appears to act as a repressor decreasing *Tcf7* expression in response to activation signals.

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P154

ESSENTIAL ROLE OF GLYCOSPHINGOLIPID METABOLISM FOR NATURAL KILLER CELL DEVELOPMENT AND FUNCTION

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Abstract Content: Super-enhancer architecture is indicative of genes that contribute to cell identity. In $CD4^+$ T cells, such regions are enriched for genes that are critical to its development and function—cytokines, cytokine receptors and transcription factors. Therefore, the identification of genes that reside within super-enhancer loci can be a clue to important factors that contribute to a cell's biology. Natural killer (NK) cells are cytotoxic innate lymphocytes that play a critical role in the clearance of both tumor and virally infected cells. To identify novel aspects of NK cell biology, we interrogated NK cell-specific super-enhancers (SEs). Along with well-known NK cell identity genes, we unexpectedly identified *Ugcg* as the top ranked SE locus in NK cells. *Ugcg*, which encodes UDP-glucose ceramide glucosyltransferase (UGCG) and catalyzes the first glycosylation step in glycosphingolipid (GSL) biosynthesis, is ubiquitously expressed among hematopoietic cells, however it is considerably higher in NK cells. In a mouse model targeting *Ugcg* expression in hematopoietic cells we found that most of the immune cell subsets developed, however there was a significant loss of NK cells. Since GSLs are critical components of the cell membrane, including lipid raft organization, cell migration and signal transduction, we set out to determine what important factors relevant to NK cell homeostasis and function are impacted by loss of UGCG activity. We found that both genetic deletion and pharmacologic inhibition of UGCG activity reduced levels of CD122, a cytokine receptor that is required for NK cell homeostasis and proliferation. Furthermore, inhibition of UGCG activity blocked NK cell mediated tumor cytotoxicity due to reduced cytolytic activity and inability to form stable immunological synapses, which led to a decline in the percentage of dead tumor cells. Taken together, the identification of SE loci in NK cells have uncovered a novel NK cell identity gene that is known to be essential for GSL metabolism. This metabolic gene is selectively required for NK cell development and further required for various characteristics of NK cell function.

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P155

ANALYSIS FOR INDUCTION MECHANISMS OF PERITONITIS REGULATED BY ADIPOSE TISSUE

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Abstract Content: Peritonitis is an inflammation of the peritoneum caused by gut perforation along with bacteria leaking and is known to be a trigger of sepsis, a clinical syndrome with multi-organ dysfunction, because of dysregulated host response to infection. It has also been known that inflammatory responses in peritonitis is followed by the immune-suppressive phase. However, the mechanisms of how the inflammatory phase turns into the immune-suppressive phase remain unclear. Here, we investigated the role of mesenteric adipose tissue, which is closely attached to the gut, in the gut perforation-induced peritonitis, by employing the peritonitis mouse model (i.e., cecal ligation and puncture; CLP). Although recent studies have shown that the adipose tissue contains various immune cells including the innate lymphoid cells (ILCs), knowledge about the immunological function of adipose tissue during peritonitis is still limited. In this study, we observed that the number of IL-7Rα$^+$ILC1 in the adipose tissue was elevated with a peak of inflammation together with the upregulation of inflammatory cytokines, IL-6 and IL-1β. In addition, the level of IFN-γ production by the induced IL-7Rα$^+$ILC1 was substantially restricted during the inflammatory stage, whereas IL-7Rα-negative ILC1 produced IFN-γ comparable to NK cells under the stimulation. Furthermore, Promyelocytic leukemia zinc finger (PLZF) KO mice showed a high susceptibility to the CLP surgery, suggesting that increased IL-7Rα$^+$ILC1 having less IFN- γ production might be involved in ameliorating peritonitis. This study thus provides important insights for adipose tissue-targeted new therapeutic strategies, that could open a window for rapid treatment of peritonitis/sepsis in the clinical area.

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P157

IRF4 CONTROLS NK CELL CLONAL EXPANSION AND DIFFERENTIATION DURING VIRAL INFECTION

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Abstract Content: Natural Killer (NK) cells are innate cytotoxic lymphocytes with adaptive-like features and share many transcriptional programs with their adaptive siblings, CD8$^+$ T cells. Over the past decade, various factors including cytokine signaling and their downstream JAK/STAT transcriptional pathways have been discovered to be important for optimal NK cell clonal expansion and effector function during mouse cytomegalovirus infection. However, additional molecular mechanisms that govern NK cell differentiation during this process remain to be revealed. In this study, we have identified the transcription factor IRF4 as a master regulator of NK cell differentiation during viral infection. Genetic ablation of IRF4 is detrimental to the generation of mature NK cells following infection, but does not affect their early effector function. Moreover, the induction of IRF4 expression in NK cells requires various signals from receptor engagement and pro-inflammatory cytokines. Finally, we demonstrate that induction of IRF4 in activated NK cells is critical to their clonal expansion and antiviral response. Altogether, these data suggest that IRF4 acts as a signal integrator that decouples effector function from differentiation during viral infection.

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P158

EMBRYONIC AND NEONATAL WAVES GENERATE DISTINCT POPULATIONS OF HEPATIC ILC1S

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Abstract Content: Group 1 innate lymphoid cells (ILCs) comprising circulating natural killer (cNK) cells and tissue-resident ILC1s are critical for host defense against pathogens and tumors. Despite a growing understanding of their role in homeostasis and disease, the ontogeny of group 1 ILCs remains largely unknown. Here, we used fate mapping and single-cell transcriptomics to comprehensively investigate the origin and turnover of group 1 ILCs. While cNK cells are continuously replaced throughout life, we uncovered tissue-dependent development and turnover of ILC1s. A first wave of ILC1s emerges during embryogenesis in the liver and transiently colonizes fetal tissues. After birth, a second wave quickly replaces ILC1s in most tissues apart from the liver, where they layer with embryonic ILC1s and persist until adulthood undergoing a unique developmental program. While embryonically-derived ILC1s give rise to a cytotoxic subset, the neonatal wave establishes a helper-like subset. Our findings uncover key ontogenic features of group 1 ILCs and their association with unique cellular identities and functions.

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P159

UNTIMELY TGFB RESPONSES IN COVID-19 LIMIT ANTIVIRAL FUNCTIONS OF NK CELLS

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Abstract Content: SARS-CoV-2 is a single-stranded RNA virus that causes COVID-19. Given its acute and often self-limiting course, it is likely that components of the innate immune system play a central part in controlling virus replication and determining clinical outcome. Natural killer (NK) cells are innate lymphocytes with notable activity against a broad range of viruses, including RNA viruses1,2. NK cell function may be altered during COVID-19 despite increased representation of NK cells with an activated and adaptive phenotype3,4. Here we show that a decline in viral load in COVID-19 correlates with NK cell status and that NK cells can control SARS-CoV-2 replication by recognizing infected target cells. In severe COVID-19, NK cells show defects in virus control, cytokine production and cell-mediated cytotoxicity despite high expression of cytotoxic effector molecules. Single-cell RNA sequencing of NK cells over the time course of the COVID-19 disease spectrum reveals a distinct gene expression signature. Transcriptional networks of interferon-driven NK cell activation are superimposed by a dominant transforming growth factor-β (TGFβ) response signature, with reduced expression of genes related to cell–cell adhesion, granule exocytosis and cell-mediated cytotoxicity. In severe COVID-19, serum levels of TGFβ peak during the first two weeks of infection, and serum obtained from these patients severely inhibits NK cell function in a TGFβ-dependent manner. Our data reveal that an untimely production of TGFβ is a hallmark of severe COVID-19 and may inhibit NK cell function and early control of the virus.

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LB-43

SEX DIFFERENCES IN NK CELLS MEDIATED BY THE X-LINKED EPIGENETIC REGULATOR UTX

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Abstract Content: Viral infection outcomes are sex-biased, with males generally more susceptible to human cytomegalovirus (HCMV) and other viral infections compared to females. These differences may reflect sexual dimorphism in immune cell composition and function. As such, it is surprising that numbers of natural killer (NK) cells, a first line of anti-viral defense, are increased in males compared to females. Here we show in mouse models and human samples that while males harbor increased NK cell numbers, they concomitantly produce less IFN-γ, a key proinflammatory cytokine for NK-mediated effector responses. This difference is not due solely to divergent levels of gonadal hormones, since these differences persist even with gonadectomy. Instead, these differences are attributable to lower male expression of the epigenetic regulator Kdm6a (UTX), a X chromosome gene which escapes X inactivation in both human and mouse NK cells. NK cell-specific UTX deletion phenocopied multiple features of male NK cells, which include increased numbers and reduced IFN-γ production. Moreover, NK cell UTX is critical for optimal anti-viral immunity, since mice with NK cell UTX deficiency show increased lethality to mouse cytomegalovirus (MCMV) challenge. Integrative ATAC-seq and RNA-seq analysis revealed a critical role for UTX in maintaining open chromatin accessibility and gene expression of Ifng, Csf2, Thy1, and other gene loci important in NK cell homeostasis and effector function. Taken together, these data implicate UTX as a critical molecular determinant of NK cell sex differences and suggest enhancing UTX function may be a new strategy to boost endogenous NK cell anti-viral responses.

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LB-44

CYCLIC FASTING INDUCES NATURAL KILLER CELL ANTI-TUMOR IMMUNITY

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Abstract Content: Natural Killer (NK) cells are cytotoxic lymphocytes that play a crucial role in host anti-tumor immunity against cancer by targeting transformed cells. Recent studies have implicated dietary restriction in the improved elimination of cancer cells, yet how immune cells contribute to this remains poorly understood. Herein, we show that a cyclic fasting diet (CFD) beginning after tumor initiation improves NK cell mediated anti-tumor responses and survival against both solid and metastatic tumors. During each fasting cycle, $CD11b^{+}$ NK cells are redistributed from secondary lymphoid organs (SLO) to the bone marrow (BM), a process governed by sphingosine-1-phospate (S1P) and its receptor $S1PR_5$. During periods of refeeding, NK cells are then rapidly redeployed to SLOs. where the total NK cell pool gains improved functionality. Enhanced effector functions are driven by a combination of mature NK cell priming in the BM, and metabolic reprogramming of immature NK cells left behind in nutrient-starved organs. Failure to enter the BM or failure to rewire metabolic pathways during CFD negates improved NK cell anti-tumor function, suggesting that both BM and SLO-derived signals are necessary to potentiate a robust response. This work uncovers a novel dietary strategy to improve tumor clearance and identifies a previously unappreciated link between dietary restriction and optimized NK cell responses.

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04. ILC2s

P160

DIETARY FIBER PROMOTES MICROBIOTA-DERIVED BILE ACIDS AND TYPE 2 INFLAMMATION

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Abstract Content: Dietary fiber can exert immunoregulatory effects through microbial fermentation products including short chain fatty acids (SCFAs). However, the influence of dietary fiber on most microbiota-derived metabolites and their role in immunoregulation remain poorly defined. Using untargeted metabolomics, we show that high fiber diet drives a significant shift in systemic levels of microbiota-derived metabolites, notably elevated levels of bile acids. This metabolomic shift is associated with type 2 inflammation characterized by production of IL-33, activation of group 2 innate lymphoid cells (ILC2s), and accumulation of eosinophils in colon and lung tissues. Administration of a single bile acid mimics high fiber diet-induced type 2 inflammation, whereas deletion of a bile acid receptor diminishes the effects of dietary fiber. Furthermore, genetic deletion of a bile acid-metabolizing enzyme in a member of the dietary fiber-associated microbiota abolishes the capacity of high fiber diet to trigger type 2 inflammation. Finally, we demonstrate that high fiber diet-induced type 2 inflammation improves immune defense against helminth infection. Taken together, these data reveal that dietary fiber triggers type 2 immune responses at barrier surfaces via microbiota-derived bile acid metabolites with direct effects on mucosal inflammation and host defense.

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P161

'BOOLEAN ILC2 MICE' – USING MULTIPLE INTERSECTIONAL RECOMBINASES TO MEDIATE IMPROVED ILC2-RESTRICTED GENE DELETION OR CELL ABLATION

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Abstract Content: The deletion of specific key genes in a single cell population or the ablation of that population is a fundamental approach to understanding the function of immune cells and their role in particular immune responses. Unfortunately, not all immune cell types express a single definitive marker. Consequently, although the use of single gene promoter-driven Cre expressing mouse lines has been informative, collateral affects can arise. To improve on existing approaches, we have sought to combine multiple recombinases Cre, Dre and Vika in 'AND' and 'AND NOT' Boolean logic gates to generate a mouse strain in which Cre expression is predominantly restricted to ILC2s. We combined *Icos* and *Il13* as drivers of the AND function and *Cd28* to drive the AND NOT function. In combination with a Rosa26-Lox-STOP-Lox-RFP allele (where fluorescent protein expression is dependent on Cre activity) we detected a high proportion of ILC2s marked by RFP. Notably, extremely few T cells or indeed any other cell type investigated were RFP-positive. Indeed, in vitro polarised Th2 cells also remained RFP-negative, supporting the indication that the *Cd28* 'AND NOT' function protects T cells that express IL-13 from Cre activity. We will discuss the use of this new model to investigate ILC2 biology.

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P162

CYTOKINE ACTIVATED ILC2 ESTABLISH AN INFLAMMATORY TISSUE MEMORY IN THE GUT

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Abstract Content: An ILC2-tuft cell circuit in the small intestine underlies helminth immunity through local ILC2 activation and dynamic epithelial barrier remodeling. We asked whether this inflammatory pathway had long term consequences on small intestinal biology. Utilizing a system of cytokine activated ILC2, we found that both ILC2 and epithelial cells underwent dynamic changes that were maintained long after resolution of the inflammatory challenge. These alterations resulted in enhanced control of worm infection, demonstrating protective immunological memory.

Finally, we have used specialized genetic tools to understand how this inflammatory memory is established and maintained in the small intestine.

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P163

TYPE I INTERFERON LIMITS ILC2 EFFECTOR FUNCTIONS THROUGH SUPPRESSION OF THE CCR8-CCL1/CCL8 AXIS

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) exert critical roles in anti-helminth immunity and the pathogenesis of allergic diseases by rapidly secreting vast amounts of type 2 signature cytokines. Type 1 interferons (IFN-I) are critical negative regulators of ILC2 effector functions and associated innate and adaptive type 2 immunopathologies. Exposure to IFN-I inhibits ILC2 proliferation as well as type 2 cytokine production, however, the mechanistic underpinnings of IFN-I-mediated ILC2 regulation remain largely elusive. Using RNA-sequencing analysis, we demonstrate that IFN-I treatment inhibits the production of the chemokine CCL1 by ILC2s. Moreover, expression of CCR8, the cognate chemokine receptor for CCL1, is downregulated upon IFN-I-stimulation *ex vivo* as well as *in vivo* upon sublethal influenza A virus infection (IAV) or upon IFN-I administration following pulmonary IL-33 challenge. In addition, expression of CCL8, the chemotactic ligand of CCR8, was suppressed in an IFN-I dependent manner in the lungs of IAV-infected mice. IFN-b potently restrained pulmonary CCL1/CCL8 expression as well as CCR8 expression by ILC2 and $CD4^+$ T cells in a model of IL-33-driven allergic airway inflammation. Thus, this study sheds new light on the underlying mechanisms of ILC2 regulation by identifying the CCR8-CCL1/CCL8 axis as a target for IFN-I-driven ILC2 inhibition.

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P164

HOIL1 REGULATES GROUP 2 INNATE LYMPHOID CELL NUMBERS AND TYPE 2 INFLAMMATION IN THE SMALL INTESTINE

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Abstract Content: Patients with mutations in *HOIL1* experience a complex immune disorder including intestinal inflammation. HOIL1 is a component of the Linear Ubiquitin Chain Assembly Complex (LUBAC). Another component, HOIP is the catalytic subunit of LUBAC that generates linear (methionine-1-linked) polyubiquitin chains. However, HOIL1 is also a functional E3 ubiquitin ligase, and its physiological functions are largely unknown. To investigate the role of *HOIL1* in regulating intestinal inflammation, we employed a mouse model of partial HOIL1 deficiency. The ileum of HOIL1-deficient mice displayed characteristic features of type 2 inflammation including tuft cell and goblet cell hyperplasia, and elevated expression of *Il13*, *Il5* and *Il25* mRNA. Inflammation persisted in the absence of T and B cells, and bone marrow chimeric mice revealed a requirement for HOIL1 expression in radiation-resistant cells to regulate inflammation. Although disruption of IL-4 receptor alpha (IL4Rα) signaling on intestinal epithelial cells

ameliorated tuft and goblet cell hyperplasia, expression of *Il5* and *Il13* mRNA remained elevated, indicating that HOIL1 functions upstream of IL4Rα in the IL-13 - tuft cell - IL-25 feed forward cycle. KLRG1^{hi} CD90^{lo} group 2 innate lymphoid cells were elevated, and remained so even when IL-4/IL-13 signaling on intestinal epithelial cells, tuft cell hyperplasia and IL-25 induction were blocked. Antibiotic treatment dampened intestinal inflammation indicating that commensal microbes are a contributing factor. Overall, we have identified a key role for HOIL1, a component of the Linear Ubiquitin Chain Assembly Complex, in regulating type 2 inflammation and ILC2 in the small intestine. Understanding the mechanism by which HOIL1 regulates type 2 inflammation will advance our understanding of intestinal homeostasis and inflammatory disorders and may lead to the identification of new targets for treatment.

Disclosure of Interest: None Declared
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TYPE 2 INNATE LYMPHOID CELL DYSFUNCTION IN SEVERE ASTHMA AND RESPONSE TO TREATMENT WITH MEPOLIZUMAB AND OMALIZUMAB.

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Abstract Content: Background:
It is evident that group 2 innate lymphoid cells (ILC2s) play an important role in asthma by promoting type 2 inflammation.
Aims:
To compare ILC2s proliferation and cytokine secretion between severe allergic and eosinophilic asthma (SA), mild asthma (MA), healthy controls (HC) and non-asthma atopic controls (NAA). Also, to demonstrate the effect of mepolizumab and omalizumab on SA ILC2s post treatment.
Methods:
HC and NAA subjects had normal lung function and no history of asthma. MA subjects had GINA step 1 or 2 asthma. SA subjects had GINA step 5 severe eosinophilic and allergic asthma. All SA subjects were on high dose of inhaled corticosteroids (ICS) median (Q1, Q3) 1440 (837, 2050) (mcg/d). Peripheral blood mononuclear cells (PBMCs) were obtained from HC n=10, NAA n=10, MA n=8 and SA n=18. ILC2s were identified and sorted by flow cytometer, cultured for 14 days with IL-2, IL-33, IL-25 and TSLP. ILC2 proliferative capacity was assessed by Ki-67 expression, surface receptors and secretion of IL-5 and IL-13 by flow cytometry on day 14 of culture. Significance was calculated using Kruskal-Wallis test.

Results:
At day 14 of culture, ILC2s from SA demonstrated increased TSLPR expression (p=<0.0001), Ki-67 expression (p=<0.0001) and cytokine release of IL-5 (p=<0.0001) and IL-13 (p=<0.0001) compared to ILC2s from HC, NAA and MA. ILC2s of SA subjects treated with mepolizumab demonstrated reduced Ki-67 expression (p= 0 .01), TSLPR expression (p= 0.01) and cytokine secretion IL-5 (p=0.003) & IL-13 (p=0.03). Subjects treated with omalizumab demonstrated reduced cytokine release of IL-5 (p=0.03) and IL-13 (p=0.04).

Conclusion:
ILC2s from SA subjects demonstrate increased TSLPR expression and cytokine secretion release compared to HC, NAA and MA subjects. Mepolizumab had more pronounced effect on ILC2s physiology compared to omalizumab.

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P166

ESTABLISHING A MODEL OF MEMORY GROUP 2 INNATE LYMPHOID CELLS IN ATOPIC DERMATITIS

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) are found in mucosal and non-mucosal tissues, such as the lung, gut and skin. In response to epithelium-derived cytokines, ILC2s produce copious amounts of type 2 cytokines, IL-5 and IL-13, initiating a cascade of reactions leading to type 2 inflammation. ILC2s have previously been identified in mouse skin and they have been implicated in atopic dermatitis (AD) in humans. We have previously described memory ILC2s in mouse lung, where ILC2s remember previous activation and respond more robustly upon challenge with an unrelated allergen/stimulus in an antigen non-specific manner. Considering that the skin is constantly exposed to various types of stimuli, we hypothesized that memory ILC2s are generated in the skin upon exposure to insults and elicit enhanced response to a secondary challenge, contributing to AD-like inflammation. To establish a model and further characterize skin ILC2s, we have investigated the kinetics of ILC responses upon topical treatment of mouse ears with a vitamin D3 analogue calcipotriol. ILCs, identified as $Lin^{-}Thy1^{+}CD103^{+}$ cells underwent a moderate expansion, reaching a peak on day 3 after the initial treatment. This was followed by a contraction phase, where ILC frequency decreased to a lower level than in naïve mice. Approximately 50% of the ILCs expressed ILC2 markers, CD25 and ICOS, while ST2 and KLRG1 were not detected. Interestingly, regulatory T cells and type 2 helper T cells gradually increased in parallel to the ILC expansion. Although the skin ILCs did not remain high in frequency for a long time after activation, whether calcipotriol treatment induces transient or permanent changes in ILCs or their environment is yet to be explored. It is conceivable that calcipotriol-induced effects on ILCs and T cells may have a potential impact on the immunological memory property of skin ILC2s.

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P167

A GUT COMMENSAL PROTOZOA REMOTELY REGULATES AN ILC2-DEPENDENT LUNG NICHE FOR ASTHMA-PROMOTING EOSINOPHILS.

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Abstract Content: The gut microbiome influences chronic inflammation of the airways via the gut-lung axis. However, causal connections between microbes and their host, including the underlying mechanisms for this phenomenon remain largely unknown. Here, we show that colonization with the gut commensal protozoa, *Tritrichomonas musculis* (*T.mu*), remotely shapes the lung immune landscape and exacerbates allergic airway inflammation. We demonstrate that colonization with *T.*mu mediates the T and B cell-dependent accumulation and activation of inflammatory group 2 innate lymphoid cells in the lungs to constitute a tripartite immune network that serves as a niche for lung eosinophils. Animals colonized with *T.mu* show severely exacerbated allergic inflammation in the airways and reveal a new protozoan-driven gut-lung axis that remotely shapes the lung immune network to potentiate chronic pulmonary inflammation.

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GROUP 2 INNATE LYMPHOID CELLS INDUCE RECURRENCE OF ALLERGIC PATHOLOGY VIA AN INNATE AMPLIFICATION CIRCUIT

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Abstract Content: Eosinophilic chronic rhinosinusitis (ECRS) is a refractory disease that causes eosinophilic infiltration of nasal mucosa, recurrent multiple nasal polyp formation and olfactory disorders. Group 2 innate lymphoid cells (ILC2s) produce IL-5 and IL-13 in response to IL-33, and induce antigen-nonspecific type 2 immune responses. ILC2s are known to accumulate in the nasal polyps of ECRS, the role of ILC2s in pathogenesis of ECRS remains unclear.

Previous research found that ILC2s induce low-affinity IgE production from B1 cells, which are innate type of B cells, through IL-4 and IL-5 production, resulting in increased survival of mast cells and basophils that exacerbate allergic responses. Mast cells and basophils are the major producers of CysLTs, which induces IL-4 production from ILC2s, suggesting that ILC2s, B1 cells, and mast/basophils constitute a positive feedback mechanism, which we named 'innate amplification circuit'. In this study, we found that ILC2s accumulated in nasal polyps of ECRS patients, predominantly correlated with IgE-positive mast cells as well as eosinophils. Single-cell RNAseq analysis revealed the presence of B1 cells and IL-4-producing ILC2s in the nasal polyps of ECRS patients. Notably, IgE production was observed mainly from B1 cells. The significant increase in ILC2s and IgE production in nasal polyps was suggestive of ILC2s contribute to low-affinity IgE production from B1 cells, causing nasal polyp formation. Furthermore, mass cytometry analysis revealed that in addition to mast cells and basophils, plasmacytoid dendritic cells (pDCs) expressed high levels of FCER1A, suggesting that pDCs, which are known to inhibit ILC2s through type I interferon production, may also be a target of IgE. Therefore, our results indicate that IgE regulated by ILC2s not only promoted mast cell survival, but also suppressed pDC function to form an enhanced amplification circuit. Collectively, our findings suggest that ILC2s, B1 cells, and mast cells form a positive feedback mechanism that determines the recurrence of nasal polyps in ECRS patients.

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IL-33 STIMULATED ILC2S REGULATE STEADY STATE AND DEMAND-ADAPTED MYELOPOIESIS

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Abstract Content: Hematopoiesis is hierarchically organized, with multipotent hematopoietic stem and early progenitor cells (HSPCs) at the top that can give rise to all hematopoietic lineages. The functionality of the HSPCs is extrinsically regulated by their surrounding microenvironment, the so-called niche. The niche is based in the bone marrow (BM) and consists of soluble and cellular factors, for example various immune cells like type 2 innate lymphoid cells (ILC2s). It has been recently shown that BM-resident ILC2s regulate HSPC recovery post 5-fluorouracil treatment. However, it is unknown if BM-resident ILC2s also regulate HSPCs during steady state or other demand-adapted conditions.

In the present study, we documented that naïve ILC2-deficient (ΔILC2) mice had a reduced BM cellularity compared to control mice, due to impaired myeloid cell counts. The BM-resident ILC2s were the only immune cell population expressing ST2 at high levels, and the BM myeloid counts in ST2- ($ST2^{-/-}$) and IL-33- ($IL\text{-}33^{-/-}$) deficient mice were reduced compared to BL/6 control animals, mirroring the phenotype of ΔILC2 mice. These data indicated that ILC2s could stimulate myelopoiesis in an IL-33/ST2 signaling dependent way. Indeed, the transfer of ST2-competent ILC2s into $ST2^{-/-}$ and ΔILC2 mice rescued the BM cellularity to levels of control mice. Furthermore, HSPCs formed significantly more colonies in an *in vitro* methylcellulose assay when they were pre-cultured with ILC2 supernatant, indicating that BM-resident ILC2s can directly modulate stem cells by soluble factors. RNA sequencing and blocking assays revealed that IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF), and amphiregulin (AREG)

were primarily responsible for this increase in the colony forming capacity of HSPCs. Mechanistically, these ILC2-secreted soluble factors induced a switch from G0 to G1 cell cycle phase in HSPCs and propelled HSPC proliferation and differentiation. To examine a condition in which the IL-33/ILC2/HSPC axis is physiologically dysregulated, we analyzed the BM of sub-lethally irradiated mice. Irradiation led to an acute increase in BM IL-33 levels. BM-resident ILC2s survived short-term post irradiation and the enforced IL-33/ST2 signaling induced increased secretion of IL-6 and GM-CSF by ILC2s. These cytokines propelled the proliferation and differentiation of HSPCs and therefore contributed to the recovery of the hematopoietic system post irradiation.
Our results indicate that BM-resident ILC2s are not only a pool of progenitors for distant barrier organs but fulfill important effector functions by themselves. During steady state, BM-resident ILC2s are activated by IL-33/ST2 signaling to produce effector cytokines, which in turn stimulate the proliferation and differentiation of HSPCs. This effect is enhanced by irradiation, pointing out that ILC2s are important regulators of HSPCs during both, steady state and demand adapted hematopoiesis.

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INNATE LYMPHOID CELLS ARE CRITICAL IN DIET AND ALCOHOL INDUCED IN STEATOHEPATITIS AND FIBROSIS

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Abstract Content: Alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) may progress to hepatitis and fibrosis. It has been shown that the number of group 2 innate lymphoid cells (ILC2s) in peripheral blood increases proportionally to the severity of fibrotic disease. In mouse models, ILC2s have been shown to be involved in the development of hepatic fibrosis in concanavalin A-induced hepatitis and carbon tetrachloride-induced liver fibrosis. However, the role of ILCs in ALD and NAFLD is still unclear. We have established an experimental murine model of chronic steatosis (fatty liver) and hepatitis. B6 mice were treated with a combination of alcohol in drinking water and a high-fat (HF) diet. This mouse model is highly relevant to human conditions as many heavy drinkers also eat fatty food. Importantly, Alcohol-HF diet-treated mice do not become obese. Alcohol-HF diet induced hepatomegaly, and histology of liver sections and Triglyceride Assay showed lipid accumulation in hepatocytes, while qPCR analysis showed upregulation of fibrosis-related genes after six weeks. The numbers of ILC2s, eosinophils, neutrophils, and macrophages in the liver were greatly increased in the treated B6 mice, showing that the alcohol-HF diet induced steatohepatitis and fibrosis. RAG1-deficient mice treated in the same way also developed steatohepatitis, indicating that adaptive immunity is not involved. In contrast, the treatment of the ILC2 deficient CD127cKO mice, generated by crossing Rora-IRES-Cre and floxed Il7ra mice, showed no increase in the liver weight and much fewer neutrophils and macrophages in the liver compared to wild-type B6 mice. Single cell RNA sequencing analysis showed significant differences in ILC2s and ILC1s between alcohol-HF diet treated B6 and CD127 cKO mice. FACS analysis also showed that ILC2s and a subpopulation of ILC1s are absent in CD127 cKO mice. These results suggest that ILC1s and ILC2s play critical roles in alcohol-HF diet induced chronic steatohepatitis and fibrosis.

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IL-10-PRODUCING HUMAN GROUP 2 INNATE LYMPHOID CELLS LIMIT GRAFT-VERSUS-HOST DISEASE AND REGULATE ALLOGENEIC T CELL RESPONSES

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Abstract Content: Identification of cells and molecules that limit harmful immune responses is critical for the development of tolerance-promoting immunotherapeutic strategies. Innate lymphoid cells (ILCs) rapidly respond to microenvironmental signals and orchestrate immune responses to maintain homeostasis. Following hematopoietic stem cell transplant (HSCT), elevated proportions of activated helper ILCs and CD56^{bright} Natural Killer cells in blood is linked to patients who do not develop acute graft-versus-host disease (aGVHD), and HSCT patients that receive ILC-rich grafts have a lower risk of developing aGVHD. Further, experimental murine studies have observed protective roles of group 2 ILCs (ILC2s) in limiting gastrointestinal GVHD. In our HSCT patient-based studies, the presence of ILC2s is associated with decreased proportions of CD4$^+$ T helper 1 (Th1) cells and protection from aGVHD, suggesting ILC2s may antagonize harmful CD4$^+$ Th1 responses in GVHD. To assess the potential of human ILC2s to limit T cell responses in adoptive cell therapies for aGVHD, we developed methods to isolate and expand human ILC2s from healthy donor blood in a manner that maintains expression of signature cytokines. Using a xenograft model of aGVHD, we show that adoptive transfer of expanded human ILC2s reduces the severity of xenograft GVHD symptoms, improves weight loss and prolongs survival of NOD-*scid* IL2Rγ^{null} mice. ILC2s limited the proliferation of allogeneic CD4$^+$ and CD8$^+$ T cells and reduced the proportion of CD4$^+$ Th1 and CD8$^+$ Tc1 cells *in vivo*, mirroring what was seen in our patient cohort. Immunohistochemistry revealed that ILC2s also limited T cell infiltration into intestinal tissue. Single cell RNA sequencing of expanded ILC populations identified IL-10 as a key cytokine differentially expressed by expanded human ILC2s, in addition to canonical ILC2 cytokines. *In vitro* studies demonstrate direct regulation of allogeneic T cells by human IL-10 producing ILC2s is mediated by a combination of IL-4 and IL-10. Collectively our findings support that human IL-10-producing ILC2s limit harmful allogeneic T cell responses and may have applications in adoptive cell-based therapies for GVHD.

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EX VIVO MODEL FOR LARGE-SCALE GENERATION OF PRIMARY MURINE AND HUMAN GROUP 2 INNATE LYMPHOID CELLS

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) regulate innate and adaptive type 2 immune responses. However, progress in our understanding of ILC2 biology has been hampered by their scarcity. Utilizing cell sorting and a defined cytokine cocktail we present here a simple, efficient, fast and reliable protocol to isolate and expand mouse bone marrow-derived ILC2s. The resulting ILC2s constitute a pure cell population with the same phenotypic and functional characteristics of cells analyzed *in vivo*. ILC2s can be frozen, shipped and thawn, and used as a novel model to study the biology of ILC2 including their positive and negative regulation. Expanded ILC2s can be transduced with viral vectors, allowing manipulation of gene expression. Furthermore, we describe a novel approach to expand human ILC2s from peripheral blood. In summary, our novel approach enables a high yield of primary ILC2s allowing genetic, cellular and molecular studies to gain an in-depth understanding of ILC2 biology critical for the development of new drugs to counter type 2 immunopathologies.

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THE PROTECTIVE ROLE OF STOMACH ILC2 INDUCED BY THE COLONIZATION OF A STRAIN S24-7

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Abstract Content: Gut microbiota has a well-documented impact on the immune development and shapes appropriate immune responses in the mucosal organs, such as the gastrointestinal tract. By contrast, the physiological significance of the stomach commensal microbiota, including its immunoregulatory functions, has barely been understood. Innate lymphoid cells (ILCs) have been identified in the intestinal tract as well as other tissues, but information about stomach-resident ILCs was lacking, especially their interaction with microbiota. Recently, we have reported that a predominant ILC2 subset among ILCs resides in the stomach and shown that their homeostasis and effector functions are regulated by local commensal communities represented by *S24-7*, which is belonging to the phylum *Bacteroidetes*. *S24-7*, also termed *Muribaculaceae*, are generally classified as a viable but non-culturable bacteria and are widely colonized in many mammalians including humans and mice. Indeed, the comprehensive microbiome analysis of feces has confirmed an increase of *S24-7* in feces from IBD patients or obese persons. These observations suggested the involvement of *S24-7* in some disease pathogenesis, however the mechanisms of how *S24-7* regulates immune responses in the stomach remain unclear. In this study, we newly showed that IL-13-producing stomach ILC2s were induced following colonization with a *S24-7* strain, whereas *Helicobacter pylori* infection led to IL-5 production from the mouse stomach.Prior colonization of *S24-7* to mice can protect from *Helicobacter pylori* infection, suggesting a novel protective function of ILC2s triggered by commensal *S24-7* colonization in the stomach.

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RETINOIC ACID DRIVES INTESTINE-SPECIFIC ADAPTATION OF EFFECTOR ILC2S ORIGINATING FROM DISTANT SITES

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Abstract Content: Adaptation of immune cells to tissue-specific microenvironments is a crucial process in homeostasis and inflammation. Here, we show that murine effector type 2 innate lymphoid cells (ILC2s) from various organs are equally effective in repopulating ILC2 niches in other anatomical locations where they adapt tissue-specific phenotypes of target organs. Single-cell transcriptomics of ILC2 populations revealed upregulation of retinoic acid (RA) signaling in ILC2s during adaptation to the small intestinal microenvironment, and RA signaling mediated reprogramming of kidney effector ILC2s towards the small intestinal phenotype *in vitro* and *in vivo*. Inhibition of intestinal ILC2 adaptation by blocking RA signaling impaired worm expulsion during *Strongyloides ratti* infection, indicating functional importance of ILC2 tissue imprinting.
In conclusion, this study highlights that effector ILC2s retain the ability to adapt to changing tissue-specific microenvironments, enabling them to exert tissue-specific functions, such as promoting control of intestinal helminth infections.

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CORTICOSTEROIDS INDUCE DIFFERENTIATION OF ILC2S TOWARDS AN IMMUNOMODULATORY PHENOTYPE CHARACTERIZED BY HIGH AMPHIREGULIN PRODUCTION

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) are major producers of type-2 cytokines and considered to be key players in the pathophysiology of various type-2 immune disorders, including asthma. In addition, ILC2s can secrete the growth factor amphiregulin to orchestrate epithelial tissue repair but also pathogenic tissue remodeling. Fortunately, asthma symptoms can be effectively controlled in many patients using anti-inflammatory corticosteroid treatment. Previously, we have reported that activation of resting CD45RA$^+$ human ILC2s is accompanied by a CD45 isoform switch, resulting in the generation of inflammatory ILC2s marked by CD45RO and steroid resistance. In this study, we show that human ILC2s start to produce and secrete amphiregulin upon dexamethasone treatment. Whereas various 'alarmin' cytokine-combinations induced amphiregulin expression in up to only 15% of ILC2s, corticosteroids provoked amphiregulin induction in ~70% of ILC2s, which is independent of additional alarmin stimulation or the type of steroid used. Strikingly, corticosteroid-resistant CD45RO$^+$ ILC2s also rapidly induce amphiregulin production upon corticosteroid exposure, despite retaining part of their type-2 cytokine production capacity. Transcriptome data mining revealed induction of amphiregulin transcription upon steroid treatment also by other lymphocyte - but not structural - cell populations such as CD4$^+$ T cells and B cells. However, production of amphiregulin by CD4$^+$ T cells upon dexamethasone treatment was substantially lower than levels produced by ILC2s. Single-cell RNA-seq and epigenomics technologies are currently employed to expose the mechanisms underlying amphiregulin induction in human ILC2s by corticosteroids. Our data suggest that the chronic production of amphiregulin by ILC2s might be an unappreciated pro-fibrotic consequence of corticosteroid therapy in patients suffering from chronic inflammatory disease that rely on frequent steroid usage.

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TYPE 2 CYTOKINES AND ILC2S CONTROL TISSUE ADAPTATIONS TO CHITIN

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) maintain tissue homeostasis by forming feedback circuits with surrounding tissue cells, often via type 2 cytokine signaling. These ILC2-tissue cell circuits can be triggered by multiple inputs; however, mechanisms linking specific external cues with endogenous tissue-derived factors influenced by type 2 immune signaling are less well understood. Here, we describe ILC2-mediated tissue adaptations to the polysaccharide chitin, a constituent of fungi, helminths, and arthropods, that promote mammalian chitinase activity. In the respiratory and gastrointestinal tracts, chitin initiates 2 cytokine production from sentinel epithelial cells and ILC2s, leading to eosinophilia, epithelial hyperplasia, and type 2 cytokine-mediated expansion of chitinase-producing cells that facilitate chitin degradation. Sustained triggering of this circuit leads to heightened basal innate type 2 immunity, tissue remodeling, and resistance to helminth infection. Exogenous chitin also alters the composition of the commensal microbiota; however, host-encoded adaptations to chitin are preserved in germ-free mice, indicating that the mammalian circuit is triggered by endogenous elements.

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IL-4/IL-13-PRODUCING ILC2 ARE REQUIRED FOR TIMELY CONTROL OF INTESTINAL HELMINTH INFECTION

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Abstract Content: Infection of mice with *Nippostrongylus brasiliensis (Nb)* serves as a model for human hookworm infection affecting about 600 million people world-wide. Expulsion of *Nb* from the intestine requires IL-13-mediated mucus secretion from goblet cells and activation of smooth muscles cells. Type 2 innate lymphoid cells (ILC2s) are a major cellular source of IL-13 but it remains unclear whether IL-13 secretion from ILC2s is required for *Nb* expulsion. Here, we compared the immune response to *Nb* infection in mixed bone marrow chimeras with wild-type or IL-4/IL-13-deficient ILC2s. ILC2-derived IL-4/IL-13 was required for recruitment of eosinophils to the lung but had no influence of systemic eosinophil levels. In the small intestine, goblet cell hyperplasia and tuft cell accumulation was largely dependent on IL-4/IL-13 secretion from ILC2s. This further translated to higher eggs counts and impaired worm expulsion in mice with IL-4/IL-13-deficient ILC2s. Overall, we demonstrate that ILC2s constitute a non-redundant source of IL-4/IL-13 required for protective immunity against primary *Nb* infection.

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ACTIVATION-INDUCED CELL DEATH OF ILC2 REGULATES CHRONIC ALLERGIC INFLAMMATION

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) are a major source of innate T_H2 cytokine in allergic inflammation. We previously showed that the activated ILC2s successively express inhibitory exhaustion markers, PD-1, IL-10, and then TIGIT during chronic allergic inflammation. Meanwhile, allergen-experienced ILC2s can survive as trained ILC2s for a long time in the tissues and provoke enhanced immune reactions to the secondary non-specific allergen exposure. However, the fate decision mechanism toward trained ILC2s or cell death remains unclear. To clarify the fate of the activated ILC2s, we performed the fate-mapping analysis of the activated ILC2s expressing TIGIT (Tigit^{FM+}ILC2s). A few percent of lung ILC2s were stably fate-mapped by TIGIT expression during chronic airway allergy and quickly disappeared after cessation of allergen exposure. Tigit^{FM+}ILC2s expressed high levels of IL-5 and IL-10 marker proteins but low levels of ILC2 signature mRNAs including *Gata3*, *Il5, Il13* and *Il10*, suggesting that they are overactivated cells losing ILC2 activity. The Tigit^{FM+}ILC2s had a short life at the trachea with chronic allergy or in vitro culture. TIGIT stimulation mediated the ILC2 death in vivo and in vitro. Finally, TIGIT blockade increased ILC2 number in the airway and deteriorated chronic airway allergy. Thus, our data establish TIGIT as a key exhaustion marker to determine the fate of ILC2s toward activation-induced cell death in chronic allergy.

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LB-45

TARGETING THE TUFT CELLS-TYPE 2 INNATE LYMPHOID CELL CYTOKINE CIRCUIT IN GASTRIC CANCER

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Abstract Content: Gastric cancer is the third leading cause of cancer-related deaths, and accounts for 900,000 deaths annually. Tuft cells are a rare subset of mucosal epithelial cells that are significantly increased during gastric

tumorigenesis and serve as a major source of IL25 within the tumour microenvironment. The production of IL25 promotes the activation of type 2 Innate Lymphoid Cells (ILC2s), and results in a feed-forward loop that promotes tuft cell development through the IL25/IL13 signal transduction pathway.
To better understand the role of tuft cells and ILC2s in gastric tumour progression, we utilized the Gp130F/F mouse model of spontaneous intestinal-type gastric cancer. We observed a significant increase in tuft cells and ILC2s in the blood and gastric tumours of Gp130F/F mice compared to wild-type (WT) controls. These results were consistent with increased Il13 and Il25 gene expression in Gp130F/F tumours compared to unaffected WT tissue. Accordingly, tuft cell ablation significantly impaired tumour growth and iILC2s in Gp130F/F mice, and reduced Il13 and Il25 gene expression within tumours.
To assess the therapeutic benefit of targeting the interaction between tuft cells and ILC2s, we treated Gp130F/F mice with either anti-IL25 or anti-IL13 blocking antibodies and observed significantly smaller tumours and reduced tuft cell numbers and ILC2s in these mice. In vitro analysis of gastric tumour organoids similarly demonstrated that treatment with anti-IL25 suppressed tumour organoid growth, while stimulation with IL13 enhanced organoid growth.
Finally, we performed single cell RNA sequencing, demonstrating the strong similarities between the tuft cell and ILC2 feed-forward loop within our Gp130F/F mice and the progression of human gastric disease.
Together, our results suggest that tuft cells and ILC2s form a positive feed-forward loop that drives gastric tumour development through an IL25/IL13 signaling cascade. Inhibition of this pathway therefore provides a promising therapeutic approach for the treatment of gastric cancer.

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07. Microbiome function and intestinal barrier

P180

DISSECTING NOVEL MECHANISMS UNDERLYING BV RECURRENCE USING A LONGITUDINAL ANALYSIS OF THE FEMALE REPRODUCTIVE TRACT IN WOMEN AFTER BV TREATMENT

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Abstract Content: Background: Bacterial vaginosis (BV) is the most prevalent vaginal condition women worldwide, and increases the risk for gynecologic and reproductive issues, and increased STI and HIV infection. Antibiotic treatment with metronidazole is largely ineffective as up to 80% of women experience recurrence of BV. BV is characterized by a decrease in optimal *Lactobacillus* species, and an increase in polymicrobial anaerobic bacteria, such as *Gardnerella vaginalis,* and is associated with vaginal inflammation. Women with BV have increased IL-1a, IL-8, IL-6 and CCL5 in the vagina, and this increased inflammation is highly associated with increased STIs and HIV infection. Further, BV-associated bacteria such as *G. vaginalis*, can also be found in vaginal microbial populations of healthy women without BV, but it unclear what role this plays in inflammation or reproductive health. Strain level variations may account for the disparities seen between healthy and BV communities with *G. vaginalis*. Currently, specific mechanisms and genotypes of *G. vaginalis* that induce inflammation are unknown, hindering diagnostics and developments of more effective treatments. This study aims to identify the mechanistic role *G. vaginalis* and other BV-associated bacteria play in vaginal inflammation and BV recurrence.
Methods: Here we used samples from an ongoing clinical prospective study of women clinically diagnosed BV through Amsel and Nugent scoring and undergo treatment with metronidazole. Vaginal swabs, cytobrush and cervicovaginal lavage (CVL) was taken at baseline, 1 month, and 6 months after metronidazole treatment. 16S and shotgun sequencing was performed on vaginal swabs at each timepoint. Vaginal cytokine concentrations assessment is underway, including molecules associated with BV such as G-CSF, IFN-γ, IL-1β, IL-6, IL-8, IL-17, MIP-1α, MIP-1β, and TNF-α, CCL5, sICAM, MCP1, and SLPI.
Results and Conclusion: The current study includes 114 patients, 44 of which have completed the 6-month sampling period; 64% of participants had recurrent BV. Vaginal 16S microbiome analysis shows women with BV had more diversity and their microbiome consisted of *Gardnerella, Prevotella, Atopobium, Megaspherea, Sneathia*, and other anaerobic genera. BV negative communities are much less diverse and are comprised of 70% *Lactobacillus* on

average (PERMANOVA: R^2=0.13, p<0.001). While the population of *Gardnerella* was much higher in BV communities, the genus remained the second most abundant in BV negative communities (25% and 10%, respectively). Three *Gardnerella* strains are more differentially abundant in BV+ microbiomes, while one is more differentially abundant in BV- patients. RNaseq and cytokine analyses are currently underway.
These data identify specific *G. vaginalis* strains which may impact BV recurrence after treatment. We will use whole genome shotgun analysis to identify specific genetic differences that may account for the discrepancies between BV- and BV+ communities with *G. vaginalis*. Assessment of cytokine concentrations that co-vary with these genes is underway to identify the relationship between *G. vaginalis* genes, inflammation, and BV recurrence in order to develop more effective therapeutic strategies for BV.

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P181

PRO-INFLAMMATORY RESPONSE TO DIETARY B-FRUCTAN FIBRES IS DRIVEN BY NLRP3 INFLAMMASOME ACTIVATION AND CYTOKINE SECRETION IN INFLAMMATORY BOWEL DISEASE PATIENTS

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Abstract Content: Background: Dietary fibers are not digested in the bowel, they are fermented by microbes, typically promoting gut health. However, IBD patients experience sensitivity to fibers. We have demonstrated that IBD patients often lack specific commensal gut microbes that are essential for fibre fermentation, resulting in an increase in unfermented fibres present in the gut. As leukocytes promote inflammation in response to fungal cell surface fibers, and altered gut microbiome has been associated with IBD, we hypothesized that a loss of fiber-fermenting microbes in IBD could lead to dietary fibers not being efficiently broken down into their beneficial biproducts, resulting in binding of intact fibers to pro-inflammatory host cell receptors.
Methods: Colonic biopsies (IBD and non-IBD) cultured *ex vivo* and cell lines *in vitro* were incubated with select dietary fibres (inulin, oligofructose, pectin, β-glucan; 5mg/mL), and immune responses (cytokine secretion [ELISA/MSD] and expression [RNA array/qPCR]) were assessed. Pathways involved in response to select fibre subtypes were plotted using STRING analysis and Cytoscape software. Validation of NLRP3-inflammasome pathway involvement was determined by treating cultures with NLRP3 (1 uM MCC950; 200uM Glyburide) and caspase-1 (50uM YVAD) inhibitors prior to treatment with fibres, and examining resultant cytokine secretion (ELISA/MSD).
Results: Human cytokine gene arrays of patient biopsies identified broad pro-inflammatory response to inulin and oligofructose, but the magnitude or direction varied depending on IBD disease activity. Distinct gene expression footprints were identified in B cell, T cell, and macrophage cells. Gene targets of interest (IL-1β, CX3CL1, IL-23A, STAT3, NLRP3, IFNλR) were validated by RTqPCR in 35 patient biopsies and various cell lines, demonstrating that pro-inflammatory markers were increased in active IBD patient biopsies in response to inulin and oligofructose compared to non-IBD; macrophages were primarily responsible for driving response. Furthermore, unfermented inulin and oligofructose induced IL-1β, IL-5, and IL-23 secretion in leukocytes and colon biopsies from pediatric Crohn disease (CD; n=38) and ulcerative colitis (UC; n=20), but not non-IBD (n=21) patients. Inhibition of NLRP3-inflamasome mitigated inflammatory pro-inflammatory response.
Conclusions: Our findings suggest that intolerance and avoidance of specific fiber containing foods in select IBD patients is associated with the inability to ferment these fibers, mediated by altered microbial functions (enzymes), leading to worsened inflammation. We further demonstrate the inflammatory response to these fibres was driven through the NLRP-3 inflammasome. Our work highlights select disease state scenarios in which administration of fermentable fibers should be avoided in IBD patients and tailored dietary interventions considered in order to increase consumption of ‘safe’ dietary fibres while reducing risk of diet-induce inflammation.

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P182

INCREASED INFLAMMATION AND GUT BARRIER DAMAGE BIOMARKERS IN HOSPITALIZED PATIENTS WITH SEVERE COVID-19.

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Abstract Content: SARS-CoV-2 is the virus responsible of COVID-19. One of the first lines of defense against viral infections is the cytokine response able to orchestrate an effective inflammatory reaction. COVID-19 causes aggressive release of pro-inflammatory cytokines in an event called “cytokine storm”, which is associated with disease severity. There is no single definition of cytokine storm, and addressing the cytokine release, and mechanisms underlying it, in the context of COVID-19 is crucial to improve therapeutic strategies. We studied cytokine profiles, gut barrier function and microbiome in hospitalized COVID-19 patients to dissect individual’s inflammatory response.
We enrolled 94 hospitalized COVID-19 patients between May and October 2020 in Minnesota, USA their age was between 20 and 88 years old (median 63); 53 male. Seventeen patients deceased, and hospitalization varied between 1 and 65 days (median 7). We cross-sectionally collected plasma, nasal, rectal, oropharyngeal swabs, and clinical information. We measured cytokines in plasma via Simple Plex (IL-1b, IL-12p70, IL-10, IL-2, IL-18, IFN-g, TNA-a, IL-6) and Milliplex (IL-8, IL-23, IL-17A). We quantified gut barrier function measuring I-FAPB, zonulin, sCD14 and LBP. We used Mann-Whitney tests to compare levels of cytokines in survived and deceased patients and in short and long hospitalized individuals. Nucleid acids were extracted from nasal, rectal, and oropharyngeal swabs,16S sequencing was performed on the V3V4 rRNA region and we studied alpha, beta and differential abundance.
Individuals deceased had higher plasma concentrations of IL-6, IL-12, and IL-10 than who survived *(p*=0.0006, *p*=0.004, *p*=0.002 respectively). Similarly, deceased people had higher level of LBP than who survived (*p*=0.01). All patients had higher levels of several markers compared to healthy controls, specifically inflammatory markers IL-18 (*p*=0.04 and *p*=0.002), TNF-a (p=0.002 and *p*=0.003), and zonulin (*p*=0.029 and *p*=0.005). Patients hospitalized the longest (from 13 to 65 days) showed higher plasma concentrations of IL-18, IL-6, TNF-a, IL-2 and zonulin (*p*=0.0036, *p*=0.0001, *p*=0.0017 and *p*=0.0028 respectively) compared to patients with shorter hospitalization times (from 1 to 5 days). Analysis of the 16S microbiome data showed significantly less bacterial richness in the nasal mucosa of patients with longer hospitalization times (*p*=0.015).
Our data provide evidence that hospitalized patients who died from COVID-19 had elevated plasma levels of several pro-inflammatory cytokines (IL-6, IL-12), a regulatory cytokine (IL-10), and biomarkers of gut barrier damage (LBP) than those who survived. In addition, patients hospitalized for longer durations had higher plasma concentration of inflammatory markers (such as IL-18 and TNF-a) and gut barrier damage (zonulin) than individuals with shorter hospital stays. These data suggest that severe COVID-19 is associated with increased systemic inflammation and gut barrier damage. We found significantly less diverse nasal microbiome in patients with severe COVID-19. Interestingly, despite increased gut barrier damage in severe COVID-19, we did not observe any significant differences in the rectal microbiome. Analysis of shotgun sequencing data and longitudinal studies are ongoing to determine cause and effect of these outcomes.

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P183

DIETARY REGULATION OF MICROBE - IMMUNE - STEM CELL INTERACTIONS IN THE INTESTINE

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Abstract Content: While it is becoming increasingly evident that nutrients may directly regulate cell fate and function, little is known about the underlying causal mechanisms. The main challenge we are addressing in the field is the fact

that nutrients vary in types, abundance and patterns of diet, and perturb interconnected modules of cells, cell networks in tissues, organs and organismal states that reciprocally influence one another. Currently there are two paradigms that posit a metabolic code in cell fate and function. The first paradigm is based on the identification of cell state-specific metabolic programs that associate with cell fate and function. The second paradigm is based on the observations that organismal metabolic states or diets influence cell fate and function in tissues and have significant impact on physiology and disease. While these paradigms suggest a key role for metabolism in cell fate and function, underlying causal mechanisms in disease states such as cancer are not well understood. In my lab, we implement a modular and systematic strategy to address these challenges, fill the gaps in existing paradigms and uncover the mechanistic basis for how nutrient metabolism, in particular fatty acid (FA) metabolism, regulates cell fate and function in cancer. Specifically, we study how dietary FAs and their metabolism affect cell fate and function through cell-intrinsic epigenetic mechanisms or by perturbing the interactions between distinct modules of cell networks including stem cells, immune cells and microbiome in the intestine. In this talk, I will highlight our recent work on dissecting how diverse FAs influence the microbiome – immune – stem cell interactions in the intestine by perturbing cytokine signaling and antigen presentation pathways.

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P184

SEX-BASED MICROBIAL ENRICHMENT IN IBD AND IMPLICATIONS FOR MUCOSAL FUNCTION AND CYTOKINES PATTERN

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Abstract Content: Introduction: Gut dysbiosis, permeability defects, and altered mucosal immune responses contribute to inflammatory bowel disease (IBD). Recent evidence suggests that sex-based differences in immune responses and disease penetration are shaped by sex-specific host-microbial interactions at the gut mucosa. Fecal microbiota transplantation (FMT) could theoretically revert dysbiosis in IBD, but its efficacy is debated, and the role of sex as a determinant of its outcome remains unclear. Thus, clarifying whether FMT has a sex-specific impact on pro-inflammatory cytokines in IBD would provide a mechanistic basis for further testing in humans. We tested the hypothesis that sex differences exist in the microbiome of IBD patients and impact on IBD severity and pathogenesis. **Methods:** To determine if sex dimorphism exists in the microbiome of IBD patients, we performed a sex- and disease-balanced nested analysis of fecal samples from 154 female (Fs) and 154 male (Ms) individuals (77 IBD and 77 healthy controls/group; mean age: 36.4±18.1 and 34.2±20.2 y; p=0.308; mean BMI 23.7±5; p=1.000; non-smokers: 70% and 66.7%; p=1.000) to identify any sex-based microbial patterns. Functionality of sex-based differences in microbiome composition was performed by FMT from ileitis-prone SAMP1/YitFc (SAMP) mouse donors raised under specific pathogen-free (SPF) conditions (showing a sex bias towards more severe disease in Fs) into same-sex or opposite-sex SAMP recipients raised under germ-free (GF) conditions, and then challenged with dextran sodium sulfate (DSS) to induce colitis. **Results:** Stool microbiome composition differed between sexes in healthy individuals, but not in IBD patients, mostly due to less alpha diversity in Ms than Fs. Using cutting-edge supervised machine learning, microbiome and age were able to correctly classify sex with a performance of 0.68 in IBD patients and 0.46 in healthy individuals based on AUC-ROC. Intriguingly, age had less relative importance in training the algorithm for sex classification in IBD compared with controls (20.0% vs 46.4%), suggesting that age-related factors, possibly sex hormones shifts in health and disease states, contribute to the findings. FMT experiments in DSS-challenged SAMP revealed significant body weight loss and increased total DAI in DSS colitic GF-SAMP receiving SPF-SAMP fecal microbiota from F compared to M donors, independent of recipient sex. Moreover, at day 26 (1wk recovery), while no differences were observed in M recipients independent of donor sex, increased endoscopic inflammation was found in GF-SAMP F recipients receiving SPF-SAMP F vs. M gut microbiota, suggesting that differences in the gut microbiome may contribute to inflammation at least in SAMP-F recipients. **Conclusion:** The results suggest a sex-specific domain in the gut microbiome in IBD patients distinct from that of healthy controls, with potential functional implications, and will be discussed in detail in addition to resulting

alterations in cytokine profiles and gut immune responses contributing to disease pathogenesis. In summary, the existence of the 'microgenderome' in IBD will allow for further dissection of potential mechanism(s) and future design of therapies based on sex differences.

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P185

CCR2-DEPENDENT MIGRATION OF CX3CR1-HI ANTIGEN PRESENTING CELLS FACILITATES ENTEROCOCCUS FAECALIS DISSEMINATION

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Abstract Content: Migratory antigen presenting cells (APCs) are critical mediators of innate and adaptive immunity in the gut and coordinate healthy commensalism with the residing microbiome. However, intracellular pathogens such as *Salmonella typhimurium* and *Citrobacter rodentium* are known to hijack migratory APCs to facilitate their dissemination throughout the body. This study aimed to explore the role of migratory APCs in the dissemination of a common commensal bacteria, *Enterococcus faecalis* (EF), which is known to cause opportunistic infections in response to antibiotic-induced dysbiosis. We reveal that clodronate-mediated phagocyte depletion resulted in reduced EF burden in the mesenteric lymph nodes (MLNs) of ceftriaxone treated mice, suggesting that migratory APCs may promote EF dissemination. We also confirmed that EF mutants that lack the gene encoding manganese-containing superoxide dismutase (*sodA*) are less adapted to intracellular survival within macrophages *in vitro* and exhibit impaired dissemination in response to ceftriaxone *in vivo*. This suggests that intracellular APC survival may serve as a mechanism by which EF disseminate in response to ceftriaxone treatment. Finally, we identified an APC subset that expresses high levels of C-X3-C Motif Chemokine Receptor 1 (CX3CR1-hi APCs) and uniquely relies on C-C Motif Chemokine Receptor 2 (CCR2) for migration to the MLNs. Chemical antagonism of CCR2 prevented CX3CR1-hi APC migration to the MLNs, and concomitantly reduced EF dissemination. Together, these data suggest that EF survive within migratory CX3CR1-hi APCs that transport EF to the MLNs in a CCR2-dependent manner, thus promoting EF dissemination during antibiotic-induced dysbiosis.

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P186

NASAL MICROBIOME STAPHYLOCOCCUS EPIDERMIDIS LIMITS IL-33 PRODUCTION IN ALLERGIC NASAL MUCOSA BY REGULATING RIPK3-MLKL-MEDIATED NECROPTOSIS

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Abstract Content: Allergic rhinitis (AR) is characterized by airway inflammation by aberrant Th2 responses initiated by inhalant allergen exposure. The epithelial cell-derived cytokine, interleukin(IL)-33 is the key player potent inducer of the allergen-provoked inflammatory process. Recently, the biological value of IL-33 was identified as a novel therapeutic target for AR at the nasal epithelial level. The nasal symbiotic microbiota is well colonized adequate times and may exert immunomodulatory functions that are beneficial to the host. However, the immunomodulatory mechanism of the nasal microbiota on allergic rhinitis has not been fully understood. The purpose of this study was to show the distinctive role of *S. epidermidis*, a major microbiome in nasal mucus, and the potential impact on suppression of T helper (Th)2 inflammation through regulating RIPK3-MLKL-mediated necroptosis and subsequent IL-33 reduction in the nasal mucosa. We investigated the differentially expressed genes (DEGs) through Bulk RNA sequencing to determine *S. epidermidis*-induced genes related to Th2 inflammation in normal human nasal epithelial

cells treated with human nasal *S. epidermidis*. Moreover, we evaluated that human nasal *S. epidermidis* could reduce IL-33 production through suppression of RIPK3/MLKL-dependent necroptosis in AR human nasal epithelial cells. We made an OVA-challenged AR mouse model using BALB/c mice and demonstrated the anti-allergic effect of human nasal *S. epidermidis* administrated intranasally. Our findings indicate that nasal microbiome *S. epidermidis* suppresses Th2 inflammation in AR via the regulation of RIPK3-MLKL-mediated necroptosis and subsequently reduces IL-33 production in the nasal mucosa.

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P187

ROLE OF INTESTINAL FUNGAL COLONIZATION IN SHAPING NEUROLOGICAL RESPONSES

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Abstract Content: Mycobiota, the fungal component in microbiota, has been suggested to play an important role in immune system regulation and disease progression. The change in composition of the mycobiota has been linked to not only gastrointestinal disorders like inflammatory bowel disease (IBD) but also several neurological diseases, such as autism spectrum disorders (ASD), Rett syndrome and schizophrenia. We characterized the compartmentalization of the fungal communities along the gastrointestinal tract and identified a distinct composition associated with either intestinal mucosa or lumen in both laboratory mice and humans. We thus assembled two consortia of mucosa- and lumen-associated fungi and investigated their effect on the mammalian host.

First, we showed that intestinal colonization with mucosa-associated fungi but not lumen-associated fungi protected mice against intestinal injury in a DSS-induced intestinal injury model. We further confirmed that the protection of gut barrier is conferred by type 17 immune response induced by mucosa-associated fungi. Moreover, the immune response is not confined locally but can be detected systemically. By examining the cytokine secretion, the colonization of mucosa-associated fungi enhances the production of IL-17A, IL-17F, and IL-22 in the gut and contributes to increased level of IL-17A in serum.

Considering that the composition of gut fungal communities is altered in neuropsychiatric conditions in humans, we next examined the effect of fungal colonization in a wide range of behavioral tests in mice and showed that mucosa-associated fungi affected mouse behavior via IL-17R-dependent signaling in neurons. We are currently investigating the role for the enteric and central nervous system and if a regional enrichment for neuron activation in the brain dictates the change of behaviors. Taken together, our work suggests the unveiled role of fungal microbiota in regulating mental health and provides the clue of potential treatments.

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INTERFERON-G-MEDIATES ANTIGEN PRESENTATION BY INTESTINAL EPITHELIAL CELLS TO CONFER PATHOGENIC INFLAMMASOME AND GM-CSF RESTRICTING CAPACITY TO T CELLS

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Abstract Content: Gut epithelium, along with a layer of mucus separates the underlying organs from over 10 trillion microbes. Modulation of this barrier is associated with alterations in inflammatory and anti-tumorigenic immunity. However, the mechanisms by which the gut epithelium affects immunity are unclear. Intestinal epithelial cells (IECs) express components of the IFN-g sensing, and the antigen presentation pathway, and SNPs in them are associated with the development of IBD. However, the function of IFN-g sensing or antigen presentation by colonic IECs is

unknown. Here, we show that colonic IECs sense IFN-g and increase antigen presentation during IBD. Antigen presentation by colonic epithelial cells to the cognate CD8 and CD4 intra-epithelial T cells induces in them a protective program characterized by ATPase production. The absence of epithelial IFN-g signaling, MHCI, or MHCII leads to an additive effect on the accumulation of extracellular ATP, that activates the NLRP3 inflammasome. Excessive IL-1b secretion consequent to inflammasome activation induces a pathogenic response in the infiltrating CD4 T cells that is characterized by GM-CSF production that underlies colitis in mouse models and humans. Overall, IFN-g mediated antigen presentation by IECs restrains pathogenic CD4 T cells to mitigate colitis.

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P189

CRISPR SCREENS IDENTIFY INTERFERON REGULATORY FACTOR 6 AS A POTENTIAL REGULATOR OF INTERFERON SIGNALING IN THE INTESTINAL EPITHELIUM

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Abstract Content: Type I IFN (IFN-α/β) and type III IFN (IFN-λ) play cell type-specific roles in mucosal antiviral immunity due partly to preferential expression of the IFN-λ receptor by epithelial cells. For example, infection of intestinal epithelial cells by murine norovirus (MNV) is controlled by IFN-λ whereas MNV infection of macrophages is controlled by IFN-α/β. The goal of this study is to use the MNV model to discover novel roles of signaling factors that differentiate between IFN-α/β responses in macrophages and IFN-λ responses in epithelial cells. To this end, we performed targeted CRISPR knockout screens using MNV-susceptible cell lines of epithelial lineage (M2C) and macrophage lineage (BV2). We designed our CRISPR screens to include gene families that encompass canonical IFN response genes and potential non-canonical family members: nuclear factor kappa B (NF-κB), Janus kinase (JAK), signal transducer and activator of transcription (STAT), and interferon regulatory factor (IRF). First, we knocked out individual candidate genes and screened for IFN-α/β or IFN-λ -stimulated protection from cell death following MNV infection. Additionally, we performed a pooled CRISPR screen for IFN-α/β or IFN-λ -stimulated control of MNV non-structural protein production. Our screens were validated by the presence of canonical genes among the top hits, including STAT1, STAT2, and IRF9. However, beyond canonical genes, both screens suggested that CRISPR knockout of IRF6 resulted in an increased IFN-stimulated antiviral response in intestinal epithelial cells but not macrophages. IRF6 is highly expressed in epithelial cells but has not been reported to regulate the interferon response in the intestinal epithelium. Prior studies of IRF6 have primarily focused on its important role in the development and differentiation of epithelial cells, specifically keratinocytes and oral epithelium, but our study suggests that IRF6 may play a role in intestinal epithelial cells beyond development, through regulation of the IFN-stimulated antiviral response. Follow up studies will define the specific transcriptional contributions of IRF6 to IFN-stimulated immunity of the intestinal epithelium.

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LB-16

COMMENSAL BACTERIA PROMOTE TYPE I INTERFERON SIGNALING TO MAINTAIN IMMUNE TOLERANCE

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Abstract Content: Type I interferons (IFN) play essential roles in numerous physiological processes, acting as central coordinators in the host response against pathogens. Upon sensing of microbial ligands, host cells rapidly activate the type I IFN response to prime innate and adaptive immune responses. Recent studies suggest tonic IFN are maintained by commensal microbes and critical in mounting an effective immune response to viral pathogens. Further,

emerging developments have extended an immunoregulatory role of type I IFN in the maintenance of immune homeostasis. Yet whether immunomodulatory bacteria from the gut microbiota operate through IFN signaling to promote immune tolerance remains largely unanswered. Here we show that commensal microbes are necessary to maintain type I IFN responses in intestinal tissues. Specifically, *Bacteroides fragilis* induce type I IFN response in dendritic cells (DCs), and this pathway is necessary for the induction of IL-10 among Foxp3+ regulatory T cells (Tregs). Upregulation of type I IFN related genes in Tregs from mesenteric lymph nodes and colonic lamina propria of mice colonized with *B. fragilis* are observed during steady-state and experimental colitis. Notably, *B. fragilis*-mediated protection during experimental colitis is abrogated in IFNAR1-deficient mice, revealing type I IFN pathways are critical for the immunomodulatory functions driven by commensal bacteria. Collectively, our findings demonstrate that commensal bacteria play an important role promoting basal type I IFN responses which are required for immune tolerance in the gut.

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08. Host defense against infections

P190

TRANSLATION CONTROL OF INNATE IMMUNITY AND VIRAL ONCOLYSIS.

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Abstract Content: Control of mRNA translation is a definite cellular feature that modulates innate anti-viral immunity. The identity and molecular characteristics of translationally regulated mRNAs functioning in host defense to viral infection is only beginning to be revealed. Discoveries on such elements will provide novel avenues for viral-based therapeutics such as oncolytic virus immunotherapies. We have examined the translatomes of murine breast cancer cells infected with three of the most clinically advanced oncolytic viruses: herpes simplex virus 1, reovirus, and vaccinia virus. In a common fashion, all three viral infections resulted in translationally de-repressed mRNAs. More specifically, we found that viral infection induces the expression of an Inpp5e mRNA variant, encoding an inositol 5-phosphatase, that lacks repressive upstream open reading frames within its 5' leader and is efficiently translated during infection, contributing to antiviral immunity by altering virus attachment. These findings uncover a role for translational control through alternative 5' leader expression and provide additional targets in the understanding of the complex virus-host translation landscape.

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P191

TYPE I AND III INTERFERONS AT THE INTERFACE BETWEEN BACTERIAL PATHOGENS AND THEIR HOST

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Abstract Content: Interferons (IFNs) are host cytokines, produced by both epithelial and immune cells in response to the detection of microbe by host receptors. IFNs are divided into 3 major families: type I IFNs mainly comprised of IFNαs and IFNβ and signal via IFN-α-Receptors (IFNARs), type II IFN only composed of IFNγ and signals via the IFN-▫▫-Receptor (IFNGR), and type III IFNs comprised of IFNλ1-4 and signal via IFN-λ-Receptors (IFNLRs). Upon binding they initiate a janus kinase (JAK)/ signal transducer and activator of transcription (STAT) signaling pathway leading to the expression of hundreds of interferon-stimulated-genes (ISGs).

IFNγ is considered the archetypal anti-bacterial IFN, while type I and type III IFNs have been historically studied for their antiviral properties. Whether type I and type III IFNs also exhibit anti-bacterial function remains poorly understood. Here, we demonstrate that type I and III IFNs protect mice against enteritis induced by the intestinal pathogens *Salmonella* enterica serovar Typhimurium and *Shigella sonnei*. We found an increased replication of *Salmonella*, accompanied by a higher pathological score, in the cecum of *Ifnlr*$^{-/-}$ mice compared to wild-type (WT) mice, suggesting that IFNλ signaling protects the tissue against *Salmonella* infection. Moreover, we found that *Shigella* colonization by a strain that cannot inhibit IFN signaling (defective-*Shigella*), was attenuated in both human colonic epithelial cells and in the cecum and colon of WT mice. In epithelial cells, the growth of defective-*Shigella* was drastically inhibited, a phenotype overcome in cells lacking IFNAR, IFNLR or both receptors. In line with that, *in vivo* colonization of defective-*Shigella* was rescued in *Ifnar*$^{-/-}$ mice, suggesting that type I IFN signaling protects against *Shigella* infection *in vivo*. These findings reveal novel functions of type I and III IFNs in anti-bacterial immunity which will serve our understanding of IFN-bacteria interactions at the gut barrier.

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P192

INTERFERON LAMBDA ALTERS IMMUNE FUNCTION DURING INFLUENZA, BACTERIA SUPER-INFECTION

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Abstract Content: Each year, influenza infections result in a significant number of mortalities, a majority of which are complicated by secondary bacterial super-infection. Primary influenza infection has been shown to increase susceptibility to secondary methicillin-resistant *Staphylococcus aureus* (MRSA) infection by altering the host immune response, leading to heightened mortality rates compared to single influenza or MRSA infection. Macrophages are important in super-infection resolution as they engulf, degrade, and present bacterial antigens to adaptive immune cells, ultimately leading to activation of lymphocytes. While the roles of interferon-(IFN)α/β during super-infection have been well characterized, type III IFNs (IFNλ) have not been as extensively studied. Data shows that IFNα/β are involved in type 17 attenuation after primary influenza infection, which indicates that IFNλ may exhibit similar inhibitory functions due to overlapping signaling pathways. Though the effects of IFNλ on epithelial cells during infection have been previously outlined, but the impacts of IFNλ on immune cells are less clearly defined. We present data supporting an inhibitory role for IFNλ *in vivo*, both by altering macrophage function and dampening type 17 immunity. Global IFNλ receptor knockout mice had lower bacterial burden during super-infection compared to wild type mice with increased levels of IL-17 and IL-22 in the airways and lung tissue. Additionally, bone marrow chimeras performed with wild type and global IFNλ receptor knockout mice and conditional IFNλR knockout mice revealed that targeted disruption of IFNλ signaling in immune cells of the lung leads to reduced bacterial burden during super-infection. Together, these data provide insights into still-emerging roles for IFNλ on lung immune cells, specifically lung macrophages. IFNλ is produced in high quantities during infection and lingers longer than other interferons, making its role during super-infection onset of great potential importance.

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P193

IMPACT OF STAPHYLOCOCCUS AUREUS COLONIZATION OF THE NASAL CAVITY ON THE OUTCOME OF RESPIRATORY VIRAL INFECTION IN THE MOUSE MODEL

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Abstract Content: The effective induction of polyclonal B cell responses is essential for mounting protective immunity against many different pathogens. *Staphylococcus aureus* (*S. aureus*) can colonize the nasal mucosa. However, it remains unclear to which extent nasal colonization affects pathogen-specific B cell responses, and specifically humoral immunity to respiratory viral infections. To first address the impact of *S. aureus* colonization on B cell development, a colony of *S. aureus* positive mice was set up and the neonatally colonized offspring was analyzed. Furthermore, adult mice were inoculated intranasally with mouse adapted *S. aureus*. In both setting, mice were persistently colonized in the nasal cavity, caecum and stool. Of note, *S. aureus* colonized neonates had a more homogenous colonization outcome than colonized adult mice. Multi-dimensional flow cytometry revealed that B cell development in the bone marrow and abundance of mature B cells in peripheral secondary lymphoid organs was not substantially affected by *S. aureus* colonization, irrespective of whether the colonization was achieved neonatally or during adulthood. Nevertheless, minor changes in the B cell subset distribution were noticed in *S. aureus* colonized mice in both the bone marrow and the peripheral secondary lymphoid organs. Next, we plan to address the impact of pre-existing *S. aureus* nasal colonization on the induction of anti-viral immunity.

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P194

LIPID-NANOPARTICLE DELIVERY OF HUMAN MRNAS ENCODING ANTI-VIRAL GENES FOR PROTECTION OF HOST AIRWAY EPITHELIUM DURING INFLUENZA A AND SARS-COV2 INFECTIONS

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Abstract Content: The development of effective mRNA vaccines was recently made possible due to breakthrough developments in mRNA delivery technology. Here, ionizable cationic Lipid-Nano-Particles (LNPs) encapsulate and protect mRNA from enzymatic degradation, and thus allow for passive cellular uptake with subsequent protein expression *in vivo*. Our approach aims to exploit this novel LNP technology in a conceptionally different manner to inhibit virus replication in airway epithelium. We have produced mRNA-LNPs to deliver full length mRNAs that encode human anti-viral gene products to human airway epithelium *in-vitro* and *ex-vivo*. We are currently in the process of determining if this approach can protect against infection with Influenza A virus and SARS-CoV2 *ex vivo* using human airway epithelium and *in vivo* in mice. This new approach could offer a significant breakthrough in anti-viral therapy, and thereby a leap forward in protection against epidemics and pandemics caused by virus.

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P195

EXPLORING THE ASSOCIATION OF IFNL4 VARIANTS WITH THE RISK OF BURKITT LYMPHOMA IN 4000 CHILDREN FROM SUB-SAHARAN AFRICA

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Abstract Content: **Background**: Burkitt lymphoma is a germinal center B cell malignancy linked to Epstein-Barr virus and *Plasmodium falciparum* infections in Sub-Saharan Africa, where the disease is endemic. Interferons are important for clearing viral and parasitic pathogens. The *dG* allele of a genetic variant *IFNL4*-rs368234815, encoding the type III interferon IFNλ4, was associated with impaired hepatitis C viral clearance. The *IFNL4-dG* allele was also associated with increased risk of several childhood infections common in Africa, including malaria. The risk of BL associated with

missense *IFNL4* variants rs117648444-G/A (P70S) and African-specific variant rs142981501-G/C (R60P) has not been studied. Using data from the Epidemiology of Burkitt Lymphoma in East African Children and Minors (EMBLEM) study and the Childhood Infections and Cancer case-control study in Malawi we explored the association of *IFNL4* genetic variants with BL.
Methods: We performed analysis in 4000 children from 4 countries – Uganda, Tanzania, Kenya, and Malawi, including 706 children (0-15 years old) with BL and 3294 age-matched controls. DNA extracted from buffy coat samples for all subjects were genotyped for *IFNL4* variants rs368234815-TT/dG (*IFNL4*), rs117648444 and rs142981501. Hardy-Weinberg equilibrium for these variants was confirmed prior to analysis. Based on genotype combinations (rs368234815 and rs117648444, or rs368234815 and rs142981501) we created IFNL4-P70S and IFNL4-R60P genotype groups, respectively. We evaluated the association between BL and *IFNL4* genetic variants rs368234815-TT/dG and genotype groups by estimating odds ratios (ORs) and 95% confidence intervals (95% CI) using logistic regression, adjusting for age, sex, country, sickle cell status, *P falciparum* positivity measured by microscopy, antigens, or PCR, and population-specific principal components derived from genome-wide analysis. A threshold of $p<0.05$ was used to assess statistical significance without adjustment for multiple comparisons.
Results: In our pooled analysis of 4000 children from 4 East African countries, the distributions of allele and genotype frequencies for the three *IFNL4* markers were in Hardy-Weinberg equilibrium, similar to what is expected for individuals from East Africa per the 1000 Genome project. In models adjusting for relevant covariates, *IFNL4* genetic variants were not statistically associated with BL risk. Specifically, for rs368234815 genotypes, compared to *IFNL4*-TT (no IFNλ4 protein), production of the IFNλ4 protein was not associated with BL risk ($aOR_{TT/dG}$ =1.15 (0.89-1.47); $aOR_{dG/dG}$=1.20 (0.92-1.55); p-trend=0.21). Similarly, we observed that compared to the IFNL4-TT group, IFNL4-P70 groups were not associated with BL risk (OR_{weak}=1.27 (0.83-1.96); $aOR_{moderate}$=1.16 (0.90-1.48); aOR_{strong}=1.17 (0.89-1.53); p-trend= 0.33). The analysis of the African-specific IFNL4-R60P groups is ongoing. Our future analysis will include additional adjustment for potential relatedness between subjects.
Conclusion: In this large study of children from Sub-Saharan Africa, in which we also controlled for local genetic diversity and malaria status, we observed null associations between IFNL4 genetic variants and risk of BL. These results suggest that clearance of viral and parasitic pathogens generally impaired by *INFL4* might not be relevant for BL carcinogenesis.

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HIGH RESOLUTION KINETIC CHARACTERIZATION AND DYNAMIC MATHEMATICAL MODEL OF THE RIG-I SIGNALING PATHWAY AND THE ANTIVIRAL RESPONSES

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Abstract Content: The pattern recognition receptor RIG-I is essential for the recognition of viral dsRNA and the activation of a cell-autonomous antiviral response. Upon stimulation, RIG-I triggers a signaling cascade leading to the expression of cytokines, most prominently type I and III interferons (IFNs). IFNs are secreted and signal in an auto- and paracrine manner to trigger the expression of a large variety of IFN-stimulated genes, which in concert establish an antiviral state of the cell. While the topology of this pathway has been studied quite intensively, the dynamics, particularly of the RIG-I-mediated IFN induction, is much less understood. Here, we employed electroporation-based transfection to synchronously activate the RIG-I signaling pathway, enabling us to characterize the kinetics and dynamics of cell-intrinsic innate immune signaling to virus infections. Using an A549 IFNAR1/IFNLR KO cell line, we were able to analyze the difference between the primary RIG-I signaling phase and the secondary signaling phase downstream of the IFN receptors. We further used our quantitative data to set up and calibrate a comprehensive dynamic mathematical model of the RIG-I and IFN signaling pathway. This model accurately predicts the kinetics of signaling events downstream of dsRNA recognition by RIG-I as well as the feedback and signal amplification by

secreted IFN and JAK/STAT signaling. We have furthermore investigated the impact of various viral immune antagonists on the signaling dynamics experimentally, and we confirmed the utility of our modeling approach to simulate and *in silico* study these critical virus-host interactions. Our work provides a comprehensive insight into the signaling events occurring early upon virus infection and opens up new avenues to study this crucial host-virus interface.

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P197

LOW OXYGEN LEVELS IN THE HUMAN GUT INFLUENCES THE IMMUNE FUNCTIONS OF INTESTINAL EPITHELIAL CELLS

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Abstract Content: Intestinal epithelial cells (IECs) lining the surface of our digestive tract are constantly exposed to both the commensal microbiota and to potential pathogens present in the lumen gut. As a consequence, to achieve homeostasis, IECs must tolerate the presence of these commensals while remaining fully immune responsive against enteric pathogens (e.g. enteric viruses). Within the intestinal epithelium, IECs are organized along a crypt/villi axis. The crypt regions are highly vascularized to provide a local normoxic oxygen environment. On the contrary, the tips of the villi are exposed to the low oxygen environment of the gut lumen (hypoxia) which is critical for the survival of the microbiota. Whether the oxygen gradient present along the crypt-villi axis can influence how IECs detect and fight enteric pathogens remains poorly characterized. In this work we evaluated how low oxygen conditions impacted virus sensing and interferon production. We could show that IECs grown under hypoxic conditions are more sensitive to viral infections compared to cells grown under normoxia. We could demonstrate that this was the result of a reduced efficacy of signal transduction downstream multiple pathogen recognition receptors leading to a severely decreased production of interferon. Under hypoxia we observed a less efficient phosphorylation and nuclear translocation of the transcription factor IRF3 which we could correlate to a decreased phosphorylation of master regulator TBK1. We identified casein kinase II as an upstream signaling component which under hypoxia leads to an impaired activation of TBK1. Together we here demonstrate for the first time that the hypoxic microenvironment of the gut regulates cytokine production. We propose that the oxygen gradient in the crypt-villus axis constitutes a strategy to maintain gut homeostasis: it renders the villi more immune tolerant to antigenic material in the lumen while allowing for full immune competency of the stem cell containing crypts.

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P198

THE DNA SENSING PLATFORM CGAS/STING IS CRITICALLY NEEDED FOR THE CONTROL OF RNA VIRAL ENCEPHALITIS

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Abstract Content: Encephalitis is an inflammatory condition of the brain parenchyma that is caused by various different infectious or immune-mediated factors. Although the syndrome is rare, severe encephalitis can result in

severe neurological manifestations and even death. As therapeutics are still very limited, it is important to find new ways to treat viral encephalitis. During infections, cells sense pathogens by pattern recognition receptors (PRRs). Upon stimulation, PRRs such as cyclic GMP-AMP synthase (cGAS) and its adapter molecule stimulator of interferon genes (STING) initiate downstream signal cascades that result in innate immune responses and protection of the host. Here we addressed whether the cGAS/STING pathway, which has mainly been studied in the context of DNA virus infections, additionally plays a role in the control of RNA virus infections of the CNS. To this end, we intranasally infected wild type (WT), STING deficient and cGAS/STING deficient mice with vesicular stomatitis virus (VSV). Interestingly, WT, STING deficient and cGAS/STING deficient mice mounted similar type l interferon responses (IFN-l) upon VSV infection, indicating that the RNA virus induced IFN-l responses independent of the cGAS/STING axis. Nevertheless, STING deficient mice showed enhanced lethality upon VSV infection when compared with WT mice, whereas cGAS deficient mice showed an intermediate phenotype. Using the ribosomal tagging approach (RiboTag), we identified microglia as the cell type within the olfactory bulb that expresses the most abundant levels of cGAS and STING under homeostatic as well as inflammatory conditions. Furthermore, histological analyses revealed that infected STING and cGAS/STING deficient mice exhibit higher CNS infiltration with myeloid cells than infected WT mice. Overall, our data highlights a critical role of STING for the control of RNA viral encephalitis.

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TYPE I INTERFERON STIMULATES NATURAL KILLER CELL SUPPRESSION OF ANTIVIRAL T CELLS

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Abstract Content: Natural killer (NK) cell suppression of T cells is a key determinant of viral pathogenesis and vaccine efficacy. This immunoregulatory process involves perforin-dependent elimination of a subset of activated CD4 T cells. We recently demonstrated that NK-cell suppression of T cells during lymphocytic choriomeningitis virus (LCMV) infection requires transient CXCR3-dependent relocation of NK cells to T cell-rich regions of secondary lymphoid tissues. This migration of NK cells is dependent on type I interferon (IFN)-dependent upregulation of CXCR3-ligand expression. Lower doses of LCMV or application of viruses (e.g., Adenoviral vector) that trigger weak IFN responses fail to induce either NK-cell migration into the T-cell zone or NK cell-mediated suppression of T-cell responses. Notably, NK-cell migration could be restored with exogenously supplied IFN-alpha. These data highlight fundamental properties of viruses and vaccine that propagate immunosuppressive functions of NK cells. Our ongoing investigations into the essential molecular mechanisms underlying migratory immunosuppressive NK-cell responses are likely to reveal targets for manipulating this activity during vaccination.

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INFLUENZA PATHOGENESIS AND TYPE 3 IMMUNITY IN THE PREGNANT NON-HUMAN PRIMATE MODEL

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Abstract Content: Pregnant women infected with influenza A viruses (IAV) are at higher risk for mortality, severe lung disease, preterm birth and stillbirth. We hypothesized that hormones elevated in pregnancy might impair the Type 3 immune response, which is critical for rapid resolution of IAV infection. We used a nonhuman primate model

(NHP; Macaca nemestrina), to investigate the temporal events in IAV pathogenesis and relationships between lung function, IAV viral load and maternal and placental immune and cytokine response in pregnant and non-pregnant NHP. Sixteen NHPs were allocated into 3 groups: 1) pregnant NHPs (N=6) receiving 7.4 x 106 plaque forming units of IAV H1N1 (A/California/04/2009) by several routes (intra-tracheal, oral, ocular, nasal), 2) adult female NHPs (N=5) inoculated by the same IAV strain, dose, and route, and 3) pregnant NHPs (N=5) receiving a saline inoculation. Peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage (BAL) samples were collected serially with a focus on 3 days post-infection (dpi), a key day in Th17 differentiation, and 5 dpi, the day of peak disease. Animals were humanely euthanized and necropsied to obtain tissues on 5 dpi. We performed RT-qPCR for IAV viral RNA, pulmonary function testing, 37-plex Luminex cytokine assays, immunophenotyping, and intracellular cytokine staining on bronchoalveolar lavage (BAL), lung, blood, amniotic fluid, and other maternal/fetal/placental tissues. Statistical analysis used Kruskall-Wallis. Pulmonary function testing on mechanically ventilated animals revealed that half the pregnant animals showed signs of respiratory failure (PaO2/FiO2 ratio <300) at peak disease. Histopathology revealed pneumonia in all animals, but extrapulmonary disease (heart, brain, spinal cord) only in pregnant NHP. Vertical transmission occurred in half (3/6) of the pregnant IAV group, as IAV viral RNA was detected in fetal tissues. Although the highest viral loads were detected in lungs and BAL, brain tissues of pregnant animals had higher viral loads than non-pregnant animals. Luminex cytokine analysis (37-plex) of lung and placental lysates and amniotic fluid revealed the following significant relationships at peak pathology (5 dpi): 1) lung: IL-12p70, BDNF and FGF2; 2) placenta: IFN-alpha, IL-10, I-TAC (CXCL11), SDF1-alpha (CXCL12), FGF2, VEGF-A; and 3) amniotic fluid: SDF1-alpha (CXCL12), VEGF-A (all $p<0.05$). Flow cytometry and intracellular cytokine staining revealed that Th17 responses (CD3+/CD4+/IL-17+/IL-22+) in PBMC and BAL were lower on 3 dpi, but higher in the lungs on 5 dpi in pregnant vs. non-pregnant NHP. In the paratracheal lymph nodes, Tc17 frequencies (CD3+/CD8+/IL-17+) were higher in pregnant vs. non-pregnant animals at 5 dpi. Notably, Treg (CD3+/CD4+/FoxP3/CD25+/CD107-) frequencies were also blunted in PBMC/BAL at 3 dpi in pregnant vs. non-pregnant but elevated in PBMC/BAL and lungs at 5 dpi. Overall, pregnant NHP had evidence of extrapulmonary disease, maternal-fetal IAV transmission, aberrant Th17/Treg responses and increased proinflammatory cytokines, chemokines, and growth factors in lungs, placenta and amniotic fluid.

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P201

SERUM TRANSCRIPTOMIC ANALYSIS OF HUMAN MONOCYTES UPON EXPOSURE TO ACUTE AND CONVALESCENT SERA FROM LYME DISEASE PATIENTS

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Abstract Content: Background. Lyme disease (LD) is an inflammatory disease in response to *Borrelia burgdorferi* (Bb) infection. It is not yet well understood why certain individuals develop an excessive inflammatory response, even after antibiotic treatment. Monocytes are main phagocytes present in the bloodstream. An enhanced innate immune responses is elicited by human monocytes following phagocytosis of live Bb.
Methods. Using a genomic approach utilized to study inflammation in autoimmune diseases, we exposed THP-1 human monocytes with sera from patients with acute and convalescent LD, as well as from healthy controls *in vitro*, and then performed RNAseq in order to study transcriptome changes by inflammatory mediators present in these sera. Furthermore, we also studied the transcriptome changes in these cells upon stimulation with live Bb. This system will control for the different cytokine-cytokine interactions present in acute LD, convalescence, and health.
Results. Compared to Healthy Control sera, when cells were exposed to Acute LD sera, we observed DE of pro-inflammatory genes including IL1A,I L6, IL7, CD80, TNSF and TNFR families, chemokine genes (CXCL3, CXCL9, CXCL10, CXCL11, CXCL13), endosomal proteins (LAMP3), type I IFN-related genes (IFNB, IRF6), and interferon dependent genes (CCR7, ISG20). In the transcriptome of cells exposed to convalescence sera, some of these genes (CXCL9, CXCL10, CXCL13, LAMP3) did not return to their baseline levels (i.e. similar to healthy controls), and genes related to non-inflammatory state of monocytes (IL13, CD1B).
Discussion. Our findings provide a thorough evaluation of co-regulated genes and pathways elicited in monocytes when exposed to inflammatory mediators present in the bloodstream during acute LD and convalescence. These DE

networks shape the subsequent responses to resolved inflammation from the first response wave to Bb in the majority of cases, or lead to a persistent chronic inflammatory state in tissue in a fraction of patients post-acute LD. Our results help to elaborate the involvement of certain inflammatory pathways during acute disease and convalescence.

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P202

MYD118 ENHANCES RLR SIGNALING BY FACILITATING G3BP-MEDIATED ANTIVIRAL STRESS GRANULE FORMATION

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Abstract Content: Myeloid differentiation primary response protein, MyD118 is a member of the growth arrest and DNA damage-inducible 45 gene family. MyD118 regulates a wide array of pivotal cellular functions including cell cycle regulation etc. Although MyD118 plays multiple regulatory functions in various signaling pathways, its specific role in antiviral immunity has not been studied. In this study, we report that MyD118 is a potent positive regulator of RLR-mediated signaling upon RNA virus infection. Cytoplasmic stress granules (SGs) are an important antiviral signaling hub that activates RLR-mediated signaling. MyD118 physically interacted with G3BPs as stress granule (SG) central node and localized in virus-induced SGs during the early time of infection. This interaction obstructs autoinhibitory electrostatic intramolecular interactions of G3BP and facilitates G3BP1-RNA interaction to stabiliz an oligomeric state of RNA-bound G3BP1. Thereby, MyD118 engender a open conformational transition of G3BP and promote the SG assembly. Ultimately, depletion of MyD118 leads to obstacle of SG formation and inhibition of antiviral responses, resulting in the enhancement of viral replication *in vitro*. Furthermore, MyD118 knockout mice were highly susceptible to RNA virus infection. Taken together, our findings indicate the critical role of MyD118 in the modulation of SG-mediated immune response to viral infection. [NRF of Korea (Grant no. 2019R1A2C2008283 and 2021R1A6A1A03045495) and KRIBB Program (KGM9942011)]

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P203

CELLULAR NUCLEIC-ACID BINDING PROTEIN RESTRICTS SARS-COV-2 BY REGULATING TYPE I IFNS AND DIRECTLY TARGETING VIRAL RNA-NUCLEOCAPSID PROTEIN CONDENSATES

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Abstract Content: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) evades antiviral immunity through the expression of viral proteins that block detection, signaling, interferon (IFN) induction, and IFN-stimulated gene (ISG) expression. Weak induction of type I IFNs is associated with a hyperinflammatory response in patients that develop severe COVID-19. Here we uncover a role for cellular nucleic acid-binding protein (CNBP) in restricting SARS-CoV-2. Typically, CNBP resides in the cytosol and, in response to RNA sensing pathways, undergoes phosphorylation, nuclear translocation, and IFNb enhancer DNA binding to turn on IFNb gene transcription. In SARS-CoV-2-infected cells CNBP coordinates IFNb gene transcription. In addition, CNBP binds SARS-CoV-2 viral RNA directly. CNBP competes with the nucleocapsid (N) protein and prevents viral RNA and nucleocapsid protein from undergoing liquid-liquid phase separation (LLPS) forming condensates critical for viral replication. Consequently, cells and animals lacking CNBP have higher viral loads and CNBP-deficient mice succumb rapidly to infection. Altogether, these findings identify CNBP as a key antiviral factor for SARS-CoV-2, functioning both as a regulator of antiviral IFN gene expression and a cell intrinsic restriction factor that disrupts LLPS to limit viral replication and spread.

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P204

IL-17-PRODUCING CD4 TISSUE-RESIDENT MEMORY T CELLS MEDIATE PROTECTIVE IMMUNITY AGAINST BORDETELLA PERTUSSIS INFECTION IN THE NASAL MUCOSA

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Abstract Content:
Whooping cough (pertussis) is a severe respiratory disease cause by the Gram-negative bacterium *Bordetella pertussis*. Despite high vaccine coverage, a resurgence of disease has been observed over recent years, highlighting the need to better understand the mechanism of protective immunity against *B. pertussis.*
We have demonstrated that CD4 tissue-resident memory T (T_{RM}) cells, a population of memory T cells that express CD69 with or without CD103, accumulate and persist in the nasal mucosa of mice following *B. pertussis* infection. CD4 T_{RM} cells were a major source of IL-17A in the nasal cavity. Furthermore, CD4 T_{RM} cells expanded locally during re-challenge and this was associated with rapid clearance of the secondary infection with *B. pertussis* from the nasal cavity. Protection against primary and secondary infection of the nasal cavity with *B. pertussis* was lost in IL-$17^{-/-}$ mice and in mice depleted of CD4 T cells. Expansion of IL-17A-producing CD4 T_{RM} cells was accompanied by infiltration of a novel neutrophil population expressing Siglec-F, which exhibited high NETosis capacity. Accumulation of Siglec-F^{+} neutrophils was reduced in IL-$17^{-/-}$ mice and mice depleted of CD4 cells. CXCL1, a chemokine required for neutrophil recruitment, was decreased in the nasal cavity of IL-$17^{-/-}$ mice, whereas intranasal IL-17A administration induced CXCL1 production in the nasal cavity. Furthermore, depletion of neutrophils significantly curtailed bacterial clearance from nasal cavity during primary or secondary infection with *B. pertussis*.
This study also revealed that respiratory T_{RM} cells induced by natural infection or immunisation with a whole cell pertussis (wP) vaccine can produce IL-17A in absence of TCR activation. We found that TRM cells could be activated directly by the cytokines IL-23 with IL-1β or IL-18 or when cultured with innate immune cells stimulated with PAMPs or the unrelated pathogen *Klebsiella pneumoniae*. Furthermore, immunisation of mice with a wP vaccine conferred non-specific protection against *K. pneumoniae* infection of the nasal cavity. These findings demonstrated that CD4 T_{RM} cells are a major source of protective IL-17A in the nasal cavity and can be maintained by non-specific activation, probably by unrelated pathogens. Immunization approaches that induce T_{RM} cells should be considered in the development of next generation pertussis vaccines that are designed to prevent transmission of *B. pertussis* in humans.

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P205

HOST-MYCOBIOME INTERACTIONS PROMOTE MACROPHAGE-MEDIATED INNATE LYMPHOID CELLS TYPE 3 ACTIVATION DURING THE RESOLUTION OF INFLAMMATION IN EXPERIMENTAL COLITIS

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Abstract Content: Recent studies have documented the complexity of the intestinal fungal community (‘mycobiome’) in mice, and clinical and experimental observations have shown that the mycobiome influences both gut health and disease, e.g., inflammatory bowel disease (IBD). In fact, prior studies have shown that Crohn’s disease (CD) patients, compared to healthy controls, harbor higher levels of intestinal *Candida tropicalis* (*Ct*), which is the major fungal species detected in the colons of colitic mice after DSS challenge. Moreover, proteins encoded by genes within IBD susceptibility loci, such as the pattern recognition receptor (PRR), NOD2, are known not only to recognize bacterial

components, but also a fungal cell wall element, chitin. In fact, the role of PRRs in regulating immunity against intestinal fungi, and how fungi influence IBD remains poorly defined. We challenged DSS colitic WT and *Nod2*$^{-/-}$ mice with *Ct* two days before DSS administration and subsequently on day 0, 3 and 6. Our data confirms previous studies that *Ct* challenge does not exacerbate colitis in DSS-treated C57BL/6 wildtype (WT) mice, however, *Ct*-infected *Nod2*$^{-/-}$ mice possess a higher fungal burden and exhibit worse colitis symptoms, such as weight loss, decreased stool consistency, and presence of blood in stools, vs. *Ct*-infected WT mice, indicating an essential and protective role for NOD2 during colitis recovery after *Ct* challenge. These data were confirmed by colonoscopy, colon length (decreased length=increased colitis severity), and histologic assessment of inflammation. When we infected WT mice with a non-IBD related fungal strain, *S. fibuligera,* following the protocolpreviously described, the recovery rate of mice infected with *S. fibuligera* showed the same trend as non-infected colitic WT mice, further suggesting that effects of NOD2 are specific to *Ct* and functional NOD2 is essential for clearance of *Ct.* Our results also show that *Ct*-infected *Nod2*$^{-/-}$ mice display a marked reduction in colonic *Il22* and *Il17*, cytokines important in maintaining epithelial barrier integrity during DSS colitis. These data were confirmed in colons of *Ct* infected DSS challenged, ileitis prone SAMP1/YitFc (SAMP) mice that were deficient in NOD2. Moreover, our *in vitro* data show, a decrease in *Il1b* and *Il23* in bone marrow-derived macrophages from SAMP *Nod2*$^{-/-}$ mice compared to WT after 2h of exposure to chitin. IL-17^{+} innate lymphoid cells (ILCs) are also known to control fungal burden during opportunistic fungal infections, in fact, our findings indicate a decrease in IL-17 production by ILCs in lamina propria in *Nod2*$^{-/-}$ vs WT mice, and even more interestingly, they show that *Ct* infection of *Nod2*$^{-/-}$ vs. WT mice results in a decreased frequency of mesenteric lymph node–derived type 3 ILCs (ILC3s), suggesting that delay in fungal clearance and recovery in *Nod2*$^{-/-}$mice may be due to the inability to mount protective type 3 immune responses. Taken together, our data suggests that NOD2 is essential to maintain gut mycobiome homeostasis and drives protective innate immune responses, via a macrophage-mediated ILC3s recruitment and IL-17 mechanism, by preventing the overgrowth of opportunistic fungi that may contribute to chronic intestinal inflammation, such as that observed in CD.

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P206

INDUCTION OF POLYFUNCTIONAL RESIDENT-MEMORY T CELLS IN THE NASAL AND LUNG MUCOSA OF INTRANASALLY IMMUNIZED ANIMALS WITH SPIKE PROTEIN COMBINED WITH A NOVEL ADJUVANT.

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Abstract Content: The first generation of COVID-19 vaccines represents a significant breakthrough in the fight against COVID-19 and has saved more than half a million lives in Europe. These first generation COVID-19 vaccines effectively prevent severe COVID-19 disease, but poorly protect against SARS-CoV-2 infection, especially with variants of concern. Furthermore, immunity wanes rapidly after Immunization, even after 2 or 3 doses of the vaccines. There is a need to develop new vaccine approaches that protect against SARS-CoV-2 infection of nasal mucosa as well as the lungs and that induce more potent immunological memory. Tissue-resident-memory T cells (T_{RM}) play a key role in long-term protection against infection of mucosal tissues.
We have assessed the immunogenicity and protective efficacy of a candidate COVID-19 vaccine comprising a recombinant SARS-CoV-2 spike trimer (S) formulated with the novel adjuvant, LP-GMP, which combines TLR2 and STING agonists. We found that intranasal (i.n.) immunization of mice with this experimental vaccine generated S-specific IgG and neutralizing antibodies in the serum, lungs and nose. It also induced CD4 and CD8 T_{RM} cells in the lung and nasal tissue and these cells produced Th1 and Th17-type cytokines, including IFN-γ, IL-17, and TNF-α, following antigen stimulation in vitro. Interestingly, a substantial proportion of these T_{RM} cells also secrete granzyme B, a marker of cytotoxic activity. Intramuscular (i.m.) immunization with the same vaccine induced potent IgG and virus neutralizing antibodies as well as systemic antigen-specific T cells that produced INF-γ and granzyme B, but did not induce T_{RM} cells in the mucosal tissues.
Using transgenic mice expressing human ACE2, we found two immunizations with our candidate vaccine comprising the S protein and LP-GMP delivered by either the i.m. or i.n. route conferred 100% protection against a lethal challenge

with SARS-CoV-2. Furthermore, the vaccine induced sterilizing immunity against infection of the lung and nose with SARS-CoV-2. Thus, while our candidate COVID-19 vaccine protected against SARS-Cov-2 infection when delivered by either parenteral or mucosal routes, only the i.n. route of immunization induces T_{RM} cells. The persistence of these polyfunctional antigen-specific tissue-resident T cells could be the key to long-term protective immunity against SARS-COV-2 infection of the upper and lower respiratory tract.

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P207

INHIBITION OF SALMONELLA ENTERICA INFECTION BY INTERFERON-STIMULATED GENES.

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Abstract Content: *Salmonella enterica* serovar Typhimurium is an intracellular pathogen that causes food- and water-borne gastroenteritis. Although in humans it usually causes a self-limiting infection, diarrhoeal *Salmonella* disease presents a huge global burden on human health. Recognition of the pathogen by the host immune system results in the production of cytokines, such as interferons (IFNs). IFNs act in an autocrine and paracrine manner to induce the expression of hundreds of interferon-stimulated genes (ISGs). As they are expressed in concert, the individual roles of many ISGs are not well understood. Furthermore, as the study of IFNs has predominantly focussed on their role during viral infection, even less is known about their effect during bacterial infections. To address this, *S.* Typhimurium infections of epithelial cells were performed following treatment with IFNs. Our data reveals that type I IFN can prevent the invasion of *S.* Typhimurium into epithelial cells and may also inhibit intracellular replication. Using a fluorescent reporter, *Salmonella* INtracellular Analyzer (SINA), and flow cytometry we have determined that treatment of epithelial cells with IFN can also alter the subcellular niche occupied by internalised bacteria. Finally, a comprehensive screen of ISGs has been carried out to identify which are able to influence *S.* Typhimurium infections of epithelial cells. This will enable us to better understand how IFNs and ISGs restrict bacterial invasion into, and replication within, host epithelial cells.

Disclosure of Interest: None Declared
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P208

SPECIES-SPECIFIC REGULATION OF HOST CELL DEATH BY VIRUS-ENCODED PROTEASES

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Abstract Content: Activation of programmed cell death during virus infection is both an antiviral mechanism and a cause for severe disease. Viruses can induce non-inflammatory or inflammatory cell death, which can result in cytokine release, immune activation, and immunopathology. However, the mechanisms driving viral and host specificity of cell death activation and antagonism are incompletely understood. Positive-sense single stranded RNA viruses, such as picornaviruses, encode proteases that are both required for the virus life cycle and are known to cleave host proteins. The goal of our research is to identify cell death-associated host targets of picornavirus proteases, determine the virus- and host-species specificity of cleavage, and determine the effect of protease-mediated cleavage on cell fate and activation of antiviral immune responses. Using an evolution-guided approach, we have identified a number of cell death-associated proteins that are targets for picornavirus proteases, including proteins involved in the highly inflammatory programmed necrotic cell death, or necroptosis, pathway. Interestingly, necroptosis-associated proteins are cleaved by picornaviral proteases in both a host-specific and virus-specific

manner, suggestive of a host-virus evolutionary arms race. Ongoing research aims to characterize the function of diverse picornavirus proteases in cell death manipulation across a range of host species, determine how cell death is regulated during infection, and identify how distinct modes of cell death contribute more broadly to the antiviral immune response. Altogether, this work will deepen our understanding of the regulation and consequences of programmed cell death during virus infection.

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P209

INTERFERON-INDUCIBLE GUANYLATE-BINDING PROTEINS CONTRIBUTE TO BACTERIAL SPECIFIC RECOGNITION AND HOST INFLAMMATION

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Abstract Content: Interferon-induced guanylate-binding proteins (GBPs) orchestrate inflammasome responses to bacterial infection. The inflammasome is an innate-immune signaling complex which is responsible for the release of interleukin (IL)-1 family cytokines including IL-1beta and IL-18 in response to host recognition of the pathogen. Certain GBPs are recruited to the bacterial membrane to facilitate inflammasome activation, however, whether GBPs can recognize specific bacteria and disrupt bacterial membrane integrity remains unclear. We used biochemical, molecular and imaging approaches to examine the molecular mechanisms of how GBPs facilitate bacteria-induced inflammasome activation. We showed that GBPs mediate pathogen-selective inflammasome activation. We found that GBP1 and GBP3 are specifically required for the release of IL-1beta and IL-18 during infection with the cytosolic bacterium *Francisella novicida*. We showed that the selectivity of GBP1 and GBP3 derives from a region within the N-terminal domain containing charged and hydrophobic amino acids, which binds to and facilitates direct killing of *Francisella novicida* but not other bacteria or mammalian cells. The pathogen-selective recognition by this region of GBP1 and GBP3 induced pathogen-membrane rupture and release of intracellular content for inflammasome sensing. Furthermore, GBP1 and GBP3 protected mice from *F. novicida* infection by restricting bacterial replication and promoting IL-18 production. Overall, our results indicate that intracellular sensing of specific pathogens by GBPs is critical for the innate immune recognition and clearance of infection.

Disclosure of Interest: None Declared
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P210

INTERFERONS PLAY A CRITICAL ROLE IN THE NATURAL IMMUNITY OF BABOONS AGAINST INFECTION WITH SIV

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Abstract Content: In natural nonhuman primate (NHP) hosts, such as African green monkeys and sooty mangabeys, SIV infection is nonpathogenic. However, in non-natural hosts such as rhesus macaques, SIV often leads to simian AIDS. Baboons represent a rarity since they widely distributed African NHPs that do not naturally harbor SIV, yet their CD4+ cells are readily infectable with SIV *in vitro*.
The mechanisms of baboon natural immunity against SIV remain unknown, but their elucidation could uncover new antiviral therapeutics. We performed transcriptomic analysis of sorted lymphocytes from baboons experimentally infected with SIV and show that infected animals upregulate interferon-stimulated genes and restriction factors such

as BST-2, but there is a concomitant marked downregulation of transcription factors in CD4 T cells. We investigated whether CD8+ cells contribute significantly to SIV restriction in two additional cohorts of four baboons each. Cohort 1 (C1) received an anti-CD8α antibody which depleted CD8+ cells. Cohort 2 (C2) received a control antibody. Following CD8+ cell depletion in C1, both cohorts were challenged with SIVmac251. C1 plasma SIV loads were significantly higher than C2 and similar to acutely infected rhesus macaques; however, PBMC proviral DNA were comparable between groups very early after infection. As CD8+ cells reappeared in C1, viral loads for both cohorts converged. While C2 baboons only experienced a slight peak of IFN-a expression at peak viremia, C1 baboons showed monocytosis, and increases in Type I and II IFN, and Type II IFN-related cytokines. We identified B cells in C1 baboons as significant producers of IFN-g, suggesting that B cells compensate for the lack of CD8+ IFN-g producing cells. As CD8+ cells returned to circulation, SIV-specific cell-mediated immune responses were stronger in C1 than C2, whereas there was no difference in humoral response between the two cohorts. Thus, in the presence of CD8 cells, interferon expression is associated with upregulation of a subset of antiviral genes in the absence of cell activation and inflammatory cytokines.
The lack of CD4 T cell activation and loss after infection, detectable antiviral immune responses, and importance of CD8 cells for durable control of viremia observed in baboons are findings also identified in HIV elite controllers (EC). EC represent less than 0.5% of all HIV-infected people, which makes them a small population in which to study mechanisms of natural control of infection. Baboons are bred for research at several institutions and may represent a potential model of EC in which to identify molecular mechanism and interventional therapies against HIV infection.

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P211

TLR-DEPENDENT PRIMING LICENSES THE MURINE NAIP-NLRC4 INFLAMMASOME TO SENSE IMMUNE EVASIVE BACTERIAL LIGANDS

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Abstract Content: Pattern recognition receptors detect broadly conserved pathogen associated molecular patterns (PAMPs) to initiate innate inflammatory responses that combat microbial infection. Pathogens like *Salmonella enterica* serovar Typhimurium (*S.* Tm) and Enteropathogenic *Escherichia coli* (EPEC) inject the flagellar motility organelle protein, flagellin, as well as structurally related type III secretion system (T3SS) structural proteins into the host cell cytosol, where they are detected by neuronal apoptosis inhibitory proteins (NAIPs). In murine cells, sensing of cytosolic flagellin, T3SS needle or T3SS rod proteins by specific NAIPs leads to assembly of a multiprotein inflammasome complex containing the nucleotide-binding domain, leucine-rich repeat-containing protein (NLR) caspase recruitment domain protein 4 (NLRC4), which activates caspase-1 to trigger IL-1 family cytokine release and inflammatory cell death known as pyroptosis. While the NAIP-NLRC4 inflammasome is a potent activator of inflammatory responses that are important for immune defense against infection, some pathogens are thought to evade NAIP-NLRC4 inflammasome detection, as EPEC flagellin and components of *S.* Tm T3SS encoded by the *S.* Tm pathogenicity island 2 (SPI-2) are thought to not activate the murine NAIP-NLRC4 inflammasome. In macrophages, NLRC4 is basally expressed, in contrast to some NAIPs or other inflammasomes, and its activation is therefore not known to be regulated at the level of NLRC4 protein expression. Here, we demonstrate that TLR priming enables murine bone marrow derived macrophages to detect immune evasive NAIP ligands, including EPEC flagellin and *S*. Tm SPI-2 T3SS structural components, suggesting that priming licenses the murine NAIP-NLRC4 inflammasome to sense bacterial PAMPs that otherwise evade detection. TLR priming of murine cells modestly increased expression of NLRC4, and ectopic expression of NLRC4 in immortalized macrophages induced pyroptosis in macrophages in response to NAIP ligands that otherwise evade detection. Altogether, our data suggest that TLR priming overcomes pathogen evasion of the NAIP-NLRC4 inflammasome by increasing availability of NLRC4 for inflammasome assembly, and provides new insight into how the innate immune system can overcome pathogen evasion strategies.

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P212

INTERFERON-LAMBDA SIGNALING REGULATES DISEASE SEVERITY AND CD8 T CELL IMMUNITY IN A MURINE MODEL OF SARS-COV-2 INFECTION

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Abstract Content: Interferon-lambda (IFN-λ) is under investigation as a human therapeutic against severe COVID-19 in clinical trials. Exogenous IFN-λ restricts SARS-CoV-2 *in vitro* and in Balb/c and C57Bl/6 (WT) murine models of infection. However, roles of endogenously produced IFN-λ in SARS-CoV-2 pathogenesis are not currently known, and the overall mechanisms by which IFN-λ modulates the induction of protective immune responses in SARS-CoV-2 infections remains to be elucidated. We find that IFN-λ receptor deficient mice (*Ifnlr1*$^{-/-}$) infected with mouse-adapted SARS-CoV-2 lose significantly more weight and have increased SARS-CoV-2 viral replication compared to WT through day 5 post infection. Intriguingly, ISG and IFN mRNA levels are not altered in the lungs of *Ifnlr1*$^{-/-}$ mice compared to WT following infection, suggesting compensatory increases in type I IFN signaling are not driving the increased weight loss or enhanced titers observed in *Ifnlr1*$^{-/-}$ mice. Global transcriptomics reveals differential regulation of several pathways involved in immune cell activation and function in *Ifnlr1*$^{-/-}$ lungs compared to WT during infection, suggesting IFN-λ critically regulates appropriate immune activation to limit SARS-CoV-2 pathogenesis. Flow cytometric analysis on day 8 post infection revealed a significant reduction in SARS-CoV-2-specific CD8 T cells in *Ifnlr1*$^{-/-}$ compared to WT, demonstrating IFN-λ signaling is critical for regulating protective T cell immunity against SARS-CoV-2. Ongoing studies are working to understand the mechanisms by which IFN-λ regulates virus-specific CD8 T cell immunity. Overall, broadening the understanding of how IFN-λ regulates SARS-CoV-2 infection, pathogenesis, and immunity will inform the utilization of IFN-λ as an immunotherapy and adjuvant.

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P213

DEFINING THE HOST AND BACTERIAL FACTORS RESPONSIBLE FOR CASPASE-1/11-INDEPENDENT CELL DEATH DURING INFECTION BY SALMONELLA ENTERITIDIS

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Abstract Content: *Salmonella enterica* is the third leading cause of death from food-transmitted diseases globally. *S. enterica* comprises over 2500 serovars which cause disease ranging from self-limiting gastroenteritis, asymptomatic carriage, and severe systemic infection. Despite wide diversity of these serovars in host-specificity, pathogenesis, and epidemiology, our understanding of the protective immune response to *Salmonella* relies on *S. enterica* serovars Typhimurium (*S*Tm) and Typhi, more specifically, a single strain of *S*Tm, SL1344. Meanwhile, *S. enterica* serovar Enteritidis has replaced Typhimurium the most common cause of salmonellosis in both the U.S. and Europe. *Despite its prominence, there are few studies on the innate immune response to SE***.** *Salmonella* utilize macrophages to replicate, disseminate, and persist within the host. Intracellularly *Salmonella* use a type III secretion system encoded by *Salmonella* Pathogenicity Island 2 (SPI-2), which injects virulence factors, known as 'effectors', into the host cytosol. These effectors manipulate the host environment and permit *Salmonella* to maintain its intracellular niche. Cytosolic innate immune sensors detect bacterial ligands, or the activity of effectors within the cytosol, which together can activate the inflammatory caspases -1 and -11 (Casp1/11). Casp1/11 are necessary for late macrophage cell death, termed pyroptosis, in response to commonly studied strains of *S.* Typhimurium. However, my studies show for the first time that multiple *S. enterica* clinical isolates of different serovars, including *S.* Enteritidis,

trigger Casp1/11-independent Casp-8 dependent cell death. My studies suggest that *S.* Enteritidis as well as other Typhimurium strains, harbor a SPI-2 factor which triggers Casp8 apoptosis. My data reveal a previously undefined pathway of apoptotic cell death distinct from the current paradigm of *Salmonella*-induced pyroptosis.

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P214

TRANSIENT DENDRITIC CELL ACTIVATION DIVERSIFIES THE T CELL RESPONSE TO ACUTE INFECTION

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Abstract Content: The precise timing of T cell priming during infection remains unclear. Here, we mapped the cellular dynamics of all immune lineages during acute infection with Listeria monocytogenes (Lm) using mass cytometry. We identified highly transient dendritic cell (DC) activation that functions as a critical temporal checkpoint for T cell fate decisions. Antigen-specific T cells that arrive late to the site of priming and miss peak DC activation acquire only memory T cell fates. Furthermore, this transient state was mediated by DC extrinsic IFNγ provided by lymphocytes. This temporal regulation of fate is recapitulated by CD8+ DCs ex vivo, suggesting that shifts in activation state of a single antigen presenting cell population alter T cell fates. These results uncover a novel mechanism for temporal regulation of T cell differentiation during a dynamic immune response to acute infection.

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P215

EXPRESSION OF P38B/MAPK11 DECREASES PRO-INFLAMMATORY CYTOKINE MRNA LEVELS AND TYPE I INTERFERON ACTIVITY DURING SARS-COV-2 INFECTION OF HUMAN LUNG EPITHELIAL CELLS

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Abstract Content: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease 2019 (COVID-19) pandemic, has caused millions of deaths in the human population since its emergence in late 2019. One hallmark of COVID-19 is the significant induction of pro-inflammatory cytokines such as interleukin-6 (IL-6), which is regulated in part by the mitogen-activated protein kinase (MAPK) cascade that mediates signals downstream of cytokine and pattern recognition receptors. The p38 family of MAPKs are among the most highly active kinases during SARS-CoV-2 infection and we have previously shown that chemical inhibition of p38 kinases in SARS-CoV-2-infected cells reduces the abundance of both IL-6 and viral mRNAs.
In order to better understand interactions between the p38 pathway and SARS-CoV-2, we performed an siRNA screen of p38/MAPK pathway components in A549 lung epithelial cells expressing the SARS-CoV-2 host receptor ACE2. We identified p38β/MAPK11, the least characterized of the four p38 isoforms, as an essential host factor for SARS-CoV-2 replication. Additionally, siRNA-mediated inhibition of p38β during SARS-CoV-2 infection results in a significant increase in pro-inflammatory cytokine mRNAs such as *tnf* and *il6,* as well as type I interferon activity. However, genetic or chemical inhibition of interferon signaling in p38β-depleted, infected cells does not improve infection levels, suggesting that the primary mechanism in which p38β inhibition prevents viral replication can be independent of interferon-mediated signaling. In parallel, we performed quantitative, unbiased phosphoproteomics analysis of SARS-CoV-2-infected cells treated with the p38 chemical inhibitor, SB203580. We identified four residues on the N-terminus of SARS-CoV-2 nucleocapsid (N) that are sensitive to SB203580. To test the significance of phosphorylation of these p38-dependent N residues, we introduced phosphoablative mutations to recombinant SARS-CoV-2. We observed that the growth of the resulting mutant virus was significantly attenuated compared to recombinant wild-type SARS-CoV-2 and induced a stronger type I interferon response. These findings indicate p38 isoforms beyond the canonical

p38α isoform need to be further characterized to more comprehensively understand cytokine-inducing signal transduction, and p38β is a promising target for antiviral drug therapies greatly needed for the treatment of COVID-19.

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P216

NRF2-DEPENDENT SILENT IMMUNITY INHIBITS SARS-COV2 AND INFLUENZA A VIRUS IN HUMAN AIRWAY EPITHELIUM

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Abstract Content: The transcription factor NRF2 has recently emerged in non-canonical anti-viral immunity. We used Crispr-activation to map the anti-viral network controlled by NRF2 effective against SARS-COV2 and Influenza A virus (IAV) - two important human pathogenic viruses. By this approach we identified a network of more than 40 NRF2-inducible genes with anti-viral activity. Several of these genes demonstrated broad inhibition of both SARS-CoV2 and IAV while other genes demonstrated specificity towards SARS-CoV2. Because anti-viral NRF2-inducible genes restrict infection in a non-inflammatory manner we label this network as being silent; – hence the term *silent immunity*. In support of our hypothesis that this network is an important part of human immunity we identified loss-of-function mutations in several NRF2-dependent *silent immunity* genes in cohorts of severe COVID-19 and of severe IAV. In conclusion, NRF2 controls an unappreciated network of non-inflammatory anti-viral genes in the human airway epithelium.

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P217

REPLICATION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN MACROPHAGES IS MODULATED BY VIRAL RNA STRUCTURE

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Abstract Content: Alphaviruses are positive sense RNA arthropod-borne viruses that cause serious disease in humans and animals. Venezuelan equine encephalitis virus (VEEV) is a neurotropic virus which causes acute encephalitis in equines and humans. Attenuated VEEV subtypes (ID, IE, II-VI) are transmitted between rodent reservoir hosts and mosquito vectors and are mostly avirulent in equines, producing only low-level viremia with little to no disease. In contrast, pathogenic VEEV subtypes (IAB and IC) are associated with increased viremia and transmission in equines and are the cause of major epidemics. We have previously shown that 5' and 3' untranslated region (UTR) RNA structures that differ amongst pathogenic and attenuated VEEV subtypes facilitate evasion of the host interferon (IFN) response. We hypothesize that additional RNA structure contributes to evasion of host IFN responses and facilitates emergence of pathogenic VEEV. To identify additional novel RNA structures important for VEEV emergence we compared the predicted structure of a pair of pathogenic and attenuated strains and identified several regions of high structural diversity throughout the genome, including within the structural ORF. We generated chimeric viruses encoding only synonymous changes in this region corresponding to the attenuated strain and compared replication kinetics in mosquito and vertebrate cell lines. We observed that chimeric viruses encoding RNA structures from attenuated subtypes were more resistant to RIG-I and MAVS dependent antiviral responses, but not MDA-5. We also observed that changes in RNA structure specifically modulated replication in macrophages but not dendritic cells. Myeloid cells are early targets of VEEV infection in vivo and recent studies have shown that a subset of macrophages are the source of IFN production, which determines pathogenic outcomes in vivo. This data provides

important mechanistic insight into how RNA structure alters host innate immune responses during VEEV infection to affect the outcome of pathogenesis.

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P218

REGULATING BASELINE ACTIVATION OF THE IFN-I RESPONSE

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Abstract Content: The induction of Type I Interferons (IFN-Is) and their subsequent antiviral state depends on the coordinated action of two major transcriptional complexes. While these have been studied in great detail, increasing evidence has emerged demonstrating the role of additional transcription factors (TFs) in fine-tuning and honing the antiviral IFN-I response. To understand the broad array of TFs involved in the coordination of the antiviral interferon response, we first generated cell lines that produce a secreted luciferase from the genomic locus of the interferon-stimulated gene (ISG) MX1. We then used these cells to screen a library of synthetic guide RNAs (sgRNAs) which targeted ~1600 transcription factors and transcriptional regulators to identify novel modulators of the interfeorn response (measured by induction of luciferase from the MX-1 locus). In addition to the known required factors - IRF3, STAT1, STAT2, and IRF9 - our screen identifed 80 other candidate molecules involved in modulating the interferon response. Of these, 9 were important for control of interferon activation in the absence of stimuli. Follow-up studies have identified the pattern recognition receptor (PRR) pathways required for and potential self-ligands that drive induction of the interferon response in the absence of these proteins. Future research will focus on the molecular mechanism by which these inflammatory self-ligands are produced and how the identfied proteins regulate the production of these self-ligands.

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P219

INTRANASAL ADMINISTRATION OF ADENOVIRUS-VECTORED VACCINE PROTECTS MICE AGAINST SARS-COV-2 INFECTION

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Abstract Content: The global pandemic of COVID-19, caused by SARS-CoV-2 infection, has affected every aspect of life and increased health and socio-economic burdens. Several vaccines have been developed to prevent the infection of SARS-CoV-2. Yet, the accumulation of mutations within the viral genome results in de novo generation of variants that reduce the effectiveness of vaccines. Further, the persistence of protection induced by vaccine becomes an issue. The purpose of this project is to develop preventative vaccines against SARS-CoV-2 infection. We developed recombinant COVID-19 vaccines using adenoviral vector platform, and tested the immunogenicity of these vaccines in mice. We found that recombinant adenoviral vaccines encoding the modified-spike antigen efficiently induced humoral and cellular immune responses in vaccinated mice, and protected hosts from lethal SARS-CoV-2 infection. We further revealed that systemic and mucosal immune responses were elicited following intranasal administration of recombinant vaccine. These results indicate that our recombinant SARS-CoV-2 vaccine has the potential to confer protective immunity against SARS-CoV-2 infection.

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P220

MECHANISMS REGULATING GRANULOMA DEVELOPMENT AND HETEROGENEITY IN HIGH- AND LOW-TRANSMISSION MYCOBACTERIUM TUBERCULOSIS INFECTIONS

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Abstract Content: The tremendous global burden of Tuberculosis (TB) is sustained due to the continued cycle of *Mycobacterium tuberculosis* (Mtb) transmission and infection. Complex host immune mechanisms induced by Mtb lead to granuloma formation, the histopathological hallmark of TB. However, the host and bacterial mechanisms that dictate Mtb containment within granulomas and those that subsequently induce granuloma necrosis and subsequent Mtb transmission remain poorly understood. In a household contact (HHC) study of TB cases from Brazil, Mtb strains were isolated from the index cases of households and categorized into High transmission (Mtb-HT) and Low transmission (Mtb-LT) based on the number of Tuberculin skin test-positive HHCs in the household. To investigate the host-pathogen interactions underlying Mtb transmission heterogeneity, these strains were studied in C3HeB/FeJ mice that develop caseating necrotic lesions as observed in human TB. We previously reported that mice infected with the Mtb-HT strain develop discrete caseating granulomas. In these mice, rapid IL-1R-dependent alveolar macrophage migration from the alveolar space into the interstitium led to early dissemination of bacteria to the lymph nodes and TH1 cell priming. In contrast, the alveolar macrophage migration and early bacterial dissemination was significantly impeded in Mtb-LT infection, promoting a TH17 cell response, diffused inflammatory lung pathology, and significantly increased bacterial burden. To further examine the lung immune cell populations that shape the distinct immune responses in Mtb-HT and Mtb-LT infections, we conducted single cell RNA-sequencing of lung cell infiltrates at 2-, 4- and 6-weeks post-infection. Of note, there was a striking increase in the neutrophil cell cluster by weeks 4 and 6 post-infection in the Mtb-LT-infected mice. Transcriptomic analysis demonstrated that in comparison to Mtb-HT-infected mice, neutrophils from Mtb-LT-infected mice upregulated pathways associated with IL-17 signaling. Our ongoing studies are focused on characterizing the kinetics and early impact of IL-17 on neutrophil recruitment, bacterial replication, and the granulomatous response to these Mtb strains. Defining these mechanisms may offer substantial opportunities for therapeutic interventions to mitigate Mtb transmission.

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P221

THE BALANCE BETWEEN GASDERMIN D AND STING SIGNALING SHAPES THE SEVERITY OF SCHISTOSOME IMMUNOPATHOLOGY

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Abstract Content: There is significant disease heterogeneity among mouse strains infected with the helminth *Schistosoma mansoni*. Here, we uncover a unique balance in two critical innate pathways governing the severity of disease. In the low pathology setting, parasite egg-stimulated dendritic cells (DCs) induce robust Interferon (IFN)β production, which is dependent on the cyclic GMP-AMP synthase (cGAS)/Stimulator of Interferon genes (STING) cytosolic DNA sensing pathway, and results in a Th2 response with suppression of proinflammatory cytokine production and Th17 cell activation. IFNβ induces signal transducer and activator of transcription (STAT)1, which suppresses CD209a, a C-type lectin receptor associated with severe disease. In contrast, in the high pathology setting, enhanced DC expression of the pore forming protein Gasdermin D (Gsdmd) results in reduced expression of cGAS/STING, impaired IFNβ and enhanced pyroptosis. Our findings demonstrate that cGAS/STING signaling represents a novel mechanism inducing protective type I IFN, which is counteracted by Gsdmd. This work was supported by NIAID grant R01 AI148656 to PK.

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P222

HIERARCHICAL CELL-TYPE-SPECIFIC FUNCTIONS OF CASPASE-11 IN LPS SHOCK AND ANTIBACTERIAL HOST DEFENSE

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Abstract Content: Caspase-11 sensing of intracellular lipopolysaccharide (LPS) plays critical roles during infections and sepsis. However, the key cell types that sense intracellular LPS and their contributions to the host responses at the organismal level are not completely clear. Here, we show that macrophage/monocyte-specific caspase-11 plays a dominant role in mediating the pathological manifestations of endotoxemia, including gasdermin D (GSDMD) activation, interleukin (IL)-1β, IL-18, and damage-associated molecular pattern (DAMP) release, tissue damage, and death. Surprisingly, caspase-11 expression in CD11c+ cells and intestinal epithelial cells (IECs) plays minor detrimental roles in LPS shock. In contrast, caspase-11 expression in neutrophils is dispensable for LPS-induced lethality. Importantly, caspase-11 sensing of intracellular LPS in LyzM+ myeloid cells and MRP8+neutrophils, but not CD11c+ cells and IECs, is necessary for bacterial clearance and host survival during intracellular bacterial infection. Thus, we reveal hierarchical cell-type-specific roles of caspase-11 that govern the host-protective and host-detrimental functions of the cytosolic LPS surveillance.
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P223

QUINONE REDUCTASE 1 TARGETS TBK1 FOR THE DOWNREGULATION OF THE HOST INNATE IMMUNE RESPONSE

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Abstract Content: TANK-binding kinase 1 (TBK1) is a key kinase of innate immunity that phosphorylates transcriptional factors to activate induction of antiviral interferons and inflammatory cytokines in response to virus infection. Here we report that the Quinone reductase 1 (QR1) regulates the innate immunity by alleviating self-association of TBK1 that is the essential step for its activation. QR1 interacted directly with TBK1, reduced oligomerization of TBK1 and finally inhibited activation of TBK1 characterized by the phosphorylation. Depletion or knockdown of QR1 increased secretion of antiviral cytokines, as well as decreased replication of RNA or DNA viruses. Consequently, QR1 knockout mice were more resistant to the lethal virus infection. Taken together, our study suggests that TBK1 and QR1 form a negative feedback loop for regulation of antiviral innate immunity. [National Research Foundation of Korea (Grant no. 2019R1A2C2008283 and 2021R1A6A1A03045495)]

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P224

CELL SURFACE BETACORONAVIRUSES NUCLEOCAPSID PROTEIN MODULATES INNATE HOST IMMUNITY

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Abstract Content: Human Coronaviruses (HCoV) have been historically experienced as common cold viruses, including HCoV-OC43, -NL63, -HKU and -229E. Over the past two decades, highly pathogenic HCoV have emerged causing severe acute respiratory syndromes (SARS), including SARS-CoV-1 in 2002, and MERS-CoV in 2012. SARS-CoV-2, the causative agent of COVID-19, has spread worldwide causing nearly a million deaths in the USA alone. Despite the unprecedented global response to SARS-CoV-2, critical aspects of HCoV biology, pathogenesis and immunomodulation remain unclear.

HCoV encode four major structural proteins: spike, membrane and envelope proteins are embedded in the viral envelope, while nucleocapsid (N) protein binds RNA. N, the most abundant viral protein expressed during infection, induces strong antibody and T cell responses. N is considered to be strictly localized intracellularly. However, cell surface expression of viral N protein is the rule, not the exception, among RNA viruses including influenza A, vesicular stomatitis, measles and respiratory syncytial viruses. Cell surface viral nucleoproteins have been reported to induce immunosuppression and also to serve as protective antibody targets.
By confocal microscopy and flow cytometry, we find that HCoV-OC43 and SARS-CoV-2 N are expressed on the cell surface of live cells in high copy numbers. N binds to infected and non-infected neighboring cells by electrostatically associating with glycosaminoglycans and is secreted via a non-canonical cellular secretory pathway. Using biolayer interferometry (BLI) we found that N binds to heparan sulfate and heparin with high affinity. BLI high-throughput screening of specific interaction between SARS-CoV-2 structural proteins and accessory factors (3a, 3b, 6, 7a, 7b, 8, 9b, 9c and 10) and 64 human cytokines, revealed high affinity interaction between N and a set of 11 chemokines, including CXCL12b. HCoV-OC43 N not only binds to those 11 chemokines, but also to an exclusive set of 6 additional cytokines. Importantly, HCoV-OC43 and SARS-CoV-2 N inhibited CXCL12b-mediated leukocyte migration in chemotaxis assays, as did SARS-CoV-1 and MERS-CoV N proteins. We also found that anti-N antibodies bound to the surface of N expressing cells can activate Fc receptor expressing cells.
These data are consistent with the idea that N displayed on the surface of CoV infected and surrounding cells play an important role in host innate and adaptive immunity to HCoV.

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P225

SARS-COV-2 IS EFFICIENTLY RECOGNIZED BY PATTERN RECOGNITION RECEPTORS IN IFN AND TNF PRIMED EPITHELIAL CELLS

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Abstract Content: SARS-CoV-2 is generally well controlled by the immune system, but it can lead to severe illness and death in a subset of cases. As SARS-CoV-2 features extraordinarily rapid replication, we and others hypothesized that differences between the patient's immediate cell-intrinsic innate immune responses are decisive in determining disease outcome. We previously reported that children's upper airway tract epithelial cells are primed for viral immune sensing resulting in more rapid and robust innate immune responses to SARS-CoV2 infection than adults. As insufficient IFN induction is determinant for permissiveness to SARS-CoV2, understanding the underlying mechanism of immune priming is crucial to mitigate disease severity.In this study, we sought to systematically determine the relevance of innate immune signaling to SARS-CoV2 and viral replication in primed epithelial cells. As would occur in children naively exposed to pathogen infection, we analyzed the cytokine profile produced by Yersinia-infected peripheral blood nuclear cells and assessed their individual contribution in priming lung epithelial cells (A549-ACE2). We found out that pre-treatment with IFN or TNF efficiently recognized incoming SARS-CoV2 by assessing IFN transcription. Using a systematic knockout approach determined that IFN- and TNF-primed A549-ACE2 is mainly dependent on MDA5 as primary sensors, with some contribution of RIG-I. Stunningly, we found that TNF induces MDA5 expression, a known ISG, in an IFN independent manner, eliciting IFN production upon SARS-CoV2 infection even in the absence of IFN downstream signaling. A double knockout of both RLR sensors, RIG-I and MDA5, leads to a complete abrogation of IFN induction and, consequently, an increase in SARS-CoV2 replication in IFN-primed cells. We confirmed SARS-CoV2 potently prevents the triggering of IFN-inducing pathways. However, this can be overcome by priming cells with IFN or TNF, putatively by upregulating the expression levels of the sensors RIG-I and MDA5 and possibly other factors. Our findings reinforce the importance of cell-intrinsic antiviral immunity components for optimal immune response to SARS-CoV-2.

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EFFECTS OF CIRCADIAN RHYTHMS ON ENTERIC VIRAL INFECTION

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Abstract Content: Much of life on earth operates on a light/dark cycle which is deeply engrained in our evolutionary history. In turn, multiple components of homeostatic and immunological pathways operate in an oscillatory manner referred to as circadian rhythms. These circadian rhythms govern molecular processes which can dictate migration patterns, immunological function, and readiness to combat pathogens. However, the intersections of these systemic pathways and how they influence infection are unclear. To further examine how circadian biology influences immune host response to a pathogen, we used coxsackievirus B3 (CVB3), an enteric virus in the *Picornaviridae* family. Our lab found that CVB3 had significantly reduced viral titers in multiple tissues when mice were orally infected in the morning (ZT0) compared to the evening (ZT12). This indicates that CVB3 infection efficiency may be under circadian control. To begin understanding what factor(s) causes this difference in infection efficiency, we are evaluating the immune responses in the lamina propria of the small intestine over time. Initial findings highlight rhythmic expression of type I interferons and downstream interferon-stimulated genes. Furthermore, immune cell numbers in the lamina propria change depending on time of day. We are developing mice lacking key circadian transcription factors in specific cell types to further interrogate this phenomenon. Our work may reveal how circadian rhythms influence control of enteric virus infection through rhythmic control of host immune responses.

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P227

INFLAMMATORY MONOCYTES PROMOTE PYOGRANULOMA FORMATION TO COUNTERACT YERSINIA BLOCKADE OF HOST DEFENSE

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Abstract Content: Granulomas are organized immune cell aggregates that form in response to chronic infection or antigen persistence. *Yersinia pseudotuberculosis* (*Yp*) blocks innate inflammatory signaling and phagocytosis, inducing formation of neutrophil-rich pyogranulomas within lymphoid tissues. Here, we uncover that *Yp* triggers pyogranuloma formation within the murine intestinal mucosa, a site not known to contain such structures. Mice lacking circulating monocytes fail to form defined pyogranulomas, have defects in neutrophil activation, and succumb to *Yp* infection. *Yersinia* lacking the virulence factors that block phagocytosis did not induce pyogranulomas, indicating that intestinal pyogranulomas form in response to *Yp* disruption of phagocytosis. Notably, mutation of a single anti-phagocytic virulence factor, YopH, restored pyogranuloma formation and control of *Yp* infection in monocyte-deficient mice, demonstrating that monocytes override YopH-dependent blockade of innate immune defense. This work reveals an unappreciated site of *Yersinia* intestinal invasion and defines host and pathogen drivers of intestinal granuloma formation.

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P228

CANDIDA ALBICANS ACTIVATION OF HOST ORAGNELLE STRESS MODULATES INFLAMMATION

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Abstract Content: *Candida albicans* is an opportunistic fungal pathogen, which causes a range of disease in humans, such as candidiasis or life-threatening candidemia. A major risk factor underlying *Candida* disease is immunocompromise, highlighting the importance of immunity in controlling *Candida* replication and dissemination. Interactions between *Candida* and host macrophages can lead to activation of the NLRP3 inflammasome, which is crucial for antifungal defense. However, the molecular principles that govern inflammasome activation during *Candida* infection are poorly understood. NLRP3 activation is thought to depend on host cell damage, thus host cell stress responses during microbial infection are intimately linked to inflammasome activation. However, host cell stress programs elicited by *C. albicans* infection are not well understood. Here, we found that *C. albicans* infection induces perturbations to macrophage mitochondria, including decreased membrane potential and leakage of mitochondrial DNA. Additionally, *C. albicans* infection triggered activation of the ER stress sensor IRE1α. IRE1α activation was dependent on the *C. albicans* transcription factor Efg1, which is required for hyphal morphology, and also the host protein Card9, which is required for C-type lectin receptor signaling. Importantly, we found IRE1α activity contributes to both mitochondrial perturbations and inflammasome activation in macrophages. Together, these results suggest that *C. albicans* induces stress responses at membrane-bound organelles and that IRE1α directs signaling to mitochondria, leading to mitochondrial damage that may contribute to inflammasome activation. These data highlight a role for organelle stress in the antifungal innate immune response.

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P229

IMMUNOMODULATORY EFFECTS OF BCG VACCINATION ON CYTOKINE AND T CELL RESPONSES TO SARS-COV-2 IN VITRO: IMPLICATIONS FOR PROTECTION AGAINST SEVERE COVID-19

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Abstract Content: In light of the beneficial off-target effects of BCG vaccination against unrelated respiratory infections, over 20 clinical trials are investigating whether BCG vaccination can protect against COVID-19. The largest is the BRACE trial (NCT04327206), in which we randomised almost 7000 healthcare workers in 5 countries to BCG-Denmark or control vaccination to determine if BCG vaccination reduces the incidence and severity of COVID-19.
BCG-induced modulation of immune responses is hypothesised to underlie its ability to protect against unrelated infections. Using whole blood samples from participants in the BRACE trial, we investigated the immunomodulatory effects of BCG on in vitro immune responses to SARS-CoV-2.
We found that BCG vaccination, but not placebo vaccination, reduced SARS-CoV-2-induced secretion of cytokines associated with severe COVID-19, including IL-6, TNF-α and IL-10. In addition, BCG vaccination promoted effector memory $CD4^+$ and $CD8^+$ T cells, and activation of eosinophils in response to SARS-CoV-2.
Conclusions: BCG vaccination induces changes in the response to SARS-CoV-2 consistent with protective immunity against severe COVID-19. This is the first insight into the mechanism by which BCG may provide protection against severe COVID-19 in humans.

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P230

RIPK1 IS DISPENSABLE FOR APOPTOSIS IN HUMAN MACROPHAGES IN RESPONSE TO YERSINIA BLOCKADE OF IMMUNE SIGNALING

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Many microbial pathogens suppress the pro-inflammatory response of innate immune cells to evade detection by their hosts. The gram-negative bacterial pathogen *Yersinia*, which causes diseases from self-limiting gastroenteritis to systemic bacteremia, utilizes a type III secretion system (T3SS) to inject effectors into host cells. These effectors disrupt NF-κB signaling, thereby preventing activation of cell-intrinsic cytokine production and anti-microbial defense. We and others have demonstrated that in murine cells, *Yersinia* blockade of NF-κB signaling triggers apoptosis, involving engagement of Receptor Interacting Serine-Threonine Protein Kinase 1 (RIPK1) and caspase-8 (Casp8). In mice, RIPK1-dependent apoptosis is required for bacterial clearance and host survival, as macrophages lacking RIPK1 kinase activity or Casp8 are defective for *Yersinia*-induced cell death, and the corresponding mice cannot control *Yersinia* tissue burdens and succumb to infection. While mice express a single Casp8 protease, humans express two orthologs, CASP8 and CASP10. Moreover, while RIPK1- or Casp8-deficient mice undergo embryonic lethality, RIPK1- and Casp8-deficient humans are born but experience autoinflammatory phenotypes accompanied by increased susceptibility to a variety of viral and bacterial infections. Here, we find that both Casp8 and 10 are activated during apoptosis of human macrophages induced by NF-κB signaling blockade. Unexpectedly, in contrast to murine macrophages, human macrophages did not require RIPK1 kinase activity or RIPK1 itself to undergo apoptosis in response to *Yersinia*-mediated blockade of NF-κB signaling. Rather, we find that the expression of the pro-survival protein, cellular FLICE-Like Inhibitory Protein (cFLIP), is downregulated during NF-κB blockade, and that the absence of cFLIP potentiates RIPK1 kinase-independent apoptosis in response to TLR or TNFR stimulation alone. Altogether, our data indicate that bacterial blockade of NF-κB triggers a human-specific apoptosis pathway in macrophages that is independent of RIPK1, activates Casp8 and 10, and is likely regulated by cFLIP.

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ANTIGENIC DRIFT IN THE RBD OF SARS-COV-2 SPIKE PROTEIN CONFERS NEUTRALIZATION ESCAPE BY SINGLE MONOCLONAL ANTIBODIES

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Abstract Content: Recombinant neutralizing monoclonal antibodies (mAbs) targeting SARS-CoV-2 spike protein present a promising COVID-19 treatment approach. However, virus mutants selected by pre-existing immunity in vaccinated and convalescent individuals may impact antivirals and vaccine effectiveness. Therefore, we studied the induction of viral mutations that lead to escape from neutralization by mAbs.
In this study, mutations in the receptor binding domain (RBD) of the SARS-CoV-2 spike protein that promote viral escape were investigated. Replicating VSV expressing wild type SARS-CoV-2 spike protein instead of the glycoprotein was passaged in the presence of single and combined mAbs *in vitro*. Viral escape mutants were Sanger sequenced, and the identified amino acid changes were mapped on published spike structures. Novel spike mutants rapidly appeared in single mAb treatments and resulted in loss of neutralization, while all combinations of two mAbs efficiently suppressed viral escape. Mapping of the identified amino acid changes on the spike structure showed distinct binding modes of the tested mAbs suggesting that these mAbs bind to different parts of the spike protein. This

observation could explain supressed viral escape in combined mAb treatment. To verify the individual mutations, single clones from all the viral mutants were isolated, and neutralization assays were performed using all the mAbs that drove the mutations. All the mutants tested conferred resistance to the mAb that they escaped from and were neutralized by the other mAbs.
To determine whether there were more possible mutations that could lead to the escape of neutralization by these mAbs, the virus was subjected to increased mAb pressure and increased virus replication events. In this setting, the recombinant VSV expressing the SARS-CoV-2 spike was subjected to high concentrations of single mAbs, and escape mutants were analysed in 96 biological replicates. Emergence of additional mutations was observed suggesting that the virus could have several escape solutions without compromising its infectivity. This also indicated that high mAb pressure on the virus leads to viral escape. High frequency of similar mutations in independent replicates was also observed suggesting that these mutations were not random and that they might have a fitness advantage for the virus. It could also be speculated that these amino acid changes might be located at a crucial mAb binding site therefore interfering with binding leading to loss of neutralization. These observations explain the high mutational potential of SARS-CoV-2 variants observed in the human population which occur due to repeated viral exposure. These studies will be further advanced to assess the antigenic consequences of these additional mutations and how this influence binding of the RBD to the human angiotensin-converting enzyme 2 (ACE-2) receptor.

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HSV-1 INHIBITS NLRP1-DEPENDENT PYROPTOSIS IN PRIMARY HUMAN KERATINOCYTES

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Abstract Content: Keratinocytes are important barrier epithelial cells that protect against cutaneous viral infections, including herpes simplex virus 1 (HSV-1). This cell type expresses Nod-like receptor protein 1 (NLRP1), an innate immune sensor that induces inflammasome-dependent pyroptosis when activated by diverse stimuli. Previous studies have demonstrated that double-stranded RNA (dsRNA), an activator of the NLRP1-infammasome, accumulates in HSV-1 infected cells, but is reduced via the RNAse activity of the virion host shutoff (vhs) protein. Consistent with these studies, we observe that infections of primary keratinocytes with recombinant HSV-1 carrying a mutation in the U_L41 gene that inhibits vhs activity result in the accumulation of dsRNA, but not pyroptosis. This observation suggests that HSV-1 may employ an additional mechanism to inhibit NLRP1 signaling in these cells.
Here, we demonstrate that HSV-1 infected keratinocytes do not respond to activators of NLRP1, resulting in a loss of IL-1b release, Gasdermin D cleavage, caspase 1 activation, and ASC speck formation. In addition, we observe a marked proteasome-dependent loss of NLRP1 in HSV-1 infected keratinocytes. We identify HSV-1 infected-cell protein 0 (ICP0), a RING-finger E3 ubiquitin ligase, as necessary and sufficient to reduce NLRP1 protein abundance. We observe no NLRP1 protein loss in keratinocytes infected with an ICP0-deficient virus thereby allowing for normal responses to NLRP1 activators in these cells. In addition, a virus carrying loss of function mutations in the RING Finger Domain of ICP0 is unable to inhibit NLRP1 signaling, suggesting that the E3-ubiquitin ligase activity of ICP0 is required for this phenotype. Together these results indicate that HSV-1 evades NLRP1-dependent pyroptosis in human keratinocytes via an ICP0-dependent mechanism.

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MAVS SIGNALING OF MICROGLIA IS CRITICALLY NEEDED TO DEFINE THE TRANSCRIPTIONAL STATUS OF CNS INFILTRATING CD8+ T CELLS IN VIRAL ENCEPHALITIS

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Abstract Content: Viral infections of the central nervous system (CNS) emerge as a global research priority. A plethora of viruses show neurotropic potential and may cause viral encephalitis. Currently, the therapeutic arsenal to treat neurotropic virus infection of the CNS is limited and many affected individuals develop severe post-encephalitis neurological sequelae or succumb to the infection.
The nasal epithelium that contains olfactory sensory neurons is constantly exposed to a broad range of viruses that may gain access to the CNS under specific conditions. Upon virus propagation to the olfactory bulb (OB), microglia undergo a morphological shift, proliferate and eventually get recruited to the site of infection in order to restrict virus dissemination within the CNS. Recent data point towards microglia cross-presenting antigens to CNS infiltrating virus-specific $CD8^+$ T cells. However, it is not yet clear how the cross-presentation capacity is regulated and which pathways coordinate that biological function in response to infection.
Our experiments revealed the importance of the adaptor molecule of RIG-I like helicases, which is termed MAVS, selectively in microglia, in promoting survival against lethal VSV infection. Selective MAVS ablation in microglia did not affect the microglia density and the leukocyte infiltration upon infection. However, microglia specific MAVS deficiency was associated with impaired gene expression of cross-presentation related genes, suggesting a loss of microglia cross-presentation capacity to T cells. To address this option, we bulk-sequenced CNS-infiltrating $CD8^+$ T cells from mice with microglia that are either MAVS competent or deficient. The data revealed that CNS-infiltrating $CD8^+$ T cells sorted from mice with a microglia-selective MAVS deficiency showed less activated gene expression profiles than $CD8^+$ T cells from control mice. In conclusion, MAVS signaling in microglia is essential for cross-presentation within the infected CNS and relicensing of CNS infiltrating $CD8^+$ T cells.

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P234

CHARACTERIZING THE DIRECT ANTIVIRAL MECHANISMS OF TYPE II INTERFERON AGAINST CORONAVIRUS INFECTION

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Abstract Content: The COVID-19 pandemic, caused by the recently emerged SARS-CoV-2, highlights our vulnerability to emerging CoVs. Novel antiviral approaches may help to protect against future emerging CoVs. IFN-γ is a pro-inflammatory cytokine that modulates both innate and adaptive immune responses. Additionally, IFN-γ has direct antiviral activities against a number of viral infections. For example, IFN-γ has been shown to potently inhibit the replication of positive-sense single-stranded RNA ((+)ssRNA) viruses, including SARS-CoV-2, although its direct antiviral mechanisms are still poorly understood. Like other (+)ssRNA viruses, CoVs induce the formation of membranous cytoplasmic replication organelles (ROs) required for viral RNA synthesis. The antiviral activity of IFN-γ against murine norovirus, another (+)ssRNA virus, has partially been attributed to the disruption of viral ROs by the induction of antiviral IFN-γ-inducible proteins known as guanylate binding proteins (GBPs). However, whether the antiviral mechanism of IFN-γ against CoVs is associated with the induction of GBP expression is currently unknown. We hypothesize that IFN-γ inhibits CoV replication by inducing the recruitment of GBPs to CoV ROs. Using the endemic human coronavirus HCoV-229E as a model, we showed that pre-treatment of A549 human lung epithelial cells with IFN-γ potently inhibits HCoV-229E infection. Furthermore, treatment with IFN-γ robustly induces GBP1 and GBP2 mRNA and protein expression in both A549 human lung epithelial cells and Huh7 human liver cells. To evaluate a specific role for GBP2 as an IFN-γ-induced effector protein, we used CRISPR/Cas9 to generate GBP2 knockout

A549 cells. Notably, the antiviral effect of IFN-γ against HCoV-229E was significantly reduced in the absence of GBP2, suggesting that GBP2 is key in mediating the antiviral effect of IFN-γ against HCoV-229E. Furthermore, GBP2 overexpression inhibits HCoV-229E replication in both A549 and Huh7 cells. Our preliminary data suggest that CoV RO formation is inhibited in GBP2-overexpressing cells, as assessed by confocal microscopy. Further evaluation of RO formation is ongoing. We are currently characterizing the mechanisms underlying restriction of CoV infection by GBP2 using site-directed mutagenesis to mutate its key domains. Overall, these findings inform our understanding of the direct antiviral mechanisms of IFN-γ against CoV infection. Furthermore, they may contribute to the development of novel immunomodulatory therapeutic strategies to protect against future emerging CoVs.

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P235

GENETIC REGULATION OF OAS1 NONSENSE-MEDIATED DECAY UNDERLIES ASSOCIATION WITH COVID-19 HOSPITALIZATION IN PATIENTS OF EUROPEAN AND AFRICAN ANCESTRIES

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Abstract Content: The chr12q24.13 locus encoding OAS1-3 antiviral proteins has been associated with COVID-19 susceptibility. Here, we report genetic, functional, and clinical insights into this locus in relation to COVID-19 severity. In our analysis of patients of European (n=2249) and African (n=835) ancestries with hospitalized vs. non-hospitalized COVID-19, the risk of hospitalized disease was associated with a common *OAS1* haplotype, which was also associated with reduced SARS-CoV-2 clearance in a clinical trial with pegIFN-λ1. Bioinformatic analyses and *in vitro* studies reveal the functional contribution of two associated *OAS1* exonic variants comprising the risk haplotype. Derived human-specific alleles rs10774671-A and rs1131454-A decrease OAS1 protein abundance through allele-specific regulation of splicing and nonsense-mediated decay. We conclude that decreased *OAS1* expression due to a common haplotype contributes to COVID-19 severity. Our results provide insight into molecular mechanisms through which early treatment with interferons could accelerate SARS-CoV-2 clearance and mitigate against severe COVID-19.

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P237

THE IMPACT OF THE CYTOKINE MILIEU IN ASCITIC FLUID FROM CHRONIC LIVER DISEASE PATIENTS: ASSOCIATION WITH IMMUNE CELL PHENOTYPE, BACTERIAL INFECTION AND CLINICAL OUTCOMES

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Abstract Content: Background and Aim:
Ascites is the accumulation of fluid in the peritoneal cavity that occurs in patients with advanced liver disease. Patients with ascites are particularly susceptible to bacterial and fungal infections of the peritoneal cavity, termed spontaneous bacterial peritonitis (SBP), that drastically increases the risk of mortality. Peritoneal macrophages are the primary sentinel population of the peritoneal cavity that are responsible for clearance of bacterial pathogens, and whose functionality is reduced in liver disease patients. It nonetheless remains unknown how the cytokine milieu of the peritoneal cavity regulates macrophage, and other immune cell phenotype, chemotaxis and phagocytic capacity. This

ongoing study aims to quantify key immunomodulatory cytokines within the peritoneal fluid to examine how they regulate immune cell phenotype, activity and clinical outcomes in advanced liver disease patients.

Methods:

Ascitic fluid (AF) was collected from patients with decompensated cirrhosis undergoing therapeutic paracentesis at Westmead and Blacktown Hospitals in Sydney, Australia. Standard biochemical and serological assays, medical imaging and histological assessment of liver biopsy were used to confirm diagnosis of liver disease and cirrhosis, including assessment of liver disease severity using Model for End-stage Liver Disease and Child-Pugh Score. Baseline and follow-up biochemistry, serology, medical history, and use of medications were available. Flow cytometry, microscopy and enzyme-linked immunosorbent assay (ELISA) were used to assess cytokines within AF, as well as AF immune cell phenotype. AF cytokine concentrations and immune cell phenotypes were associated with clinical outcomes.

Results:

Pro/anti-Inflammatory cytokines TNF, IL-1β, IL-10 and TGFβ, interferons (IFNs) β, γ, λ1, λ3, chemokines CXCL10, CCL19 and CCL21 as well as sCD163 and S100A8/A9 were measured from patient ascitic fluid. Inflammatory cytokines as well as markers of macrophage and neutrophil activation were associated with SBP and poorer clinical outcomes. In particular, CCL19 became elevated as patients neared death. Among peritoneal immune cell populations, a CRIg+ CD206+ tissue-resident macrophage population was characterized, consistent with an alternatively activated phenotype demonstrating a function consistent with antimicrobial responses. We identified autofluorescent retinol as a novel marker for these cells, and a likely driver of their phagocytic functionality.

Conclusion:

The characterization of unique AF cytokine signatures may provide clinical markers that could prove clinically useful for the diagnosis of patient decompensation or in the early diagnosis of SBP. Determining how the cytokine profile effects immune cell populations within the peritoneal cavity can guide novel treatments to improve patient outcomes.

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P238

REGULATION OF CORONAVIRUS NSP15 CLEAVAGE SPECIFICITY BY VIRAL RNA STRUCTURE

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Abstract Content: Over the past two decades, several highly pathogenic human coronaviruses (CoVs) have emerged. Like many viruses, the interferon response plays a key role in determining the pathogenesis of CoVs. Consequently, CoVs have evolved to evade the host immune response. In particular, the viral endonuclease (nsp15) is critical for immune evasion by cleaving viral RNA to reduce PAMP accumulation and thus prevent activation of the interferon response. Though nsp15 is established as a 3'uridine-specific endonuclease, other determinants of cleavage specificity have not been explored. We predict that RNA structure dictates specificity of nsp15 cleavage. However, the structural features of the substrates that nsp15 cleaves has not been thoroughly investigated. In this study, we examined the role of RNA secondary structure in modulating SARS-CoV-2 nsp15 endonuclease activity.

Using an *in vitro* endonuclease assay, we observed that thermodynamically stable RNA structures were protected from nsp15 cleavage relative to RNAs lacking stable structure. Moreover, we observed that nsp15 cleaves specific uridines in the SARS RNA element S2M suggesting that RNA structure may be important for presentation of uridine nucleosides to nsp15 for cleavage. To explore the specific structures that are important for nsp15 cleavage specificity, we designed mutant S2M RNAs and examined their nsp15 binding and cleavage efficiency. In conjunction with structural analysis of these RNAs, these data will provide mechanistic insight into the RNA structure determinants of nsp15 cleavage efficiency and immune evasion.

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P239

ANT2 FUNCTIONS AS A NEGATIVE REGULATOR IN THE DNA SENSING PATHWAY VIA STING AND PROTECTS HEPATOCYTES FROM CHEMOTHERAPY-DRIVEN LIVER DAMAGE

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Abstract Content: Elimination of damaged cells that may cause cancer is critical event in tumor immunity. Stimulator of interferon genes (STING) adaptor protein plays important role for the DNA sensing pathway triggered by DNA virus infection and DNA damage inducing regents to harness type I interferon (IFN) and inflammatory cytokine. However, the DNA damage induced-regulation of STING and subsequent signaling events remain to be fully clarified. Here, ANT2 was identified as a novel STING interacting protein and upregulated by DNA damage. ANT2 suppressed STING activation but not TLRs, RIG-I and NLRP3 inflammasome signaling pathway through counteracting the interaction of cyclic GMP-AMP (cGAMP) with STING. Knockout of ANT2 in mice liver displayed increased level of doxorubicin-induced liver damage marker and inflammatory cytokine *in vivo*. Thus, our results demonstrate that ANT2 functions as a critical negative regulator in the DNA sensing pathway via STING and protects hepatocytes from chemotherapy-driven liver damage, which may have therapeutic implications.

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P240

INFLAMMATION INDUCED BY DISTINCT TYPE I INTERFERON SIGNALING IS INVOLVED IN FUNGAL CLEARANCE AND OUTCOME DURING ASPERGILLUS PULMONARY INFECTION

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Abstract Content: Background: It is estimated that between four and 8 million people worldwide suffer from respiratory infections caused by the fungus *Aspergillus fumigatus* (*Af*). Each year over 300,000 of those cases are due to invasive pulmonary aspergillosis (IPA) in patients with suppressed immune systems. The recent rise in the number and severity of cases of both influenza and SARS-CoV-2-infected patients acquiring IPA suggests that viral infection can create a transiently suppressed immune environment permissive to fungal infection. We recently discovered that differential type I interferon (IFN) signaling, via the type I IFN receptor 2 (IFNAR2) subunit of the IFNAR1/2 heterodimeric receptor, regulates damage responses during pulmonary infection, allowing for an environment permissive to infections, specifically to *Af* infection. As clinical outcome to *Af* is associated with host tissue damage, this suggested involvement of IFNAR2 signaling in *Af* susceptibility.
Results: To determine the distinct roles for IFNAR2 and IFNAR1 in regulating both damage and clearance during *Af* infection, we used a murine pulmonary infection model. We determined the components and extent of the damage response using proteomic, histological, and molecular approaches. We found that absence of IFNAR2 (*Ifnar2*$^{-/-}$ mice) resulted in increased inflammation, morbidity, and damage in the lungs (from both myeloid and epithelial/endothelial cells) in response to *Af*, while absence of IFNAR1 (*Ifnar1*$^{-/-}$ mice) did not. Specifically, this included significantly increased levels of pro-inflammatory cytokines, Pneumonitis, lactate dehydrogenase (cell damage), and albumin (vascular leakage). We also found that the *Ifnar2*$^{-/-}$ mice were better able to clear conidia initially, compared to wild type (WT) and *Ifnar1*$^{-/-}$ mice. However, this early clearance did not prevent invasive disease from developing in the *Ifnar2*$^{-/-}$ mice as infection progressed, which was likely driven by the increased level of damage and inflammation that occurred early during infection. Importantly, we found that by altering the inflamed environment of the *Ifnar2*$^{-/-}$ mice early during *Af* infection, via antibody neutralization of TNFα, we were able to reverse not only the morbidity, but also the fungal burden in these mice back to WT levels.
Conclusions: Together, our results begin to establish IFNAR2's role in regulating host damage responses to *Af* and suggest that aberrant type I IFN signaling contributes to an *Af* permissive environment through regulation of inflammation. Further, our results indicate that while IFNAR2 is regulating damage, that IFNAR1 is involved in

mediating clearance of *Af* during pulmonary infection and suggests that IFNAR2 regulates host-mediated damage, while IFNAR1 regulates pathogen-mediated damage. Understanding the mechanisms involved in IFNAR regulation of damage and anti-fungal immunity could inform design of better treatments aimed at minimizing damage in patients with IPA.

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P241

THE EFFECT OF AIR-LIQUID INTERPHASE CONDITION ON THE PRODUCTION AND SECRETION OF TYPE 3 INTERFERON IN NASAL EPITHELIUM AGAINST RESPIRATORY VIRAL INFECTION

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Abstract Content: Interferons (IFNs) are central to antiviral defence in our body. Type 3 IFN discovered in 2003 are the most recent addition to the IFN family. It is the earliest and predominant IFNs produced during influenza virus infection. However, it is not clear how it is regulated differently from type 1 IFN. Therefore, we sought to investigate the mechanism to selectively induce the production and secretion of type 3 interferon in nasal epithelium in response to respiratory viral infection. Primary human nasal epithelial cells (HNEpCs) were differentiated under air-liquid interface (ALI) condition for at least 14 days. The fully differentiated cells were divided into 2 groups: exposed to ALI and liquid-liquid interface (LLI) condition for 24 hours. Then, the cells were treated with Polyinosinic:polycytidylic acid (poly I:C, 50ug/ml) for 24 hours. The media were collected and total RNA was isolated using RNeasy Mini kit (Qiagen, CA, USA). The mRNA expression and secretion level of type 3 IFN was significantly upregulated in the HNEpCs exposed to LLI followed by poly I:C compared to those exposed to ALI. RNA sequencing showed transcription factor NUPR1 was significantly increased in the HNEpCs under LLI compared to ALI. In the present study, we revealed only type 3 IFN were significantly induced against poly I:C under the disrupted ALI condition. Furthermore, transcription factor NUPR1 could selectively regulate the production and secretion of type 3 IFN.

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COMPARISON OF T-SPOT SARS-COV-2 AND MABTECH IFN-GAMMA ELISPOT KIT IN SARS-COV-2 VACCINEES AND WHO RECOVERED FROM SARS-COV-2 INFECTION.

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Abstract Content: **Introduction:** Neutralization by antibodies induced from vaccination (using wild type) significantly reduced to omicron variant. However, T cell responses are relatively conserved. In this study, we compared the IFN-gamma response of T cells using T-SPOT SARS-CoV-2 (Oxford Immunotech, UK) with Mabtech IFN-gamma ELISPOT (Mabtech Ab, Sweden) and anti-SARS-CoV-2 IgG (Abbott, USA) in both vaccinees and subjects recovered from SARS-CoV-2 omicron infection.
Materials and Methods: Thirty-four vaccinees who got 3rd vaccination (25 ChAdOx1-S and 9 Bnt162b2) and 24 subjects recovered from SARS-CoV-2 omicron infection after 3rd vaccination (15 ChAdOx1-S and 9 Bnt162b2) were included. IFN-gamma responses of T-cells were measured using T-SPOT COVID (Oxford Immunotec, UK) and Mabtech IFN-gamma ELISPOT kit (Mabtech Ab, Sweden). Spike and nucleocapsid peptide pools of SARS-CoV-2 wild type and B1.1.529 (Omicron) (JPT, Germany) were used for stimulation. Humoral response was measured using Anti-SARS-CoV-2 IgG II Quant (Abbott, USA).
Results: T-SPOT COVID spike and nucleocapsid results were significantly correlated with Mabtech IFN-gamma ELISPOT (r = 0.547, 0.697, 0.870, 0.894 for spike of wild type, omicron, and nucleocapsid of wild type, omicron,

respectively). Abbott anti-SARS-CoV-2 IgG was significantly correlated with T-SPOT COVID spike and nucleocapsid or Mabtech IFN-gamma ELISPOT using spike of wild type, omicron, nucleocapsid of wild type, and omicron (r = 0.353, 0.624, 0.408, 0.392, 0.590, 0.616, respectively). However, in subgroup analysis, anti-SARS-CoV-2 IgG was not correlated with T-SPOT or Mabtech in vaccinees.
Conclusion: T-SPOT SARS-CoV-2 was correlated with Mabtech IFN-gasmma ELISPOT kit. Anti-SARS-Co-2 IgG was correlated with both ELISPOT kits only in recovered subjects not in vaccinees. Considering the importance of T cell response in omicron infection and no correlation of T cell response with antibody levels in vaccinees, both anti-SARS-CoV-2 antibody and T cell response should be measured for determination of immune correlates of protection in vaccinees.

Disclosure of Interest: None Declared
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P244
CELL TYPE SPECIFIC RESPONSE TO VIRAL INFECTION IN THE HUMAN GASTROINTESTINAL TRACT
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Abstract Content: Human intestinal epithelial cells (hIECs) are arranged as a monolayer of cells and provide the first line of defense against invading pathogens. Upon viral infection hIECs upregulate type I and III interferons to control the infection. These interferons lead to the induction of hundreds of interferon stimulated genes (ISGs) that aid in the clearance of the virus and protection of uninfected cells. While hundreds of ISGs have been described using mainly enterocyte models, whether all cell types in the human gastrointestinal tract upregulate the same ISGs following enteric virus infection has yet to be addressed. To evaluate how each cell type in the human ileum and colon respond to virus infection we employed human mini-gut organoids as a model. These mini-gut organoids are primary non-transformed cells that are composed of all major cell types found in the human gastrointestinal tract. We confirmed that mini-gut organoids supported the infection of the enteric virus astrovirus and upregulated both type I and III interferons following infection. Using both multiplex RNA FISH and single cell RNA sequencing (scRNA-Seq) following astrovirus infection, we were able to determine the cell type specific response to virus infection. Our results show that astrovirus had a broad tropism and was able to infect all major cell types in our organoid model. Additionally, our analysis showed that each cell type upregulated a unique pattern of ISGs to combat astrovirus. Interestingly, we could also show that each cell type in the human ileum also expressed cell-type specific basal ISGs. Together our results illustrate that each individual cell type has a unique ISG pattern that can help us understand virus tropism and virus clearance mechanisms used by hIECs.

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NRF2 CONTROLS ANTIVIRAL GENES IN THE AIRWAY EPITHELIUM
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Abstract Content: NRF2 is a transcription factor that regulates the expression of antioxidant genes and inflammatory responses.
We demonstrate that a subset of NRF2 genes, which are highly expressed in the airway epithelia, act antiviral towards Influenza A virus (IAV). Overexpression of these genes in HUH7 cells by CRISPR activation reduced IAV replication. Using human airway Air-liquid interface cell cultures, we investigated the expression of this subset of NRF2-dependent genes through celluar maturation from basal cell stage to fully differentiated epithelium cells. This was done by qPCR and Western Blotting using samples harvested at different stages of cellular development. The expression of these genes increased vastly through differentiation on both RNA and protein level.

We are trying to identify the antiviral mechanisms of these NRF2 subset genes and the mechanisms that control the increase in expression through differentiation.

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A TLR4-INDEPENDENT CRITICAL ROLE FOR CD14 IN INTRACELLULAR LPS SENSING

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Abstract Content: Intracellular sensing of LPS is crucial for antimicrobial defense and sepsis. Noncanonical inflammasome sensing of cytosolic LPS via caspase-4/11 triggers gasdermin-D mediated pyroptosis and the release of cytokines and alarmins. During infection, LPS associated with bacteria or bacterial outer membrane vesicles gains access to the cytosol. However, free LPS can access the cytosol *in vivo*. The mechanism by which free LPS gains access to the cytosol and activate the caspase-11-noncanonical inflammasome *in vivo* mostly remains elusive. CD14, a GPI-anchored glycoprotein, binds and transfers LPS to the TLR4-MD2 complex. While the role of CD14 in TLR4-mediated host responses to extracellular LPS is clear, its role in the noncanonical inflammasome sensing of cytosolic LPS is poorly defined. Here, we found that cytosolic LPS-induced GSDMD cleavage, IL-1β, IL-18 and DAMP release, pyroptosis and lethality were remarkably impaired in CD14-deficient mice, but not in TLR4-deficient mice. By using *Cd14*$^{-/-}$ and *Casp11*$^{-/-}$ mice strains on a *Tlr4*$^{-/-}$ background, we have decoupled CD14's role in caspase-11 activation from its known function in TLR4 signaling. Mechanistically, CD14 mediates caspase-11 activation by facilitating the internalization of LPS in a TLR4-independent manner. Overall, our findings highlight a new TLR4-independent role of CD14 in noncanonical inflammasome activation by intracellular LPS *in vivo*

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CALMODULIN REGULATES HOST PATTERN RECOGNITION AND IS TARGETED BY BACTERIAL PATHOGENS

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Abstract Content: Effective innate immunity requires recognition of Pathogen-Associated Molecular Patterns (PAMPs) by Pattern Recognition Receptors (PRRs) leading to the production of pro-inflammatory cytokines. Beyond the core machinery, regulation of pattern recognition depends on additional context-dependent cues, many of which remain undefined. Calmodulin (CaM) is an intracellular sensor of calcium which at low cytosolic concentrations adopts an inactive form termed apo-CaM. Following rising concentrations of calcium, CaM changes conformation such that it activates several downstream proteins including Calmodulin-dependent kinase (CaMK) and Protein tyrosine kinase 2 beta (Pyk2). The role of CaM in immunity, and specifically in modulating pattern recognition is established in plants, but its role in mammalian immunity remains poorly defined.

Here, we demonstrate that infection of THP-1 macrophages with diverse bacterial pathogens leads to phosphorylation of CaMK and Pyk2 to comparable levels observed with purified LPS treatment. Activation of CaM during infection fine-tuned innate sensing of pathogens, as pre-treatment of cells with the CaM inhibitor W7 significantly reduced the activation of both IFNβ expression, and the NF-κB pathway both following infection, and treatment with purified Toll-Like Receptor ligands. During infection with the major gastrointestinal pathogen *Shigella sonnei,* CaM activation was inhibited by the Type 3 Secretion System (T3SS). The T3SS was also able to inhibit the degradation of IκB, and the phosphorylation of TBK1. Inhibition of CaM partially underpinned this phenotype as pre-treatment with W7 led to comparable levels of TBK1 phosphorylation between both WT and T3SS-deficient *Shigella*.

Lastly, we identify a *Shigella* protein secreted by the T3SS which potently inhibits CaM. Deletion of this effector protein from *Shigella* leads to enhanced signalling downstream of TLR4 following infection, highlighting a novel virulence strategy of this enteropathogen.

In conclusion, these data demonstrate that CaM is a critical modulator of innate sensing, and a target for bacterial pathogens during infection.

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P249

CASPASE-8–CASPASE-3 AXIS INHIBITS CYTOKINE RELEASE BY INACTIVATING GSDMD DURING INFECTIONS

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Abstract Content: Programmed cell death (PCD) is essential for the innate immune response, which serves as the first line of defense against pathogens. Caspases regulate PCD, including subsequent cytokine release and immune responses. Caspase-8 specifically plays multifaceted roles in PCD pathways including pyroptosis, apoptosis and necroptosis. However, because caspase-8–deficient mice are embryonically lethal, little is known about how caspase-8 coordinates different PCD pathways under physiological conditions. Here, we report anti-inflammatory role of caspase-8 during bacterial or viral infection (i.e. influenza A virus, Herpes simplex virus-1 or *Yersinia pestis*). We generated viable mice carrying an uncleavable version of caspase-8 (*Casp8*$^{DA/DA}$). We demonstrated that caspase-8 autoprocessing was responsible for activating caspase-3, thereby suppressing gasdermin D (GSDMD)-mediated pyroptosis and inflammatory cytokine release during these infections. We also found that apoptotic and pyroptotic pathways were activated at the same time during these infections, which enabled the cell-intrinsic anti-inflammatory function of the caspase-8–caspase-3 axis. Our findings suggest a general immunological consequences of caspase-8–coordinated PCD crosstalk under infection conditions.

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P250

E3 UBIQUITIN LIGASE SEVEN IN ABSENTIA HOMOLOG-1 TARGETS SPECIFIC DEUBIQUITINASE FOR THE POSITIVE REGULATION OF HOST INNATE IMMUNE SIGNAL

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Abstract Content: SIAH (Seven in Absentia Homolog), an E3 ubiquitin ligase is mammalian homolog of SINA (Seven in Absentia), a *Drosophila* protein which first identified as involved in the development of the *Drosophila* eye. With a highly conserved N-terminal RING domain, SIAH1 consists of two zinc finger domains and a substrate-binding domain. It is mainly involved in cellular stress responses, but SIAH1 is associated with a broader range of cellular processes including neural functions, hypoxia, DNA damage response, and cell cycle regulation. In this study, we report that E3 ligase SIAH1 as a novel positive regulator of host innate immune signaling upon viral infection. SIAH1 interacted directly with USP19 which is known to be involved in deubiquitinating Beclin1, TRAF3, and TRIF for downregulation of the interferon (IFN) signaling pathway during the early phase of infection and catalyzed K-27-linked ubiquitination of 3 Lysine residues on USP19, which resulted in its degradation. Ultimately, knockdown of SIAH1 inhibited type I interferon signaling and enhanced viral replication. Collectively, these data provide a clear understanding of the

molecular mechanism of SIAH1 mediated positive regulation of host immune response to virus infections by targeting USP19. [NRF of Korea (2019R1A2C2008283 and 2021R1A6A1A03045495), KRIBB Program (KGM9942011)]

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P251

4-OCTYL ITACONATE REDUCES INFLUENZA A REPLICATION BY TARGETING THE NUCLEAR EXPORT PROTEIN CRM1

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Abstract Content: Activation of macrophages with inflammatory stimuli leads to the accumulation of the metabolite itaconate. Both anti-bacterial and anti-viral effects have been described for itaconate derivatives, such as 4-Octyl itaconate (4-OI). We have revealed that 4-OI inhibits replication of multiple Influenza A virus (IAV) strains by restricting nuclear export of viral ribonucleoproteins (vRNPs), a key step in the IAV life cycle. This nuclear retention is achieved by deactivation and subsequent degradation of the host cell Chromosomal Maintenance 1 protein (CRM1), also known as Exportin 1 (XPO1), a protein exploited by IAV during replication. 4-OI-mediated inactivation of CRM1 resulted in accumulation of the IAV nucleoprotein (NP) as well as the natural CRM1 cargos p53 and p65, in the nucleus in epithelial cells. In depth investigation for the mechanism through which 4-OI acts on CRM1 showed that 4-OI modified a key cysteine in the cargo binding pocket on CRM1 at position 528. Alkylation of this cysteine by the antiviral compound leptomycin B (LMB) or other selective inhibitors of nuclear export (SINEs) has previously been shown to deactivate CRM1. Using a cell line in which the cysteine at position 528 in CRM1 was substituted by a serine, we demonstrated that modification of this residue was indeed the cause for the observed inhibitory effect induced by 4-OI on CRM1 function. Overall, this study demonstrates a mechanism through which 4-OI directly interferes with the lifecycle of CRM1-dependent viruses such as influenza, supporting the use of itaconate derivatives as novel antiviral therapeutics.

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P252

INNATE IMMUNE SIGNALING IN TROPHOBLAST AND DECIDUA ORGANOIDS DEFINES DIFFERENTIAL ANTIVIRAL DEFENSES AT THE MATERNAL-FETAL INTERFACE

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Abstract Content: Infections at the maternal-fetal interface can directly harm the fetus and induce complications that adversely impact pregnancy outcomes. Innate immune signaling by both fetal-derived placental trophoblasts and the maternal decidua must provide antimicrobial defenses at this critical interface without compromising its integrity. Here, we developed matched trophoblast and decidua organoids from human placentas to define the relative contributions of these cells to antiviral defenses at the maternal-fetal interface. We demonstrate that trophoblast and decidua organoids basally secrete distinct immunomodulatory factors, including the constitutive release of the antiviral type III interferon IFN-λ2 from trophoblast organoids and differentially respond to viral infections through the induction of organoid-specific factors. Lastly, we define the differential susceptibility of trophoblast and decidua organoids to

human cytomegalovirus (HCMV) and the transcriptional and immunological responses of these organoids to HCMV infection. Our findings establish matched trophoblast and decidua organoids as ex vivo models to study vertically transmitted infections and highlight differences in innate immune signaling by fetal-derived trophoblasts and the maternal decidua.

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P253

YERSINIA TYPE III-SECRETED EFFECTORS EVADE HUMAN CASPASE-4-DEPENDENT INFLAMMASOME ACTIVATION IN INTESTINAL EPITHELIAL CELLS

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Abstract Content: *Yersinia* are gram-negative zoonotic bacteria responsible for significant disease burden in humans, ranging from recurrent disease outbreaks (yersiniosis) to pandemics (*Yersinia pestis* plague). *Y. pseudotuberculosis* (*Yptb*) is widely prevalent within farmed livestock globally and causes gastrointestinal illness and mesenteric lymphadenitis in immunocompetent humans after ingestion of contaminated meat. *Yersinia* uses its type three secretion system (T3SS) to inject virulence factors termed *Yersinia* outer proteins (Yops) into the host cytoplasm in order to subvert essential components of innate immune signaling. However, T3SS activity triggers host immune pathways, including formation of immune complexes known as inflammasomes. Inflammasomes can cleave and activate caspases, leading to inflammatory cell death and cytokine release aimed at containing infection. The requirement of different inflammasomes, as well as the role of Yops in suppressing inflammasome activation, have been studied extensively in macrophages. However, interactions between *Yersinia* and inflammasomes in intestinal epithelial cells (IECs), the primary site of gastrointestinal *Yersinia* infection remain poorly defined. We found that in human IECs, several *Yptb* effectors work in concert to block T3SS-dependent-inflammasome activation. Notably, *Yptb* lacking the antiphagocytic Yops (YopE and YopH), as well as YopK, a known inhibitor of inflammasome activation, induced robust inflammasome activation in IECs. Deletion of both YopE and YopH led to increased *Yptb* internalization into IECs. We found that caspase-4, which detects cytosolic LPS, is fully required for the inflammasome response to *Yptb* in IECs. Additionally, both caspase-1 and caspase-8 partially contribute to the inflammasome response. Together, our results reveal insight into how IECs employ inflammasomes to sense and respond to *Yersinia* infection and how *Yersinia* is able to evade this response.

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P254

HBV VACCINATION NON-RESPONSIVENESS IS ASSOCIATED WITH THE EXPRESSION OF HLA-DQ2 AND THE PRESENCE OF AUTOANTIBODIES

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Abstract Content: With 95% efficacy, the hepatitis B virus (HBV) vaccination is very effective. However, 5% of all vaccinated individuals do not mount protective antibody responses. In order to understand the phenomenon of vaccination non-responsiveness, we initiated an observational study. We performed longitudinal flow cytometry-based

immunophenotyping of immune cells in the blood before and after a vaccination with Engerix-B. Furthermore, we did HLA typing and collected information about preexisting conditions and medication usage for all study participants.
Analysis of the HLA alleles revealed an accumulation of the MHC-II allele HLA-DQ2 in the group of non-responders. Based on in silico testing of the capacity of DQ2 to present peptides derived from the HBV surface antigen (HBsAg), we hypothesized that DQ2 is a poor presenter of HBsAg-derived peptides. Nevertheless, the deamidation of glutamic acid potentially can create new peptides that would facilitate the presentation of HBsAg-derived peptides presented by DQ2. Tissue transglutaminase 2 (TG2) is known to deamidate glutaminic acid and is expressed in many tissues, including muscle tissue. To address whether reduction of deamidation due to the presence of anti-TG2 autoantibodies impairs peptide presentation and reduces the induction of T cell help, which would result in poor antibody production and vaccination non-responsiveness, we determined anti-TG2 antibodies in plasma samples. Overall, we detected sub-clinically elevated anti-TG2 antibody levels in the group of non-responders, while one non-responder showed even clinically relevant levels of anti-TG2. Furthermore, we established a T cell stimulation assay using peptides with the deamidated amino acids in comparison with peptides derived from the original version of the HBsAg to address our working hypothesis. We compared the T cell responses of DQ2 positive donors with donors expressing MHC-II alleles that are known to be associated with HBV vaccination high-responsiveness to the two different peptide versions.
In summary, first results support our hypothesis that certain MHC-II alleles and autoantibodies play an important role in causing HBV vaccination non-responsiveness.

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LB-17
RAVER 1 CONTROLS SPLICING OF RIPK1 MRNA AND REGULATES CELL DEATH AND INFLAMMATION MEDIATED BY CASPASE-8

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Abstract Content: Pathogens and cytokines can trigger cell death and inflammation via RIPK1 and caspase-8. Previously we have described that upon TAK1 or IKKß inhibition by Yersinia effector protein YopJ, RIPK1 and caspase-8 drive the cleavage of pore-forming molecule Gasdermin D (GSDMD), which results in cell death, accompanied by the release of interleukin (IL)-1ß and IL-18. However, many aspects are unclear on how the RIPK1-caspase-8 axis is regulated during inflammatory cell death. Here we identified splicing factor Raver1 as a new regulator that controls RIPK1 mRNA splicing, expression and function in apoptosis, pyroptosis and inflammation. Macrophages derived from Raver1-deficient mice have diminished cell death and activation of RIPK1, caspase-8, GSDMD, caspase-1, and IL-1ß induced by Yersinia bacteria, or TAK1 blockade plus LPS or TNF/IFNγ cytokines. Loss of Raver1 resulted in alternative RIPK1 mRNA splicing, leading to reduced protein levels as well as increased expression of a truncated inactive splice variant that could bind to RIPK1 and inhibit its ability to induce cell death. Consequently, Raver1-deficient mice are highly susceptible to Yersinia infection and have reduced ability to control bacterial replication. Together, our study suggests that Raver1 controls a narrow set of innate immune responses and emphasizes a crucial role of this splicing factor in tuning inflammatory processes.

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LB-18
INTERACTION BETWEEN MIR-15/16 AND THE LONG NONCODING RNA MALAT1 REGULATES T CELL RESPONSES

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Abstract Content: Proper induction of cytotoxic T cells via the T cell receptor and the costimulatory receptor CD28 is essential for adaptive immunity against viruses, many intracellular bacteria and cancers. Through biochemical analysis of RNA:protein interactions, we uncovered a novel non-coding RNA circuit regulating these facets of cytotoxic T cell biology composed of the long non-coding RNA Malat1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) and the microRNA family miR-15/16. miR-15/16 is a widely and highly expressed tumor suppressor miRNA family important for cell proliferation and survival. miR-15/16 also play important roles in T cell responses to viral infection, including the regulation of antigen-specific T cell expansion and T cell memory. Comparative Argonaute-2 high throughput sequencing of crosslinking immunoprecipitation (Ago2 HITS-CLIP, or AHC) combined with gene expression profiling in normal and miR-15/16-deficient T cells revealed a large network of several hundred direct miR-15/16 target mRNAs, many with functional relevance for T cell activation, survival and memory formation. Among these targets, the long non-coding RNA Malat1 contained the single strongest miR-15/16-dependent AHC signal in T cells. This binding site was also among the strongest lncRNA:miRNA interactions detected in the T cell transcriptome. We used CRISPR targeting with homology directed repair to generate mice with a 5-nucleotide mutation in the miR-15/16 binding site in Malat1. This mutation interrupted Malat1:miR-15/16 interaction, and enhanced the repression of other miR-15/16 target genes, including CD28. Interrupting Malat1 interaction with miR-15/16 decreased cytotoxic T cell activation, including the expression of IL-2 and a broader CD28-responsive gene program. Accordingly, Malat1 mutation diminished memory cell persistence following LCMV Armstrong infection. This study marks a significant advance in the study of long noncoding RNAs in the immune system by ascribing cell-intrinsic, sequence-specific *in vivo* function to Malat1. These findings have implications for T cell-mediated autoimmune diseases, antiviral and anti-tumor immunity, as well as lung adenocarcinoma and other malignancies where Malat1 is overexpressed.

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LB-19

T-CELL DERIVED INTERFERON GAMMA IS REQUIRED FOR RESTRICTION OF MYCOBACTERIUM TUBERCULOSIS IN THE INFECTED MURINE LUNG

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Abstract Content: Although both T cells and IFNγ are critical for immunity against *Mycobacterium tuberculosis* (*Mtb*), adoptive transfer studies in *Mtb*-infected mice have suggested that T cell-derived IFNγ plays only a minor role in immunity against pulmonary *Mtb*. We sought to identify IFNγ-independent T cell pathways that mediate *Mtb* control in the lung. In contrast with prior results, our experiments using both T cell chimeric and adoptive transfer mouse models show that control of *Mtb* bacterial burdens in the lung is dependent on T cell-derived IFNγ. We propose several reasons for the discrepancies between our results and previous work, including increased survival and proliferation of IFNγ-/- T cells compared with WT T cells. To understand macrophage programming in the presence of T cells that can or cannot produce IFNγ, we analyzed lung macrophages in WT, IFNγ-/-, and TCRβδ-/- T cell-chimeric mice using RNAseq and flow cytometry. Absence of T cell-derived IFNγ led to skewing of both infected and bystander macrophages to an M2 alternatively activated phenotype, as well as decreased or delayed recruitment of monocyte-derived macrophages to the lung. Together, these results are consistent with an essential role for T cell-derived IFNγ in establishing protective macrophage programs in the *Mtb*-infected lung.

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LB-20

BASOPHILS SUPPORT OPTIMAL INTESTINAL TH2 CELL RESPONSES

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Abstract Content: Type 2 inflammation is characterized by production of the cytokines IL-4, IL-5 and IL-13 and promotes clearance of gastrointestinal helminths, which infect over 2 billion people worldwide. During infection with the helminth *Trichuris muris*, $CD4^+$ T cells are an essential source of type 2 cytokines that promote parasite clearance, and basophils accumulate at the infection site. Basophils are a rare innate immune cell population that have previously been suggested to interact with $CD4^+$ T cells during helminth infection. However, basophils are not critical antigen presenting cells *in vivo*. Thus, how basophils influence $CD4^+$ T cell function is not fully defined. Our previous work shows that the Notch signaling pathway regulates basophil gene expression programs during infection and drives basophil localization proximal to $CD4^+$ T cells in the inflamed intestine. Mice lacking basophil-intrinsic Notch signaling have reduced frequencies of $Gata3^+$ T helper 2 cells and abrogated $CD4^+$ T cell production of type 2 cytokines, associated with impaired worm clearance. Blockade of Notch signaling in basophils impacts $Gata3^+$ T cell viability and proliferation, while Th2 cell transcriptional programming remains intact. Further, *in vitro* coculture experiments demonstrate that basophils directly promote a Th2 phenotype in $CD4^+$ T cells from *T. muris*-infected mice in a contact-dependent manner. This leads us to hypothesize that direct contact with basophils is required to promote Th2 cell survival and function, likely driving maintenance of Th2 cell fate at a post-transcriptional level. This work provides a greater understanding of how productive basophil-T cell interactions drive fulminant type 2 immunity in the intestine and could provide new therapeutic targets for modulating Th2 function at mucosal surfaces.

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LB-21

THE LACK OF IL-17 RECEPTOR C LIMITS HOST RESISTANCE AND SUSTAINS TYPE 17 INFLAMMATION DURING AIRWAY CHRONIC INFECTIONS BY PSEUDOMONAS AERUGINOSA

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Abstract Content: **Background:** The exaggerated inflammation and tissue damage, associated to persistent infections, such as *Pseudomonas aeruginosa*, are central pathological features of Cystic Fibrosis (CF) disease. The interleukin(IL)-17 inflammatory pathway represents an emerging key player in CF pulmonary disease. In this regard, the IL-17 pathway, through IL-17 receptors A (IL-17RA) was already described. Here, we aimed at dissecting the role of IL-17RA mediated by the interaction with other IL-17 receptors (IL-17RC, IL-17RB, IL17RE) in modulating host resistance against chronic lung infection by *P. aeruginosa* in murine models.
Methods: C57BL/6N mice were infected with *P. aeruginosa* CF clinical strain (AA43) embedded in agar beads and the levels of IL-17 cytokines (IL-17A, IL-17F, IL-17E, IL-17B and IL-17C) were evaluated by ELISA. To directly address the contribution of each IL-17Rs (IL-17RC, IL-17RB, IL-17RE), three knockout (KO) mice for each IL17R were generated by CRISPR/Cas9 technology. Chronic *P. aeruginosa* infection was established in the *Il17rc-/-, Il17rb-/-, Il17re-/-* mice and we evaluated bacterial burdens and host defense, such as proinflammatory cytokines (ELISA) and lung infiltrating cells (FACS), at the early (2 days) and advanced (28days) phase of chronic infection.
Results: We found that chronic infection by *P. aeruginosa* was characterised by the presence of neutrophilic inflammation (CD11+, GR-1hi), T cell recruitment and sustained levels of IL-17 cytokines during the early (2-days) and late (28-days) phases of chronic respiratory infection. Moreover, we exploited KO murine models for Il17rc, Il17rb, Il17re genes in order to address the mutual contribution of each IL-17R to the host resistance. Among Il17rc-/-, Il17rb-/-, Il17re-/- mice, only the lack of Il17rc induced higher bacterial burdens in comparison to wt control, after 2 days and after 28 days post challenge. At the advanced stage of chronic infection, we observed a significantly higher incidence of chronic infection in Il17rc-/- mice in comparison to wt control group. After 28 days post challenge, infected Il17rc-/- mice showed a significantly elevated neutrophilic recruitment in the airways than wt control mice. Concurrently Il17rc-

/- mice were characterized by the presence of CD4 T cells secreting IL-17A and high levels of KC, G-CSF and IL6 cytokines in the overall lung.
Conclusion: Our data demonstrated that, among IL-17 receptors, only lack of IL-17RC may contribute to alter host resistance and promote pulmonary type 17 inflammation during CF chronic infection by *P. aeruginosa*.
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LB-22
IMMUNOLOGICAL HISTORY OF THE LUNG GOVERNS INNATE RESISTANCE TO SARS-COV-2
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Abstract Content: SARS-CoV-2 infection results in diverse outcomes ranging from asymptomatic infection to fatal disease. Co-morbidities, age, host genetics and various other factors can alter susceptibility to infection, but little is known about how the lung microenvironment at the time of exposure impacts outcome. Here we utilize multiple mouse models of respiratory infection and inflammation to understand the impact of recent pulmonary immune history on SARS-CoV-2 (SCV2) replication. Mice previously infected with *Mycobacterium tuberculosis* (Mtb) exhibit 1-3 log reduction in viral titers within one to three days following SCV2 infection. Previous acute pulmonary infection with *Staphylococcus aureus* or Influenza A virus (IAV) preceding SCV2 exposure similarly decreases viral titers 1-2 logs, and even ovalbumin/alum-induced asthma constrains early viral loads. Moreover, a single intranasal administration of toll-like receptor (TLR) agonists prior to infection suppresses early SCV2 viral replication. All these models induce quantitative and qualitative changes to lung resident myeloid cells, and upregulation of interferon (IFN) response markers on pulmonary epithelial cells. Protection mediated by TLR agonist pre-treatment is partially dependent on both type-I-IFN and TNF, suggesting that induction of multiple innate signaling pathways prior to infection can suppress subsequent SCV2 viral loads. Transcriptional analysis of TLR agonist pre-treated lungs also reveals activated macrophage gene expression profiles preceding SCV2 infection. This data suggests that diverse immunological stimuli can non-specifically, yet profoundly, impact SCV2 replication and suggest a potential tissue-resident macrophage (TRM)-epithelial crosstalk in this protective anti-viral state. Thus, SCV2 may benefit from immunologically quiescent environments, indicating that the outcome of SCV2 infection may be highly dependent on the individual's pulmonary immune status at the time of viral exposure.
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LB-23
INTERFERON LAMBDAS ARE EXPRESSED IN TUBERCULOSIS GRANULOMAS AND PROMOTE MACROPHAGE ANTIMYCOBACTERIAL ACTIVITY
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Abstract Content: Granulomas are the pathologic hallmark of *Mycobacterium tuberculosis* (Mtb) infection and their ability to limit Mtb growth depends on a balance of granuloma-level pro- and anti-inflammatory cytokine expression. Hence, identifying the cytokines that regulate protective and detrimental inflammation is important for development of new therapeutics for TB. Type III interferons (IFNλs) may regulate inflammation in tuberculosis (TB), yet little is known

about their expression in granulomas, relationship to disease progression, and whether they differ from type I IFNs (IFNα/β), which are associated with exacerbated TB. We found that IFNλs were expressed in non-human primate lung granulomas and IFNλ content negatively correlated with the bacteria load per granuloma. We found that neutrophils and macrophages were the dominant IFNλ1- and IFNλ4-expressing cell types in granulomas. Interestingly, IFNλ1 and IFNλ4 differed in their subcellular localization, with IFNλ4 showing greater nuclear localization in macrophages than IFNλ1, whereas IFNλ4 was localized in the cytoplasm of neutrophils. Intranuclear IFNλR1 localization was also noted in granulomas and *in vitro* work showed that IFNλ1 stimulation induced nuclear translocation of IFNλR1, suggesting that IFNλ signaling occurs in these lesions. Relatedly, *in vitro* work with monocyte-derived macrophages showed that IFNλ1 upregulated pro-inflammatory gene and protein expression that was distinct from IFNλ4 and IFNα/β. IFNλ1 pre-treatment increased macrophage control over bacterial transcriptional activity in an Mtb reporter strain and this control was accompanied by increased lysosomal acidification. These results suggest that IFNλs may have unique and unappreciated roles in TB that include regulation of macrophage antimycobacterial activity.

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LB-24
YIN AND YANG OF DELTA VERSUS OMICRON

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Abstract Content: Since the identification of SARS-CoV-2 back in 2019, the world has been ravaged by many surges with different variants of concern , varying in transmissibility and infectiousness. Looking back into the trajectory of pandemic, Alpha (B.1.1.7) and Delta (B.1.617.2) had been the most dominant variants circulated worldwide, with Omicron subvariants (BA.1.1.529, BA.1, BA.2, BA.3, BA 4/5) outcompeting them currently. The vaccines have fared exceptionally well by reducing the severity of infections with hospitalisation rates and fatalities. But the virus has outsmarted by constantly evolving new variants which evade neutralisation antibodies and T cell responses. BA 4/5 is presently the most infectious variant circulating around. Constellation of mutations in RBD and NTD of spike protein in Omicron variant has been the prime reason for evasion of neutralizing antibodies and therapeutic monoclonals. With passage of time more mutations incurred, ensuing in more evading (sotrovimab sensitive to sotrovimab resistant) subvariants like, BA 4/5. BA 4/5 showed reduced neutralization by serum from individuals vaccinated with triple dose, compared to BA 1/2 – substantiates it further.
Lesser spike protein cleavage and fusogenicity, lesser hypercoagulability and cell death with reduced usage of TMPRSS2 coupled with enhanced binding with ACE2 – attributes to low severity and high transmissibility of Omicron in comparison to Delta.
Initially, the Omicron variant (BA.1.1.529) was found to be extremely less sensitive to neutralization by antibodies than Delta and other variants in convalescent patients and m-RNA vaccinated patients (double dose). Booster regimen of triple dose was found to confer more protection to Omicron variant, albeit less than Delta. Depleting $CD8^+$T and $CD4^+$T in B cell deficient mice had higher viral burden with respect to control- which is suggestive of T cell mediated protection in Omicron infections. Indeed, $CD4^+$T/ $CD8^+$T cell functionalities and corresponding memory response was not much changed for Omicron variant. Interestingly, reports also state more patients with prior infection and/or vaccination had drastic reduction (more than 50%) in T cell response with Omicron spike than Delta spike (21% versus 9.7%). Similar trend was observed with booster vaccinations too.
The surge of Omicron juxtaposed with spring and summer- the seasons for allergy, with pollen as the major allergen. Individuals with auto-antibodies against type 1 IFNs has been known to be predisposed to COVID-19 severity. IFN-α/β are known to negatively regulate Th2 function (key driver of allergic response) by IgE mediated mast cell and basophil degranulation. Hence, seasonal changes can perhaps play a role in the recent COVID-19 surge too. Interestingly, TMPRS22 level is positively regulated by Th2 response indicating a new infection threat by a variant using TMPRS22 extensively.
With waning humoral and cellular immunity against the Omicron variant, bolstering the nasal mucosal immunity by enrichment of tissue resident memory T cells and B cells and circulating T/B cells with multiple booster dosages might

be a good intervention strategy. Upper respiratory tract (URT) dysbiosis might be an important phenomena in the disease pathogenesis as the virus seeds initially in the URT. Hence, restoring URT microbiota could be helpful as an intervention. Lastly, endosomal acidification inhibitors coupled with anti-allergic could be helpful for variants less using TMPRS22

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LB-25

CALPROTECTIN IS INVOLVED IN EOSINOPHIL EXTRACELLULAR TRAPS IN CHRONIC RHINOSINUSITIS

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Abstract Content: ***Background****: Calprotectin is an antimicrobial peptide primarily secreted by neutrophils. Furthermore, calprotectin secretion increases in patients with chronic rhinosinusitis (CRS) with polyps (CRSwNP) and positively correlates with neutrophil markers. However, eosinophils have a dominant role in the pathophysiology of CRSwNP. Therefore, this study investigated calprotectin expression in eosinophils and eosinophil extracellular traps (EETs) and explored associations between tissue calprotectin expression and the clinical findings of patients with CRS.*
Methodology/Principal*: We included 63 participants with CRS, classifying them based on their Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC) score. Then, we performed haematoxylin and eosin staining, immunohistochemistry and triple immunofluorescence with calprotectin, myeloperoxidase and major basic protein (MBP) with tissues from 12-week-old eosinophilic CRS mice and the participants. Finally, we analysed correlations between calprotectin and the clinical data.*
Results*: Calprotectin positive cells co-localised with MBP-positive cells in eosinophilic CRS mouse and human tissues. Calprotectin was also involved in EETs and neutrophil extracellular traps. The number of calprotectin-positive cells in the tissue positively correlated with the number of tissue and blood eosinophils. Additionally, calprotectin in the tissue correlated with the olfactory function, Lund-Mackay computed tomography and JECSREC scores.*
Conclusions*: Both neutrophils and eosinophils expressed calprotectin. Calprotectin may be essential for the innate immune response based on its EET involvement. Therefore, calprotectin expression in CRS tissue could be a disease severity biomarker.*

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LB-26

REPRESSION OF THE ARYL HYDROCARBON RECEPTOR PREVENTS OXIDATIVE STRESS AND FERROPTOSIS OF INTESTINAL INTRAEPITHELIAL LYMPHOCYTES

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Abstract Content: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that buoys intestinal immune responses. AHR induces its own negative regulator, the AHR repressor (AHRR). Here we show that AHRR is vital to sustain intestinal intraepithelial lymphocytes (IEL). AHRR deficiency reduced IEL representation in a cell-intrinsic fashion. Single-cell RNA-sequencing revealed an oxidative cell stress profile in *Ahrr*$^{-/-}$ IEL. *Ahrr* deficiency unleashed AHR-induced expression of CYP1A1, a monoxygenase that generates reactive oxygen species, increasing redox imbalance, lipid peroxidation and ferroptosis in *Ahrr*$^{-/-}$ IEL. Dietary supplementation with selenium or Vitamin-

E to restore redox homeostasis rescued *Ahrr*$^{-/-}$ IEL. Loss of IEL in *Ahrr*-deficient mice caused susceptibility to *Clostridium difficile* infection and dextran sodium sulfate-induced colitis. Inflamed tissue of IBD patients showed reduced *Ahrr* expression that may contribute to disease. We conclude that AHR signaling must be tightly regulated to prevent oxidative stress and ferroptosis of IEL to preserve intestinal immune responses.

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LB-27
DURING ASPERGILLUS INFECTION, MONOCYTE-DERIVED DCS, NEUTROPHILS, AND PLASMACYTOID DCS ENHANCE INNATE IMMUNE DEFENSE THROUGH CXCR3-DEPENDENT

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Abstract Content: *Aspergillus fumigatus*, a ubiquitous mold, is a common cause of invasive aspergillosis (IA) in immunocompromised patients. Host defense against IA relies on lung-infiltrating neutrophils and monocyte-derived dendritic cells (Mo-DCs). Here, we demonstrate that *Aspergillus*-infected murine Mo-DCs and neutrophils recruited plasmacytoid dendritic cells (pDCs) to the lung by releasing the CXCR3 ligands, CXCL9 and CXCL10, in a Dectin-1/Card9- and type I and III interferon-signaling dependent manner, respectively. During aspergillosis, circulating pDCs entered the lung in response to CXCR3-dependent signals. Using a targeted pDC ablation strategy, we found that pDCs were essential for host defense in the presence of normal neutrophil and Mo-DC numbers. Although interactions between pDC and fungal cells were not detected, pDCs regulated neutrophil NADPH oxidase activity and conidial killing. Thus, pDCs, prototypical anti-viral cells, participate in innate immune crosstalk that underlies mucosal antifungal immunity and act as positive feedback amplifiers of neutrophil effector activity against inhaled mold conidia.

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09. Regulation of host physiology and pathology

P255
IL-13 INDUCTION OF HYALURONAN AS A DRIVER OF ACUTE LUNG DISEASE

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Abstract Content: We previously reported that Interleukin-13 (IL-13) is associated with the need for mechanical ventilation in COVID-19 patients, and that individuals prescribed Dupilumab, a mAb that blocks IL-13 and IL-4 signaling, had less severe disease. Using a mouse model of SARS-CoV-2, we demonstrated an immunopathogenic role for IL-13 during acute viral infection. While SARS-CoV-2 infection in mice induced only moderate type 2 immune activation, blockade of IL-13, led to a significant reduction in hyaluronan synthase 1 (*Has1*) expression and reduced accumulation of the polysaccharide, hyaluronan (HA) in the lung. These findings are consistent with increasing evidence for HA in lung pathology in Covid-19 and in other acute viral respiratory infections. Here we describe delivery of recombinant IL-13 to the lung, demonstrating the ability of this cytokine to directly induce accumulation of HA in the lung and airways. The role of HA in lung pathology is multi-factorial. As a very large hydrophilic molecule, HA promotes water retention in the lung, but also has a wide range of immune regulatory functions, influenced by cross-linking with matrix-associated molecules and interactions with HA receptors, such as CD44. Indeed, blockade of CD44, reduced mortality in SARS-CoV-2 infected mice. While IL-13 has known roles in the regulation of other extra-cellular matrix (ECM) components such as collagen and mucins, the induction of HA suggests that IL-13 has an even broader role in ECM regulation. We are using IL-13 delivery to unravel the mechanistic pathways behind our discovery of this new function for IL-13.

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P257

ELUCIDATING THE ROLE OF NUCLEATED RED BLOOD CELLS TO UNCOVER KNOWLEDGE OF IMMUNE REGULATION DURING PREGNANCY

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Abstract Content: Red blood cells (RBCs) are not typically considered active mediators of immune responses. This dogma stems from the fact that RBC precursors discard organelles as they mature, thus losing the ability to alter gene expression in response to stimuli. In contrast, in non-mammalian vertebrates, mature RBCs retain their organelles and orchestrate immunological processes. Intriguingly, nucleated RBCs (nRBCs) circulate in human fetuses and neonates. Due to high evolutionary pressure for successful reproduction, circulation of nRBCs during pregnancy is likely important. However, the role of nRBCs *in utero* remains unknown, leaving a critical gap in knowledge. To define the role of human nRBCs during pregnancy, I queried single cell RNA-sequencing data and found that transcriptomics support nRBCs as putative mediators of antimicrobial and immunotolerant responses.
Pregnancy is an astounding immunological feat that requires overriding our intrinsic immune response to mount defense against non-self. Mechanistic details underlying this immune shift are largely undiscovered. In line with my hypothesis that nRBCs contribute to this immune shift and mediate fetal tolerance, I found that conditioned media from nRBCs induces differentiation of regulatory T cells *in vitro*, suggesting that nRBCs modulate immune activity via a secreted factor. I found that nRBCs express several soluble immunomodulatory factors including cytokine Growth/Differentiation Factor-15 (GDF15). Future studies aim to define the contributions of GDF15 to nRBC-mediated immunosuppression.
I also found that human nRBCs express molecular machinery required for pathogen recognition and response. Unexpectedly, I found that nRBCs constitutively express MHC Class II and co-stimulatory molecules, a hallmark of specialized antigen-presenting cells, as well as pattern recognition receptors capable of detecting pathogens and initiating an antimicrobial response. These data suggest a putative functionality for nRBCs in mediating innate and adaptive immunity *in utero*. I am currently investigating the antimicrobial immune properties of nRBCs.
Taken together, these findings shed light on an unexpected orchestrator of fetal immune activity. A better understanding of the fetal immune system has potential to help us understand health and disease during pregnancy, as a neonate, and likely every stage of life afterward.

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P258

IL-38 MODULATES SUPEROXIDE PRODUCTION DURING OXIDATIVE STRESS IN KERATINOCYTES

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Abstract Content:
Introduction: Interleukin (IL)-38 is constitutively expressed in epidermal keratinocytes and has shown broad anti-inflammatory properties, raising hopes for its potential therapeutic utility. However, its functions and molecular mechanisms of action remain incompletely understood. A yeast-two-hybrid screen identified 4 potential IL-38 interaction partners, which are structurally or functionally linked to stress granules. Among them, peroxiredoxin (PRDX) 1 and PRDX2 are also key regulators of redox homeostasis. The natural exposition of skin to oxidative stress

and the well-documented role of reactive oxygen species in inflammation led us to explore the role of IL-38 during oxidative stress in an immortalized normal human keratinocyte (NHK) cell line.
Methods: Protein interactions were assessed by co-immunoprecipitation. Menadione (75 µM) was used to induce oxidative stress in NHK cells overexpressing IL-38 (NHK/38) or the irrelevant bacterial protein β-galactosidase (NHK/β-Gal). Protein subcellular localization was explored by cellular fractionation and Western blotting, and by immunostaining. Oxygen consumption rate (OCR) and superoxide production were measured respectively using the Seahorse platform and a colorimetric WST-1 assay. *In silico* modelling was based on the human IL-38 crystal structure (PDB: 5BOW), and the protein conformational space was analyzed through accelerated molecular dynamics. Disulfide bridges were mapped in recombinant human IL-38 by combined top-down and bottom-up nanoLC-MS/MS.
Results: We confirmed interaction of IL-38 with PRDX1 and PRDX2 in HEK293 cells and validated our *in vitro* oxidative stress model in NHK/38 cells by monitoring PRDXs dimerization. Upon menadione treatment, IL-38 relocalized from the cytoplasm of NHK/38 cells to the nucleus and to a cytoskeletal/insoluble fraction. Immunostaining revealed relocalization of IL-38 to stress granules, where it co-localized with Ras GTPase-activating protein-binding protein 1 (G3BP1). To assess the function of IL-38 during oxidative stress, we compared NHK/38 and NHK/β-Gal cells. IL-38 overexpression reduced the menadione-induced conversion of oxygen into superoxide. Scanning of the human IL-38 sequence revealed 2 low complexity regions (LCRs) that might mediate its assembly into stress granules. These LCRs are not intrinsically disordered in the IL-38 crystal, which represents a reduced form of the protein. However, disulfide mapping and *in silico* modelling revealed the formation of 2 disulfide bridges in IL-38, of which one induced conformational changes that increased the surface exposition of the 2 LCRs. Oxidation sensitive cysteines may thus act as redox switches to modify the conformation and regulate the cellular function of IL-38.
Conclusion: Our results link intracellular IL-38 to structures and proteins involved in stress responses and highlight effects of IL-38 in keratinocytes during oxidative stress. IL-38 protein itself appears to undergo redox modifications influencing its structure and biochemical properties. Overall, these observations suggest a role for IL-38 in modulating cellular stress responses in the epidermis.

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THE COMPLEX ALLERGEN HOUSE DUST MITE (HDM) DRAMATICALLY INCREASES THE ABUNDANCE OF THE AUTOPHAGY CARGO ADAPTER SQSTM1 IN MACROPHAGES AND ENGAGES AN ENDOPLASMIC RETICULUM-ASSOCIATED NON-DEGRADATIVE AUTOPHAGY MECHANISM.

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Abstract Content: Autophagy is an essential homeostatic process directing lysosomal degradation of cytosolic components. Autophagic mechanisms have been linked to allergic asthma and macrophage phenotypes, however the contribution of degradative autophagy to these processes is unclear. We evaluated the direct effect of the major human allergen, house dust mite (HDM), on responses in macrophages. We observed an increase in the number of LC3+ foci (autophagosomes) in bone-marrow derived macrophages (BMM) in a manner similar to the autophagy inducer Torin 1. Further, electron microscopy revealed an increase in double membraned vesicles in response to HDM and Torin 1. While Torin 1 induced a specific increase in lipidated LC3, treatment with HDM caused equal increases in both non-lipidated and lipidated LC3. Torin 1 stimulated autophagic degradation as measured by reduction of p62/SQSTM1. In contrast, SQSTM1 levels increased dramatically in response to HDM. The increase was blocked by actinomycin D and cycloheximide, indicating that new gene transcription and protein synthesis are required for the HDM-induced effect. An increase in SQSTM1 is often interpreted as a block in autophagic degradation in lysosomes. To determine whether HDM suppressed autophagic flux, we utilized RAW 264.7-Difluo mLC3 cells expressing a chimeric LC3 protein B that is fused to green fluorescent protein (GFP) and red fluorescent protein (RFP). GFP signal is quenched in acidic environments while RFP signal is not. Treatment of cells with Torin 1 or deprivation of amino acids resulted in formation of LC3 puncta that were largely GFP-, RFP+ indicative of delivery of LC3 to the acidic amphisome and ultimately the lysosome. However, HDM induced LC3-puncta that remained GFP+ and RFP+. In addition, inclusion of HDM had no effect on the loss of GFP signal induced by amino-acid deprivation

or Torin 1 indicating that HDM does not block classic, degradative autophagy. HDM has been shown to induce endoplasmic reticulum (ER) stress in epithelial cells and ER stress has been linked to an increase in SQSTM1. To evaluate ER-participation, we performed double staining for calreticulin, a unique ER luminal resident protein, and SQSTM1 in HDM-treated BMM. We observed that calreticulin and SQSTM1 colocalized in foci induced by HDM treatment while there were no obvious calreticulin and SQSTM1 foci in the control and Torin 1 treated cells. Taken together, these results suggest that HDM dramatically increases the abundance of SQSTM1 in macrophages and engages an ER-associated, non-degradative autophagy mechanism.

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P260

INTESTINAL EPITHELIAL RESPONSES TO IL-17 IN HUMAN INTESTINAL ORGANOIDS

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Abstract Content: BACKGROUND: Th17 cells and their main secreting cytokine interleukin-17A (IL-17) are considered as the main pathogenic factors in inflammatory bowel diseases (IBDs). However, anti-IL-17 neutralizing antibodies, a theoretically curative medication for IBDs, paradoxically aggravated intestinal inflammation. The mechanisms by which it mediates the protective and pathologic effects of IL-17 remain unclear in the intestinal epithelium.
METHOD: The intestinal epithelial responses induced by IL-17 was evaluated using the human small intestinal organoid (enteroid) model.
RESULTS: Organoid-forming efficiency, cell viability and proliferation of enteroids were decreased in proportion to the concentration of IL-17, which did not differ between the enteroids derived from controls and patients with Crohn's disease. Bulk RNA-sequencing revealed the enrichment of secretion signaling in IL17-treated enteroids. Among its components, PIGR was up-regulated significantly as the concentration of IL-17 increased, resulting in IgA transcytosis and protective role against pathogens. The IL-17-induced cytotoxicity was predominantly mediated by pyroptosis with activation of CASP1 and cleavage of GSDMD. Single-cell RNA- sequencing identified pyroptosis occurred actively in intestinal stem cells (ISCs) and enterocytes. Anti-IL-17 antibody, izekizumab, completely restored IL-17-induced cytotoxicity, but suppressed mucin secretion and IgA transcytosis. CASP1 inhibitor, Ac-YVAD-cmk, restores cytotoxicity induced by IL-17, without impairing its beneficial effects.
CONCLUSION: IL-17 induces pyroptosis of ISCs and enterocytes, as well as mucin secretion and PIGR-induced IgA transcytosis. Paradoxical gastrointestinal effects of IL-17 neutralizing antibodies may be associated with inhibition of mucin secretion and IgA transcytosis. The inhibition of pyroptosis using the CASP1 inhibitor prevents the cytotoxicity induced by IL-17 without compromising its beneficial effects.

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P261

IMMUNE CELL-ENRICHED SINGLE-CELL RNASEQ OF BRAIN PARENCHYMAL CELLS FROM ALZHEIMER'S DISEASE MOUSE REVEALED INCREASED INFILTRATION OF CD8+ T CELLS WITH ALTERED GENE EXPRESSION IN CHOROID PLEXUS CELLS.

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Abstract Content: Alzheimer's disease, a progressive neurological disorder, is the most common cause of dementia. However, treatment strategies targeting amyloid beta have not been as successful as expected. Recently, the role of neuroinflammation and adaptive immune response in the pathogenesis of Alzheimer's disease is getting more attention. Here, we performed immune cell-enriched single-cell RNA sequencing of the brain parenchymal cells from the 12-month-old 5xFAD mice, an Alzheimer's disease mouse model, and a total of 9,925 single cells was analyzed. T cells and choroid plexus cells show a clear difference in population between 5xFAD mice and the littermate control. The altered gene expression pattern was observed in choroid plexus cells of the 5xFAD mice showing higher expression of MHC-I / MHC-II related molecules and IFN-γ stimulated genes than the cells from the control mouse. Subsequent sub-clustering of T cells in 5xFAD mice revealed various T cell subtypes. CD8+ resident memory T cell (TRM), a memory T cell subtype characterized by tissue retention marker *Itgae and Cd69*, is the most prevalent type of T cell in 5xFAD mice. This study suggests a marked alteration of immune status in the brain of the Alzheimer's disease model and opens possibilities for the modulation of adaptive immune responses as a potential clinical target for Alzheimer's disease.

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INTERLEUKIN-17 GOVERNS HYPOXIC ADAPTATION OF INJURED EPITHELIUM

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Abstract Content: Epithelial barriers limit the penetration of disease-causing environmental agents. Organismal survival thus impinges on rapid barrier re-establishment following damage. Epithelia are surveilled by an arsenal of lymphocytes that rapidly respond to injury. If and how extrinsic microenvironmental signals from repair-associated lymphocytes intersect with primitive cellular stress responses during tissue repair remain incompletely defined. To tackle this question, we employed scRNA-seq, spatial transcriptomic, and mouse genetic strategies to track communications between lymphocytes and wound edge epithelium. We uncovered that injury-induced hypoxia is insufficient to trigger hypoxia inducible factor 1 alpha (HIF1a) in damaged epithelium. Instead, multimodal analyses, and functional in vivo studies reveal that interleukin (IL)-17A, emanating from pre-existing skin resident RORgt^{+} cells, is necessary and sufficient to activate HIF1a in normoxia and chronic hypoxia. Protein kinase B (AKT) and ERK1/2 signaling proximal of IL-17RC receptor activates mammalian target of rapamycin (mTOR) pathway and consequently HIF1a activation. The IL-17A–HIF1a transcriptional axis drives functional program of glycolytic metabolism in damaged epithelium. Epithelial-specific loss of IL-17RC, HIF1a, or blockade of glycolysis impairs migration and derails tissue repair. Our findings underscore the role of inflammatory cytokines in directing epithelial migratory, and metabolic programs to expedite healing and illuminate the immune cell–derived inputs in cellular adaptation to hypoxic stress during tissue repair.

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ALLEVIATES HEREDITARY ANGIOEDEMA BY PROTECTING ENDOTHELIUM AGAINST BK-INDUCED INTERENDOTHELIAL CELL GAP FORMATION WITH CU06-1004

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Abstract Content: Over released of vasoactive peptide bradykinin (BK) due to mutation in SERP ING 1 gene is the leading cause of hereditary angioedema (HAE). BK directly activates endothelial cells and increases vascular permeability by disrupting the endothelial barrier, then causes angioedema involving the skin, gastrointestinal tracts, and submucosal tissues of the upper airway in patients. The various pharmacological treatment options were became available during last decade; however, they are yet limited and poses huge economic burden to patients. To optimize HAE management, more therapeutic options need to be develop; therefore, we evaluated the prophylactic effect of CU06-1004, an endothelial dysfunction blocker, in BK-induced hyperpermeability and furthermore in C1-INH-HAE murine model. To investigate the effect of CU06-1004 against BK in vivo, we pre-treated the drug and induced vascular leakage on WT mice through intravenous injection of BK. As a result, CU06-1004 protected mice from BK-induced hyperpermeability on capillaries and post-capillary venules by reducing interendothelial gap formation. Consistent with in vivo findings, CU06-1004 protected the HUVEC monolayer against BK-induced endothelial barrier disruption and intercellular gap formation. The drug inhibited VE-cadherin phosphorylation and actomyosin contraction by suppressing Src and MLC activation. Furthermore, we examined the efficacy of CU06-1004 in the C1-INH-HAE murine model (SERPING1 KO) and showed that the administration of CU06-1004 significantly alleviates increased vascular permeability in SERPING1 KO mice. Taken together, CU06-1004 significantly ameliorates vascular hyperpermeability in the C1-INH-HAE murine model by protecting endothelial barrier function against BK stimulation. Therefore, protecting endothelium with CU06-1004 may become a potential therapeutic approach for C1-INH-HAE patients to prevent angioedema.

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DIFFERENTIALLY EXPRESSED GENES IDENTIFIED IN HUMAN ABDOMINAL AORTIC ANEURYSM (AAA) LESIONS

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Abstract Content: AAA is a complex multifactorial and life-threatening immunological disease. We examined certain gene expression profiles in AAA lesions from patients with AAA vs normal aortas by cDNA microarray and real-time quantitative RT-PCR (qRT-PCR). Microarray comprised of 588 genes primarily of the cardiovascular system was used, and results were validated by qRT-PCR. Student's t-test was used and candidate genes were further considered using the log2 ratio fold change. 35 of 588 genes were differentially expressed, with either log 2 ratio of AAAs/Controls ≥1 (upregulated; 20 genes) or log 2 ratio of AAAs/Controls ≤-1 (downregulated; 15 genes) in AAA lesions vs. normal aortas. The differential expression of 25 of these 35 genes (71%) was statistically significant. The expression of the following genes was upregulated in AAA vs normal aortas: MMP-9, TIMP3, apolipoprotein D (APOD), APOE, calcium and integrin-binding protein (CIB), collagen 1α1 subunit (COL1A1), COL6A3, COL15A1, decorin, endoglin, H19, ICAM1, ICAM2, membrane-associated phospholipase A2, monocyte chemotactic protein 1, phospholipid transfer protein, placental thrombin inhibitor (PTI), PDGFR β subunit, urokinase-type PA, and von Willebrand factor. The expression of the following genes was downregulated in AAA vs normal aortas: acetyl-CoA acyltransferase (ACAA), α-actinin 1 cytoskeletal isoform (ACTN1), amiloride-sensitive epithelial sodium channel β (SCNEB), atrial natriuretic peptide receptor A (ANPA), biglycan, cardiac phospholamban (PLB), COL4A4, COL11A2, endothelial PA inhibitor-1, extracellular superoxide dismutase, galectin-1, LDL receptor-related protein 1, GAP junction α-1 protein (GJA1),

integrin α8 (ITGA8) and MUC18. The differential expression in AAA lesions vs normal aortas of the following genes is reported for the first time. Six genes were upregulated and the results were statistically significant: APOD, CIB, COL6A3, COL15A1, ICAM2 and PTI. Eight genes were downregulated: ANPA, ACTN1, GJA1, SCNEB, PLB and ACAA, COL11A2, ITGA8, and the results were statistically significant only with the first five. Remaining results confirmed previous reports. Agreement in general was observed between qRT-PCR and microarray results. A number of pathways are involved in the pathogenesis of AAA.

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P265

FIBROBLASTS-PRODUCED INTERLEUKIN 11 CONFERS RESISTANCE TO DEXTRAN SULFATE SODIUM (DSS)-INDUCED COLITIS IN MICE

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Abstract Content: Intestinal homeostasis is tightly regulated by epithelial cells, leukocytes, and stromal cells, and its dysregulation is associated with inflammatory bowel diseases. Interleukin (IL)-11, a member of the IL-6 family of cytokines, is produced by inflammatory fibroblasts during acute colitis. However, the role of IL-11 in the development of colitis is still unclear. Herein, we showed that fibroblast-produced IL-11 ameliorated DSS-induced acute colitis in mouse models. We found that deletion of *Il11ra1* or *Il11* rendered mice highly susceptible to DSS-induced colitis compared to the respective control mice. The number of apoptotic epithelial cells was increased in DSS-treated *Il11ra1*- or *Il11*-deficient mice. Moreover, we showed that IL-11 production was regulated by reactive oxygen species (ROS) produced by *Lysozyme M*-positive myeloid cells. These findings indicate that fibroblast-produced IL-11 plays an important role in protecting the mucosal epithelium in acute colitis. Myeloid cell-derived ROS contribute to the attenuation of colitis through the production of IL-11.

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P266

NEURONAL INTERLEUKIN-13 AND ITS RECEPTOR ARE INVOLVED IN SYNAPTIC AND NEURONAL PHYSIOLOGY

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Abstract Content: Cytokines are no longer considered mediators uniquely involved in immune-system coordination: some cytokines secreted by neurons control the reactive state of microglia and astrocytes in the brain. Roles for neuronal cytokines beyond the neuro-glia crosstalk have not been reported. cytokines. Here we report the characterization of IL-13 (and its receptor IL-13R1a) as synaptic proteins involved in synaptic plasticity and neuroprotection, a function unrelated to the established role in humoral immunity, allergy and responses to parasites. We have used single-molecule in situ hybridization to show that IL-13 is highly expressed in neurons, and super-resolution confocal and STED microscopy and subcellular fractionation to demonstrate that IL-13 is enriched in presynaptic terminals where IL-13Ra1 is clustered in post-synaptic densities in mice, rats and human brain samples. Phospho-proteomics and electrophysiology revealed that IL-13 drives the phosphorylation of multiple pre- and post-synaptic proteins, notably glutamate receptors, resulting in increased synaptic activity and post-synaptic excitation. IL-13 activates multiple transcriptional programs in neurons, most notably CREB and STAT6, which are instrumental in inducing immediate-early genes and anti-apoptotic responses. Importantantly, neuronal IL-13 is upregulated by Traumatic Brain Injury in mice as well as in humans (as shown by the elevation in human injured cerebral cortex and

in human CSF from TBI patients). Chemogenetic approaches show that elevation of IL-13 is dependent on neuronal firing but plateau quickly if forced activity is imposed. We used label-free holotomography microscopy to show that IL-13 reduces the vulnerability of neurons to cytotoxic injuries; surprisingly, IL-13 is effective at low but not at high doses. Thus, IL-13 is a previously unrecognized neuronal cytokine acting as physiological modulator of synaptic function, glutamate receptor expression and neuronal plasticity, and playing neuroprotective roles upon neuronal injury.

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P267

DYSREGULATED TISSUE NICHE POTENTIATES RESIDENT LYMPHOCYTES TO SUPPRESS AN INTERFERON-SENSITIVE STEM CELL RESERVOIR IN EMPHYSEMA

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Abstract Content: Aberrant tissue-immune interaction is the hallmark of diverse chronic lung diseases. Here we sought to define these interactions in emphysema, a progressive disease characterized by infectious exacerbations and loss of alveolar epithelium. Single cell analysis of human emphysema lungs revealed the expansion of tissue-resident lymphocytes (TRLs). Murine studies identified a stromal niche for TRLs that expresses *Hhip*, a disease-variant gene downregulated in emphysema. Stromal-specific deletion of *Hhip* induced the topographic expansion of TRLs in the lung that is mediated by a hyperactive hedgehog-IL7 axis. 3D immune-stem cell organoids and animal models of viral exacerbations demonstrated that expanded TRLs suppressed alveolar stem cell growth through Interferon gamma. Finally, we uncovered an interferon-sensitive subset of human alveolar stem cells that is preferentially lost in emphysema. Thus, we delineate a novel stromal-lymphocyte-epithelial stem cell axis in the lung that is modified by a disease-variant gene and confers host susceptibility to emphysema.

Disclosure of Interest: None Declared
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P268

A NOVEL ROLE FOR INTERLEUKIN-22-SIGNALLING IN MODULATING MUCOSAL EPITHELIAL CELL ANTIGEN PRESENTATION MACHINERY TO CONTROL DISEASE

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Abstract Content: The mucosal surfaces of the gastrointestinal and respiratory tracts are strategically shielded with versatile epithelial cells which constitute a physical barrier that segregate mucosal immune system from the luminal microbiota and importantly regulate host tolerance and immunity. Major Histocompatibility complex (MHC) II is dynamically expressed on these epithelial cells and plays key roles in tissue-resident immune cell activation, epithelial cell differentiation and intestinal tumorigenesis. The expression of MHC II is induced in response to inflammation, parasitic infections, and upon exposure to microbiota and reported to be increased in inflammatory bowel disease (IBD). However, the regulation of epithelial cell-specific MHC II during homeostasis is yet to be explored. We have discovered a novel role for IL-22 in suppressing epithelial cell MHC II via the regulation of endoplasmic reticulum (ER) stress, using animals lacking the Interleukin-22-receptor (IL-22RA1), primary human and murine intestinal and respiratory organoids, and a murine model of infection (pneumovirus) or with epithelial cell defects (*Muc2* missense mutation). Animals lacking IL-22RA1 have significantly higher MHC II expression on small intestinal and respiratory epithelial cells at baseline. IL-22 directly downregulated Interferon-γ-induced MHC II on primary mucosal epithelial

cells by modulating the expression of MHC II antigen A alpha (*H2-Aα*) and Class II transactivator (*Ciita*), a master regulator of MHC II gene expression. Using chemical-induced and spontaneous mouse models of IBD (colitis), we show that in non-infectious inflammatory disease, IL-22-induced suppression of MHC II expression leads to an improvement in the paracellular barrier permeability, restoration of goblet cells, reduced inflammation and attenuated disease. However, during acute infection of respiratory mucosa, IL-22-mediated suppression of epithelial cell-MHC II expression leads to an aberrant immune response, suppression of cellular stress, increased mucus secretion, severe pathology, and increased mortality. Our work highlights the context dependent role of IL-22 and the potential implications for future IL-22-based therapeutics as long-circulating recombinant IL-22 are currently in clinical trials for several indications including inflammatory bowel disease.

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P269
IDENTIFICATION OF NOVEL REGULATORS CONTROLLING TRISTETRAPROLIN STABILITY

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Abstract Content: Fine-tuning of innate immune signaling is essential to restore homeostasis and to avoid the development of inflammatory disorders. In this context the RNA binding protein Tristetraprolin (TTP) plays a central role as gatekeeper of cellular homeostasis by promoting the degradation of several proinflammatory cytokines transcripts. Indeed, mice lacking TTP develop profound inflammatory syndromes.
TTP activity and stability is critically controlled by its phosphorylation status and by the intrinsically unstructured nature of the protein itself. Indeed, TTP, as well as many intrinsically disordered proteins (IDPs), is rapidly degraded in a proteasome- dependent manner. However the molecular mechanism and the factors mediating its degradation have remained elusive.
In order to identify factors controlling TTP stability, a genome-wide CRISPR/Cas9- genetic screen will be performed. The main premise of the designed gain-of-signal screening strategy is that cells, in which genes mediating TTP degradation are knocked-out, will show an increased stability of a mCherry-TTP reporter. The genes targeted in these populations with an increased fluorescent signal will be determined, providing candidates for TTP degradation.
The molecular mechanism controlling TTP turnover will then be elucidated, providing molecular insights in how TTP is negatively regulated through its coordinated degradation.

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LB-28
DIETARY PROTEIN SHAPES THE PROFILE AND REPERTOIRE OF INTESTINAL CD4+ T CELLS

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Abstract Content: During routine eating, massive quantities of diet-derived antigens are absorbed through the intestine which must be tolerated by the immune system. However, the polyclonal $CD4^+$ T cell response to food remains largely unexplored. To characterize physiological intestinal T cell responses to food protein, we developed a protein antigen-free solid diet which we either supplemented with a single food protein or compared to complex chow. We found that at steady state, chow diet promotes epithelial adaptation and cytotoxic programming of intestinal $CD4^+$ T cells, a pathway which is further boosted by signals from the microbiota. In a polyclonal setting, exposure to dietary

proteins induced public clones of mature intestine-adapted CD4⁺ T cells, indicating that food antigen-specific intestinal CD4⁺ T cells are generated in the course of eating. Finally, we show that protection against cholera toxin-induced food allergy was associated with increased intestinal influx and clonal expansion of regulatory T cells (Tregs), while development of food allergy was associated with influx of pro-inflammatory Th17. Altogether, these findings suggest that tightly regulated maintenance of cytotoxic epithelium adapted CD4⁺ T cells in addition to Tregs may be critical for preventing inappropriate immune responses to food and subsequent disease.

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LB-29
A CONSERVED ANTIGEN DERIVED FROM COMMENSALS OF THE BACTEROIDETES PHYLUM DRIVES REGULATORY IMMUNE RESPONSES IN THE INTESTINE

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Abstract Content: Considering the highly diverse immune repertoire and equally diverse microbiome, a variety of intestinal T cell responses against commensals and pathobionts with specific functions are expected. Intestinal T lymphocytes can differentiate into functional subtypes with regulatory or effector functions in response to the microbiome. Two subsets of CD4⁺ T lymphocytes can regulate adaptive immunity at the intestinal mucosa: peripherally induced regulatory T cells (Treg) and CD8αα-expressing intraepithelial lymphocytes (CD4IELs). The development of CD4IELs depends on the microbiota. However, the identity of the microbial antigens recognized by CD4⁺ T cells that can differentiate into CD4IELs remains unknown. We identified β-hexosaminidase, a conserved enzyme across commensals of the Bacteroidetes phylum, as a driver of CD4IEL differentiation. In a mouse model of colitis, β-hexosaminidase–specific lymphocytes partially protected against intestinal inflammation, in a Treg cell-independent manner. Thus, commensal-specific lymphocytes that can recognize a variety of abundant commensals of the Bacteroidetes phylum can also elicit a regulatory immune response at the intestinal mucosa. Defining the T cell responses against different commensals is essential to understand how they can each contribute to the maintenance of intestinal homeostasis.

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LB-30
MATERNAL IL-33 SIGNALING IS ESSENTIAL FOR UTERINE TISSUE REMODELING AND PREGNANCY PROGRESSION IN MICE

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Abstract Content: IL-33 is a pleiotropic cytokine with central roles in tissue remodeling and homeostasis. During pregnancy, the uterus undergoes rapid and dramatic changes fundamental for pregnancy progression. The maternal immune system is a critical player in regulating uterine tissue remodeling. Clinical studies have linked many pregnancy

complications in women with an abnormal expression of IL-33 and its receptor, ST2. However, it remains unclear what are the cellular and molecular mechanisms by which IL-33 may support pregnancy progression. Here, we demonstrate that, in mice, maternal IL-33 signaling is essential for proper uterine tissue remodeling and immune cell function in early gestation. IL-33 KO dams show defective decidualization and uterine vascular tissue remodeling. Further, embryos from IL-33 KO dams exhibit developmental defects, including delayed embryogenesis, increased resorptions, and intrauterine growth restriction in late gestation. We identify that in the early pregnant uterus, the primary IL-33 cellular sources are decidual endothelial and stromal cells and myometrial fibroblasts, whereas ST2 is expressed by many immune cells involved in Type 2 immunity. Critically, we find that uterine lymphocytes and M2 macrophages from IL-33 KO dams show impaired Type 2 cytokine responses that coincide with the tissue remodeling defects in early pregnancy. Our results reveal a regulatory pathway involving IL-33 signaling that is crucial for pregnancy progression in mice and provides potential mechanisms of how maternal IL-33 signaling may support human pregnancy.

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LB-31

MECHANISMS OF CASPASE-8 SUPPRESSION OF THE RIPK1-TBK1 AXIS IN HOMEOSTATIC TYPE I INTERFERON PRODUCTION AND REGULATION

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Abstract Content: Type-I interferons (IFNs) are an essential component of the innate immune response that are upregulated in response to infection. However, a low level of basal, or tonic, IFN expression is also critical to maintain tissue homeostasis. While the regulation of type-I IFN production during infection is well studied, the mechanisms controlling this basal expression and its impacts on homeostasis remain enigmatic. Here, we report the role of caspase-8 as a negative regulator of type-I IFN production during homeostasis in vitro and in vivo. Caspase-8 deficiency led to hyperactivation of basal type-I IFN signaling across multiple cell types and tissues and provided naturally heightened resistance to viral infection with norovirus. Mechanistically, the absence of caspase-8 allowed for an interaction between RIPK1 and TBK1, leading to enhanced TBK1 activation and aberrant type-I IFN production under homeostatic conditions. Loss of RIPK1 in caspase-8–deficient cells normalized TBK1 phosphorylation, IFN production and IFN signaling, thereby sensitizing cells to viral infection-induced inflammasome activation and inflammatory cell death, PANoptosis. In vivo, the elevated type-I IFN signaling, as well as the previously established early onset lymphadenopathy at 8 weeks of age, in caspase-8–deficient mice were reduced upon deletion of RIPK1 or type-I IFN neutralization. Overall, our study identified a mechanism which constrains tonic type-I IFN production and signaling during homeostasis. These results advance our understanding of tissue homeostasis and IFN regulation in infectious, autoimmune and autoinflammatory diseases, providing new insights for the development of disease treatments.

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P270

DNA DEMETHYLASE TET2 MEDIATES EPITHELIAL-IMMUNE CROSSTALK DURING COLON TUMORIGENESIS

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Abstract Content: Ten Eleven Translocation (TET) enzymes are executioners of epigenetic imprinting via DNA demethylation of CpG motifs. CpG islands are enriched in the proximity of tumor modulator genes and hypermethylated in various cancers, however, the function of DNA demethylation or its executioners in colon cancer is unknown. We found that the expression of DNA methylases is increased in colon tumors, while the expression of

TET2, but not other demethylases is significantly reduced. Further, the expression of *TET2* is a strong prognostic marker for survival in patients with colorectal cancer. The expression of TET2 and its enzymatic product, hydroxyl-methyl cytosine (OH-me-C) was also reduced in tumors in mice subjected to azoxymethane- dextran sodium sulfate (AOM-DSS), a model of colitis-associated colorectal cancer (CAC). Within the tumor, DNA demethylation negatively correlated with epithelial cell proliferation, and *Tet2*$^{-/-}$ mice subjected to AOM-DSS exhibited an increased tumor burden. Further, WT mice supplemented with alpha-ketoglutarate, a cofactor of TET2 exhibited significantly reduced tumor burden, and mice with catalytically inactive TET2 have increased tumor burden demonstrating that the tumor-restrictive function of TET2 is mediated through its DNA demethylase activity. Using lineage-specific deletion, we found that TET2 expression within the epithelial (*Tet2*^{IEC}) but not the hematopoietic cells were required to restrict colon tumorigenesis. Colon cancer induced by mutagen exposure alone was unaffected in *Tet2*^{IEC} mice, suggesting that epithelial TET2 controls response to mucosal injury to restrict CAC. Tumors from TET2-deficient mice have reduced Th1 and increased Th17 inflammatory signature, and TET2-deficient IECs exhibit increased activation of STAT3 and higher expression of Serum amyloid A3 (SAA3). Using genetic, biochemical and *in vivo* depletion approaches, we demonstrate that in IECs, TET2 restricts IL-6-STAT3 signaling to control the production of SAA3. The absence of TET2 in IECs, therefore, imparts an inflammatory program within CD4 T cells characterized by increased production of IL-17A and GMCSF, and depletion of CD4^{+} T cells, IL-17A or GM-CSF reduced the tumor burden in *Tet2*$^{-/-}$ mice. Overall, these data demonstrate that in response to mucosal injury, epithelial TET2 restricts tumorigenic inflammation to control colon cancer. Therefore, modulation of TET2-mediated DNA demethylation may present a novel and effective therapeutic strategy in colon cancer.

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P271

ELEVATED CEREBRAL INTERFERON-ALPHA MEDIATES NEUROTOXICITY THROUGH THE MICROVASCULATURE

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Abstract Content: Excessive production of interferon-alpha in the brain leads to a severe, complex and often fatal neuropathological phenotype that is the hallmark of several inflammatory and degenerative diseases of the central nervous system. Here we combine single-cell RNA sequencing with a mouse model of brain-targeted interferon-alpha production to identify endothelial cells as a major facilitator of interferon-alpha neurotoxicity. Elevated intracerebral interferon-alpha led to upregulation of endothelial-specific interferon-regulated genes and pathways which caused a complex microangiopathic phenotype characterised by upregulation of adhesion molecules, aneurysms and loss of blood-brain barrier integrity. Deletion of the interferon-alpha receptor in endothelial cells restored the microangiopathic phenotype, but also prevented widespread brain damage, revealing the brain's vasculature as a gatekeeper for interferon-alpha neurotoxicity. Thus, we propose that targeting interferon-driven microangiopathy, prior to the onset of diffuse brain disease, may represent an important neuroprotective strategy for a spectrum of human diseases characterised by excessive intracerebral interferon-alpha signalling.

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P272

GUT-INNERVATING NOCICEPTORS REGULATE THE INTESTINAL MICROBIOTA TO PROMOTE TISSUE PROTECTION

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Abstract Content: Pain is a hallmark of many chronic inflammatory conditions including Inflammatory bowel diseases (IBD), however whether pain-sensing neurons influence the development or severity of intestinal inflammation remains poorly defined. Employing chemogenetic silencing or pharmacological ablation of TRPV1$^+$ nociceptors, we observed more severe inflammation and defective tissue-protective reparative processes in a murine model of intestinal damage. Dysregulated intestinal homeostasis was associated with alterations in the composition of the intestinal microbiota in mice following disrupted nociception, which was sufficient to transfer exacerbated intestinal damage and inflammation in wild-type recipient mice. Mechanistically, levels of substance P, a nociceptive neuropeptide, was decreased upon nociception disruption and delivery of substance P promoted tissue-protective effects exerted by TRPV1$^+$ nociceptors in a microbiota-dependent manner. Finally, dysregulated expression of nociceptor-associated genes was observed in intestinal biopsies from IBD patients. These findings collectively demonstrate a functional link between pain sensing, the intestinal microbiota, and the restoration of intestinal homeostasis.

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10. Autoimmunity

P273

EXPLORING A POTENTIAL ANTI-TUMOR MICROENVIRONMENT IN GIANT CELL ARTERITIS: A PILOT STUDY

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Abstract Content: Objectives: Giant cell arteritis (GCA) is a chronic vasculitis characterized by T-helper-1-associated cytokines interferon gamma (IFG and interleukin-12 (IL-12) and folate-receptor-beta (FRB)+ macrophages with potential anti-tumor properties that can explain why GCA patients have significant longevity and reduced rates of certain cancers. We explored whether cancer incidence is lower in treated GCA than controls and whether this is associated with FRB expression and its associated autoimmune microenvironment.
Methods: GCA subjects and negative controls selected by ACR/pathologic criteria were compared and reviewed for tumor outcomes and immunohistochemical expression of folate receptor beta (FRB), interferon-gamma (IFG), interleukin-12 (IL12) and CD3 in temporal artery biopsies (TAB).
Results: A total of of 40 GCA and 40 controls were assessed. Both groups showed similar follow-up durations (76.9 vs 70.1 years; NS), times-to-incident cancer (21 vs 62 mos.;NS) and deaths from cancer (5% vs 17.5%;NS). GCA subjects were older (77.2 vs 69.7 yrs.; p= 0.004) and incidence of cancer was lower than controls (25% vs. 47.5%; p=0.036). FRB expression was restricted to macrophages and correlated with IFG, IL12 and CD3. The expression of all proteins were significantly higher in GCA without incident cancer compared to controls with cancer (FRB 4 vs 2.3;p=0.006, IFG 3 vs 1;p=0.005, IL12 1.5 vs 0;p=0.003, CD3 79 vs 0;p=0.004). This FRB-IFG-IL12-CD3 network persisted in repeat biopsies. Conclusion: GCA demonstrated a lower risk of cancer and was associated with a chronic FRB+-macrophage/Th1-polarized anti-tumor autoimmunity.

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P274

INGESTED (ORAL) ADRENOCORTICOTROPIC HORMONE (ACTH) INHIBITS INTERLEUKIN-17 IN THE CENTRAL NERVOUS SYSTEM IN THE MOUSE MODEL OF MULTIPLE SCLEROSIS, EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Abstract Content: BACKGROUND: Experimental autoimmune encephalomyelitis (EAE) is an inflammatory autoimmune disease of the central nervous system (CNS) that resembles multiple sclerosis (MS) and provides a useful animal model for the evaluation of mechanisms of action for potential immunomodulatory therapies. We have previously shown that oral adrenocorticotropic hormone (ACTH) decreased interleukin (IL)-17 in the gut lamina propria (LP) and the spleen, increased $CD4^+$ $FoxP3^+$ T regulatory (T_{reg}) cells and IL-10 in the spleen during EAE in the C57BL/6 mouse. However, we did not investigate the specific cellular alterations of pro-inflammatory and anti-inflammatory factors in the CNS.
OBJECTIVE: Determine if oral ACTH would have a similar clinical effect on inflammatory cytokines in the gut and define specific cellular effects in the CNS in an alternative strain of mice.
DESIGN/METHODS: SJL/J mice were immunized with proteolipid (PLP) peptide 138-151 and gavaged with scrambled ACTH (scrambled melanocyte stimulating hormone [scrambled α-MSH]) or ACTH 1-39 during ongoing disease.
RESULTS: Ingested (oral) ACTH attenuated ongoing clinical EAE disease, increased T_{reg} cells and IL-10 production in the LP, and increased $CD4^+$ T_{reg} cells and decreased $CD4^+$ and γδ IL-17 production in the CNS.
CONCLUSIONS: Ingested ACTH attenuated EAE clinical disease by increasing IL-10 in the gut associated lymphoid tissue (GALT) and decreasing IL-17 in the CNS.

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P275

CAN WE TREAT INFLAMMATORY ARTHRITIS BY 14-3-3ZETA

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Abstract Content:
Inflammatory Arthritis (IA) affects millions of people worldwide, and its preclinical intervention has been a significant focus of IA clinical research. We recently showed that 14-3-3ζ KO animals develop early-onset severe arthritis in pristane-induced arthritis (PIA). 14-3-3ζ immunization during the pre-symptomatic phase significantly suppressed arthritis in wild-type (WT) and 14-3-3ζ KO animals. Significant suppression of Interleukin-1b levels was observed in the 14-3-3z-treated animals. The MicroCT images and measurements of 14-3-3ζ-immunized bones show an increase in trabecular bone density and cortical thickness, a particular effect on the bone mineral density was noticed. To understand the molecular mechanism, we investigated how 14-3-3ζ supplementation promotes bone quality *in vitro*.

The 14-3-3ζ global KO Lewis rats generated using CRISPR/Cas9 were subjected to pristane-induced IA. The rats were immunized with 14-3-3ζ during the pre-symptomatic phase. Rat femur and tibia were harvested and processed for MicroCT and immunostaining. Primary rat bone marrow-derived mesenchymal cells were isolated from Wt and 14-3-3z KO rats and cultured to study osteoblast differentiation. The recombinant 14-3-3ζ protein was used to examine the effect on osteoblast differentiation. RNA extraction was performed to measure collagen 1 using RT-qPCR, while total collagen was measured using the Chondrex Sirius Red Total Collagen Detection Kit. Alizarin Red was used to stain for the total mineral deposition, and Von Kossa stain was performed to examine calcium deposits on the bone sections.

Our results validate the recently reported finding of 14-3-3ζ's role in bone growth and quality in vivo and in vitro. Our new results show that collagen synthesis is positively regulated by 14-3-3ζ *in vivo* and *in vitro*. The Masson's trichrome-stained trabecular bone sections show 14-3-3ζ presence significantly affected collagen expression in the cortical and trabecular bones. Similarly, primary mesenchymal cells in exogenous 14-3-3ζ show increased expression of collagen1 transcript and total collagen protein. Notably, an increase in the mineral density was observed when 14-3-3ζ was supplemented during osteoblast differentiation. Similar results of increased mineral deposits on the 14-3-3ζ-immunized animals further reinforce our previous finding that endogenous 14-3-3ζ plays an essential role in bone formation and quality.

Our results show that 14-3-3z is a novel regulator of bone homeostasis and a suppressor of IA.

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P279

IL-17 DEPENDENT RNA BINDING PROTEINS AND CROSSTALK WITH TRANSCRIPTION: A LESS EXPLORED PATH TO UNDERSTAND AUTOIMMUNE DISORDERS OF KIDNEY

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Abstract Content: Dysregulation of IL-17 signaling pathways contributes to pathology in many autoimmune diseases. Emerging data implicate involvement of IL-17 in autoimmune kidney disease models in human and mouse. IL-17 signaling in renal tubular epithelial cells (RTECs) is essential to mediate kidney damage in a mouse model of experimental autoantibody mediated glomerulonephritis (AGN). Multiple RNA-binding proteins (RBPs) in the IL-17-signaling cascade are crucial to regulate the stability and fate of IL-17 dependent transcription factors, such as C/EBPs and IκBξ. These in turn impact the expression of kidney injury markers (IL-6, Lcn2) that drives pathology in AGN. For example, the m6A 'reader', IGF2BP2 (IMP2) drives the renal pathology through stabilization and upregulation of C/EBPβ and C/EBPδ. Conversely, the endoribonuclease, Regnase-1 restricts AGN in an IκBξ -dependent manner. In all these settings, the RBPs act in non-hematopoietic renal compartment to regulate the pathogenicity, specifically RTECs. Thus, crosstalk between RBPs and transcription factors was evidenced to influence kidney damage through upregulation of pro-inflammatory mediators. To understand the specific targets of transcription factors in the larger context of kidney disease, we are using KO mouse models and analysis of human kidney to further understand the mechanism at play in this disease. In-depth investigation on these RBP-regulated transcription factors in the context of renal damage may ultimately be a promising therapeutic target to treat autoimmune disease of kidney.

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P280

ANTIBODY-MEDIATED DEPLETION OF PROGRAMMED DEATH 1-POSITIVE (PD-1+) CELLS

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Abstract Content: The role and function of PD-1 immune checkpoint in cancer and autoimmune disorders has been intensively investigated recent years. Cells that express PD-1 receptor (termed as PD-1-positive cells) are drawing ever-increasing attention. In cancer research, PD-1-positive T cells are employed to suppress tumors; on the other hand, PD-1-positive tumor cells have been reported as initiator of tumors as well as one factor that cause the resistance to PD-1 blockade therapy. In autoimmune diseases, PD-1-positive lymphocytes were found to infiltrate tissues and connected with disease progression. However, there still remains large ambiguity and unknown in the role and function of PD-1 positive cells in cancer and autoimmunity. One of the reason of such ambiguity is the method chosen by researchers. Up to now, all such researches investigated the role of PD-1 positive cells by either PD-1 blockade or PD-1 gene regulation. They failed to investigate PD-1 positive cells as a whole. To address this issue, we unprecedentedly designed and generated a depleting antibody (D-αPD-1) that is able to deplete PD-1 positive cells as a tool to study PD-1 positive cells. D-αPD-1 has the same variable domain as an anti-PD-1 blocking antibody (RMP1.14 clone) we cloned previously, and its constant domain was designed based on the Fc of IgG2a. D-αPD-1's binding capacities with both PD-1 molecule and FcγRIV, a mouse immuno-activating Fc receptor, were verified. The cell depletion effect of D-αPD-1 was confirmed in vivo using a PD-1^{+} cell transferring model. EL4 cells that are PD-1 positive were then transferred into mice followed by single-dose treatment of D-aPD-1 and D-aPD-1's inhibition effect on EL4 tumor initiation was measured. Compared with other groups of mice without D-aPD-1 treatment that have

median survival days of approximately 30 days, D-aPD-1 treated group survived the entire study up to 60 days, which indicates the depleting effect of D-aPD-1 on PD-1 positive cells *in vivo*. Furthermore, we also investigated the working mechanism of D-aPD-1 and proved that D-αPD-1 depleted PD-1 positive tumor cells by antibody-dependent cell-mediate phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) mechanism. These results altogether proved the specificity and depleting efficacy of D-αPD-1 on PD-1 positive cells. The results also highlighted the potential of D-αPD-1 as a robust tool to study PD-1+ cells in cancer and autoimmune diseases and a therapeutic for these diseases.

Disclosure of Interest: None Declared
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P281

EFFECT OF AN 8-WEEK YOGA-BASED LIFESTYLE INTERVENTION ON MARKERS OF IMMUNOSENESCENCE AND INFLAMMATORY CYTOKINE PROFILE IN ACTIVE RHEUMATOID ARTHRITIS PATIENTS: A RANDOMIZED CONTROLLED TRIAL

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Abstract Content: Background: Apart from usual medical therapy, recovery of patients with Rheumatoid Arthritis (RA) in which quality of life (QoL) is greatly compromised is dependent on several physical and psychological factors. RA patients serve as a useful model system for studying immunological ageing molecular processes. RA T cells have several hallmarks of cellular aging; most importantly, they accumulate damaged DNA. With advancing therapeutic options, achieving a state of remission has become the treatment goal in rheumatoid arthritis. This trial was designed to explore the effect of yoga based lifestyle intervention (YBLI) on markers of immunosenescence and inflammatory cytokine profile in active RA group compared with usual-care control group.

Methods: A total of 134 subjects were randomized into 2 groups: YBLI group and usual care control group which were assessed pre (Day 0) and post (8 weeks) intervention for acute phase reactants (ESR, CRP), inflammatory cytokines (IL-6, IL-17A, TNF-α, TGF-β, soluble HLA-G); subset population of Th17 and T reg cells; cellular health – oxidative stress (ROS, TAC), DNA damage (8OHdG), and cellular aging – telomerase activity and telomere length. Parameters of disease activity, quality of life (QoL), and disability index and pain acuity were assessed by various scales.

Results: YBLI participants showed significant improvements in disease activity ($p<0.05$***), disability index ($p<0.05$***), pain acuity ($p<0.05$***) and QoL ($p<0.05$***) over the control group. In YBLI group, there was reduction in mean levels of CRP ($p<0.05$***), ROS ($p>0.05$), 8OHdG ($p<0.05$**), TNF-α ($p<0.05$**), IL-6 ($p<0.05$**), IL-17A ($p<0.05$**) and ESR ($p<0.05$*) and elevation in TAC ($p<0.05$**), sHLA-G ($p<0.05$**) and TGF-β ($p<0.05$**) at 8 weeks compared to baseline level (day 0). The Th17 population showed a significant decrease whereas Treg population showed a significant increase in yoga group. Telomere length ($p>0.05$) did not show any significant change after YBLI.

Conclusion: The present study demonstrated that YBLI including yoga practice results in regression of inflammatory processes by reducing inflammatory cytokines in active RA patients. YBLI has significantly reduced pain perception, disability quotient, and disease activity and improved quality of life. Yoga reduces the rate of immunological aging as seen by reduction in aged Th17 cell population and aged Treg cell population. Yoga maintained telomere length by increase of telomerase enzyme levels, hence yoga aids in slowing the rate of biological aging, hence can be beneficial as an adjunct therapy.

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P283

IL-17A-SECRETING ΓΔ T CELLS ARE REGULATED BY PD-1 IN A MODEL OF CENTRAL NERVOUS SYTEM AUTOIMMUNITY

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Abstract Content: Multiple sclerosis (MS) is a chronic demyelinating autoimmune disease of the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) is the mouse model for MS and is mediated by the pro-inflammatory cytokine IL-17A. The main sources of IL-17A in EAE are Th17 cells and γδ T cells. Programmed cell death protein 1 (PD1) is an inhibitory receptor expressed by T cells and engagement of this receptor negatively regulates T cell function. The impact of PD-1 expression on CD4 T cell function has been thoroughly investigated and exploited in the clinic through immune checkpoint inhibitors as immunotherapeutics for cancer. However, the impact of PD-1 expression on γδ T cell function has not yet been described. In this study, we have examined the role of PD-1 in regulating γδ T cell function in the development of EAE. Treatment with anti-PD-1 neutralizing antibody throughout EAE significantly enhanced disease onset and severity. We found that $CD27^-$ γδ T cells express the highest levels of PD-1 in the lymph node and peritoneal exudate cells (PEC) of naïve mice. Addition of anti-PD-1 to lymph node cells from mice with EAE enhanced IL-17A production by $CD27^-$ γδ T cells following activation with IL-1β and IL-23. The anti-PD-1-induced increase of IL-17A was reversed in the same system using lymph node cells from mice that lack γδ T cells. Treatment with anti-PD-1 neutralizing antibody during EAE significantly increases the proportion of $CD27^-$ γδ T cells in the lymph node when compared with lymph nodes from isotype control-treated mice. The results suggest that anti-PD1 enhances proliferation of $CD27^-$ γδ T cells and thereby increases IL-17A production *in vivo* which leads to the exacerbation of disease in the EAE model. These findings elucidate a novel regulatory mechanism that constrains IL-17A-producing γδ T cells and modulates the development of autoimmune disease.

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P284

THE CTRP3-ADIPOR2 AXIS REGULATES THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY SUPPRESSING TH17 CELL DIFFERENTIATION

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Abstract Content: C1q/TNF-related protein (CTRP) including CTRP3 have a complement C1q-like domain, and play multiple roles in lipid metabolism, bone metabolism and immune response. Previously, we showed that the mRNA expression of CTRP3 is highly augmented in joints of autoimmune arthritis models and CTRP3-deficiency exacerbates collagen-induced arthritis in mice. However, the mechanisms how CTRP3 regulates immune response still remain to be elucidated. In this study, we showed that CTRP3 was highly expressed in IL-17-producing helper T (Th17) cell, a key player for the development of autoimmune diseases, and Th17 cell differentiation was augmented in CTRP3 deficient mice. Th17 cell differentiation was suppressed by CTRP3 and this suppression was abolished by the treatment with a receptor antagonist against AdipoR2, but not AdipoR1. The development of multiple sclerosis-model, experimental autoimmune encephalomyelitis was enhanced in CTRP3 deficient mice associated with increase of Th17 cell population. CTRP3 inhibited antigen-induced IL-17 production from T cells by affecting both T cells and dendritic cells. These results show that CTRP3 is an endogenous regulator of Th17 differentiation, suggesting that the CTRP3-AdipoR2 axis is a good target for the treatment of Th17 cell-mediated diseases.

Disclosure of Interest: None Declared
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P286

ROLE OF CYTOKINES IN ACQUIRED APLASTIC ANEMIA : A COMPARATIVE STUDY OF BONE MARROW AND BLOOD PLASMA LEVELS

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Abstract Content:
Introduction: Acquired aplastic anemia (aAA) is an autoimmune bone marrow failure disorder. Postulated pathology involves destruction of hematopoietic stem cells by T lymphocytes and effector cytokines such as interferon gamma (IFN-γ). Apart from this IL-4 and IL-17A may play important roles. We investigated the plasma levels of IFN-γ, IL-4 and IL-17A cytokines in peripheral blood (PB) and bone marrow (BM) of untreated aAA patients and healthy controls. Further, the patients were categorized as per disease severity.

Method: Heparinized PB and BM paired samples collected from 20 untreated aAA patients; 15 PB and 10 BM samples from healthy controls. Patients categorized as non-severe aplastic anemia (NSAA), severe AA (SAA), very severe AA (VSAA). Sample centrifuged at 2000 rpm for 10 minutes for plasma isolation. Plasma levels of IFN-γ, IL-4 and IL-17A measured by quantitative enzyme-linked immunosorbent assay (ELISA). Mann-Whitney U test and Pearson correlation coefficient used for statistical analysis.

Results: Increased level of IFN-γ, IL-4, IL-17A observed in PB and BM plasma of aAA patients (1803±570, 48.78±17.8, 135.4±7.65 & 1938±402, 57.54±32.66, 142.3±13.77) than controls (840.7±244.9, 28.74±2.61, 90±11.76 & 560.7±102.7, 26.13±5.91, 62.67±18.04) respectively. Among patients, IFN-γ, IL-4, IL-17A levels were higher in PB and BM plasma of VSAA+SAA (2238±484, 60.04±19.51, 141.3±5.957 & 2224±328, 78.61±34.32, 152.2±12.42) compared to NSAA patients (1367±179.3, 37.52±2.58, 129.5±3.27 & 1651±227.6, 36.48±9.36, 132.3±4.96) respectively. Significant correlations found between laboratory parameters and IFN-γ and IL-17A cytokine levels of patients.(All p values ≤ 0.05).
No difference in cytokine levels between patient PB and BM plasma.

Conclusion: The study highlights increased plasma level of IFN-γ, IL-4 and IL-17A in PB and BM of aAA patients than healthy controls. The findings suggest that enhanced levels of pro-inflammatory cytokines: IFN-γ and IL-17A create an inflammatory environment in aAA. The anti-inflammatory cytokine IL-4 may not be able to diminish the established inflammatory setting. The disparity between the cytokine levels varies with disease severity.
Additionally, significant correlations between laboratory parameters and cytokines in patients depict assault by the cytokines at stem cell level. Thus, injury by cytokines and disruption of cytokine stability appears to take part in acquired aplastic anemia pathogenicity.
Table 1: Significant Pearson correlation coefficient (r) between patient peripheral blood plasma cytokines and laboratory parameters. (P ≤ 0.05)

Blood Plasma Cytokines	Absolute neutrophil count	Hemoglobin	Platelet count	Retic%
Interferon-γ	r = -0.5183	r = -0.5900	r = -0.5421	r = -0.5924
Interleukin-17A	r = -0.4540	r = -0.5606	r = -0.5188	r = -0.4606

Disclosure of Interest: None Declared
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P287

INTERFERON LAMBDA SIGNALING RESTRAINS DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALITIS

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Abstract Content: Multiple sclerosis (MS) is a chronic autoimmune diseases caused by immune cell-mediated demyelination of neurons in the central nervous system (CNS), resulting in highly variable symptoms, ultimately

leading to paralysis and loss of vision. MS is the commonest inflammatory and neurodegenerative disease, which affects the CNS and correlates with dysregulation of immune system. Therefore, there is an imminent need for understanding of mechanism(s) that lead to inflammation and immune dysregulation. Type III interferons (IFNs) or IFNλ play an important part in regulation of inflammation. We examined the development and progression of experimental autoimmune encephalitis (EAE) in *Ifnlr1 knockout (Ifnlr1$^{-/-}$)* and wild type (WT) mice following immunization with MOG_{35-55} peptide (MOGp). We found that *Ifnlr1$^{-/-}$* mice develop significantly more severe EAE than WT littermates. Overall mortality was 47% for *Ifnlr1$^{-/-}$* and 25% for WT. The day of onset of EAE was similar for both WT and *Ifnlr1$^{-/-}$* mice suggesting that early T cell activation is similar in both WT and KO mice. To obtain an insight into the cell population in which IFNLR signaling is most important for restraining EAE, we passively transferred encephalitogenic T cells generated from WT or *Ifnlr1$^{-/-}$* mice into naïve WT or *Ifnlr1$^{-/-}$* mice. We found that the genotype of donor encephalitogenic T cells had no impact on EAE disease. However, *Ifnlr1$^{-/-}$* recipients that received WT encephalitogenic T cells developed more severe EAE than WT recipients. From the results, we inferred that expression of IFNLR1 on innate immune cells was required to restrain EAE induced by encephalitogenic T cells. We further determined that loss of IFNLR1 expression in macrophages promoted expansion of myelin-peptide reactiveTh17 cells. Overall, from these results we propose that IFNλ signals in innate immune cells, such as macrophages and neutrophils is necessary to restrain autoimmune neuroinflammation through a mechanism that involves regulating the expansion of Th17 cells.

Disclosure of Interest: None Declared
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LB-32
THE ROLES OF CLEC1A IN THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND COLITIS

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Abstract Content: Clec1A belongs to the C-type lectin receptor (CLR) family, which recognizes pathogen-associated molecular patterns of pathogens. Clec1A was reported to recognize pathogenic fungi, Aspergillus fumigatus, through the carbohydrate recognition domain, and plays a protective role in the host defense. However, Clec1A does not contain any known signaling motifs and no adaptor has been detected yet, suggesting that Clec1A may have other mechanism for signal transduction. The conservation rate of Clec1A amino acid sequences between mouse and human is 69.4% identity. Similar to human data, *Clec1a* is expressed in endothelial cells as well as myeloid cells, although the expression levels are much lower in myeloid cells. Here we found that *Clec1a$^{-/-}$* mice developed milder symptoms upon experimental autoimmune encephalomyelitis (EAE) induction. Expression of inflammatory cytokine including *Il17a*, *Il6*, and *Il1b* was greatly decreased in *Clec1a$^{-/-}$* mice. Interestingly, in dextran sodium sulfate (DSS)-induced colitis model, *Clec1a$^{-/-}$* mice developed more severe symptoms in a microbiota-independent manner. The number of polyps increased in azoxymethane (AOM)/DSS-induced colon tumorigenesis model. These results suggest that Clec1A plays an important role in the regulation of immune system and intestinal homeostasis.

Disclosure of Interest: None Declared
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11. Cytokine-related interventions/therapies

P289
INTERFERON EPSILON LIMITS OVARIAN CANCER METASTASIS VIA TUMOUR-EXTRINSIC MECHANISMS

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Abstract Content: The novel type I interferon, interferon epsilon (IFNε), is a unique cytokine which is constitutively expressed by epithelial cells under hormonal regulation in the female reproductive tract (FRT). IFNε is known to be protective against FRT infections[1], however its role as an anti-tumour cytokine is under investigation. High grade serous ovarian cancer (HGSOC) is a cancer of the FRT which is frequently characterised by extensive metastasis throughout the peritoneal cavity, and carries a five year survival rate of <50%. Preliminary research has suggested that IFNε is protective against HGSOC metastasis through both intrinsic action on tumour cells, and extrinsic action via immune and stromal cells. However, the relative contribution of these intrinsic and extrinsic effects to the observed anti-tumour efficacy of IFNε is unclear. Moreover, analysis of human HGSOC tumours indicates a potentially high prevalence of resistance/insensitivity to type I IFNs which may limit treatment response to IFNε in a clinical setting.

Here, the role of extrinsic anti-tumour activity by IFNε was investigated in a syngeneic mouse model of HGSOC. An IFN-insensitive ID8 mouse ovarian tumour cell line was generated via CRISPR-Cas9 knock-out of the type I IFN receptor subunit, IFNAR1. Mice were injected intraperitoneally (i.p.) with wild-type (WT) or IFNAR1$^{-/-}$ ID8 cells, and treated with i.p. PBS or IFNε thrice-weekly for 6 weeks. Analysis of disease scores, including tumour burden, ascites volume and peritoneal hemorrhaging, revealed that IFNε treatment effectively limited HGSOC metastasis and disease progression, with no loss of efficacy observed in mice bearing IFNAR1$^{-/-}$ ID8 tumours versus WT. Furthermore, IFNε treatment in mice bearing both WT and IFNAR1$^{-/-}$ ID8 tumours was associated with marked alterations in peritoneal immune cells. PBS-treated mice displayed significant infiltration, expansion, and activation of immunosuppressive immune cells subsets, alongside a global inihibition of immune cell proliferation - features which were reversed with IFNε treatment. Moreover, IFNε-treated mice displayed a greater frequency of activated anti-tumour immune cells such as CD8^{+} T cells. Together, these results indicate that the primary mechanism of action of IFNε in HGSOC is tumour-extrinsic, mediated through activity in immune and stromal cells, and is sufficient for effective tumour control versus IFN-insensitive tumours.

[1]Fung, K. Y. *et al.* Interferon-ε protects the female reproductive tract from viral and bacterial infection. *Science (80-.).* 339, 1088–1092 (2013).

Disclosure of Interest: None Declared
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P290

NONVIRAL DELIVERY STRATEGIES OF THE CRISPR SYSTEM FOR IN VIVO GENE EDITING AND CANCER IMMUNOTHERAPY

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Abstract Content: *In vivo* gene editing based on the clustered regularly interspaced short palindromic repeats (CRISPR) system has been challenging due to the complexity and barriers within the physiological microenvironment. We have focused on the development of efficient delivery methods of the CRISPR-associated protein 9 (Cas9) ribonucleoprotein for non-viral gene editing. We introduce a chemical tailoring approach by creating Cas9-polymer conjugates, which readily complex with single guide RNA (sgRNA) and stabilizing DNA, to form nano-assembled RNP (NanoRNP) complexes. NanoRNP shows greatly enhanced internalization and gene editing in melanoma cells in vitro, while showing low cytotoxicity. NanoRNP was designed to target PD-L1 for blockade of the immune checkpoint, which upon treatment to orthotopic melanoma model in mice resulted in major suppression of the tumor growth by modulation of the immune microenvironment. We also further developed a recombinant form of Cas9 protein by incorporation of unnatural amino acids (Cas9-UAA), which can be bioorthogonally functionalized *in situ* with bioactive molecules. We show that co-treatment of Cas9-UAA and antibody with chemoselective functionality allows efficient and target-specific delivery of the Cas9 ribonucleoprotein to Her2-positive breast cancer cells. We anticipate that the current strategies can be usefully applied as a therapeutic genome editing platform for treatment of various types of cancer, such as melanoma, breast cancer, and ovarian cancer.

Disclosure of Interest: None Declared
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P291

A NOVEL ALLOSTERIC MODULATOR OF IL-6R, HSJ633, REDUCED PRETERM BIRTH AND INFLAMMATION IN MICE

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Abstract Content: Preterm birth (PTB) is one of the main causes of neonatal mortality and morbidity. Current studies have shown that neonatal morbidity in PTB is linked to increased levels of IL-6 in amniotic fluid, fetal blood and gestational tissues (GT) and that IL-6 increases uterine activating proteins' expression leading to PTB. A small peptide, HSJ633, developed in our lab inhibits selectively IL-6-induced STAT3 phosphorylation and LPS-induced PTB in mice. We hypothesize that IL-6 induces damages to fetal tissues, and that inhibiting the IL-6 receptor using our nanopeptide, HSJ633, will improve birth outcome and prevent fetal injury.
CD1 pregnant mice were injected with LPS (10µg/kg i.p.) at gestational day (GD) 16 in presence or absence of HSJ633 (1mg/kg/12h), Tocilizumab (TOC; 10mg/kg/12h), or vehicle. Prematurity, fetal mortality and morbidity rates were evaluated. Neonates' intestines, lungs, and brain were collected at P1 to evaluate IL-1 and IL-6 concentrations by ELISA. HEK-Blue IL-6 cells were treated with IL-6 (0.1µg/ml) in presence or absence of HSJ633 (1µg/ml) and TOC to determine the activation of signaling pathways by Western Blot. STAT3 QUANTI-Blue assay was performed to assess the concentration of HSJ633 that inhibits 50% of STAT3 activation. We injected FITC-HSJ633 (1mg/kg/12h) in our PTB murine model at GD18 and tissues (placenta and fetus) were collected 4h post-injection. Fluorescence was evaluated to determine HSJ633's localization.
Our peptide allowed PTB rates to drop from 80% (LPS group) to 25% ($p<0.05$, n=12). This also influences neonatal survival which is 30% in the LPS group compared to 75% in the HSJ633 group ($p<0.05$, n=12). Moreover, the pups' birth weight in the HSJ633 group is the same as the sham group, i.e. 1.5g, whereas neonates in the LPS group weigh 1.0g ($p<0.05$, n=12). Moreover, HSJ633 decreased proteins' concentrations in comparison to the LPS group (IL-1 and IL-6) in the brain and lungs. This reduction was comparable to the sham group ($p<0.05$, n=4). We did not see a significant difference in the intestine for both cytokines. We then investigated HSJ633's mechanism of action in HEK-Blue IL-6 cells treated with IL-6 and HSJ633 where we demonstrated that HSJ633 reduced the activation of STAT3 by 100% ($p<0.05$) but not p38, AKT and ERK (ns, n=3). In addition, the injection of a STAT3 inhibitor in a murine LPS-induced PTB model reduced PTB by at least 50% (n=1, preliminary result). Furthermore, we found that 12 nM of HSJ633 is sufficient to inhibit 50% of STAT3 activation in HEK-Blue IL-6 cells (IC50 = 12nM) ($p<0.05$, n=4). Fluorescence analysis of HSJ633-FITC revealed its presence in the placenta on both fetal and maternal sides in the presence of inflammation (LPS stimulus) ($p<0.05$) (n=3).
Collectively, our data shows that HSJ633 antagonized the activity of IL-6R in a LPS-induced PTB model by inhibiting STAT3 activation, and improved birth outcome by increasing survival and preserving neonatal organ integrity. These findings highlight the importance of IL-6 in PTB and uncover in vivo pharmacological efficacy of a novel IL-6R modulator. HSJ633 is a promising new therapeutic prototype in the prevention of PTB.

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GUANYLATE-BINDING PROTEINS (GBPS) PROVIDE A HOST-INSPIRED ROADMAP FOR THE DESIGN OF SYNTHETIC ANTIMICROBIAL PEPTIDES

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Abstract Content: Guanylate-binding proteins (GBPs) are a family of interferon-inducible GTPases that are critical components of innate immunity. We recently found that mouse GBP1, in addition to the previously reported GBP2 and GBP5, targets and kills intracellular *Francisella novicida*, and protects mice from *F. novicida* infection. These observations suggest that GBPs carry specific antimicrobial features that could be harnessed into therapies to combat bacterial infections. To test this hypothesis, we interrogated the sequence of GBP1 to predict regions that have high antimicrobial probability. We synthesised four putative antimicrobial peptides ($GBP1^{28-67}$, $GBP1^{209-238}$, $GBP1^{424-452}$, $GBP1^{558-577}$; superscripted number indicates the amino acid position) and investigated their ability to kill *F. novicida*. Remarkably, $GBP1^{28-67}$ exhibited antimicrobial activity against *F. novicida* based on colony-forming unit (CFU) assays and flow cytometry (IC_{50} of 1.1 μM). Confocal microscopy revealed that FITC-tagged $GBP1^{28-67}$ localised to the bacterial membrane and resulted in the release of cytoplasmic DNA suggesting bacteriolysis. Electron microscopy confirmed that $GBP1^{28-67}$ treated *F. novicida* had a marked loss of membrane integrity and expulsion of intracellular content. Furthermore, we show that $GBP1^{28-67}$ is specific for bacteria and does not induce toxicity in mammalian cells. Lastly, we investigated the antimicrobial spectrum $GBP1^{28-67}$. Using CFU assays, we examined the viability of a range Gram-positive and Gram-negative bacteria following $GBP1^{28-67}$ treatment and found that $GBP1^{28-67}$ exhibited antimicrobial activity against *Moraxella catarrhalis* and *Neisseria meningitidis*, but not against *Citrobacter rodentium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella* Typhimurium, *Bacillus cereus*, *Listeria monocytogenes,* and *Staphylococcus aureus*. Collectively, our study identified that $GBP1^{28-67}$ is a highly pathogen-selective, and non-toxic antimicrobial that could be developed into a bona fide therapy for bacterial infections. Our findings provide evidence that innate immune proteins such as GBPs are a source of antimicrobial peptides that can be used to develop novel therapies to help combat the growing issue of antimicrobial resistance.

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P294

PRECLINICAL VALIDATION OF ALPHASEPT, AN ENGINEERED HUMAN CYTOKINE, AS A NEXT GENERATION IMMUNOTHERAPY TO TREAT SEPSIS

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Abstract Content: Sepsis is an indication with an enormous unmet medical need. In western countries, more people die from sepsis than from the most common types of cancer combined. There is no effective and causative treatment available yet. To address this unmet medical need, we are developing alphaSEPT. alphaSEPT is an engineered human immune signaling molecule. It has immunomodulatory properties. In the case of indications caused by an immune system out of control these functions are of central therapeutic importance. Sepsis is such an indication: Our immune system overreacts to an infection and then breaks down. Patients with sepsis die either from multi-organ failure or from secondary infections as the sepsis progresses.
For the first time, alphaSEPT offers an approach that is advantageous in the initial as well as advanced sepsis phase. Studies with the mouse protein in mice showed a significant reduction in mortality from sepsis. Currently, alphaSEPT is being preclinically validated as a next-generation biopharmaceutical to treat sepsis and other indications caused by an immune system out of balance. We will present the molecular basis and current development status of alphaSEPT.

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P295

IL-2 IS INACTIVATED BY THE ACIDIC PH ENVIRONMENT OF TUMORS ENABLING ENGINEERING OF A PH-SELECTIVE MUTEIN

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Abstract Content: Cytokines interact with their receptors in the extracellular space to control immune responses. How the physicochemical properties of the extracellular space influence cytokine signalling is incompletely elucidated. Here, we show that the activity of interleukin (IL)-2, a critical cytokine in T cell immunity, is profoundly affected by low pH (~ 6.5), limiting IL-2 signalling in a IL-2Rα-dependent manner. Generation of lactic acid by tumours limits STAT5 activation, effector differentiation and anti-tumour immunity by $CD8^+$ T cells and renders high-dose IL-2 therapy poorly effective. Directed evolution by yeast display enabled selection of a pH-selective IL-2 mutein (Switch-2). Switch-2 binds the IL-2 receptor subunit IL-2Rα with higher affinity, triggers more potent STAT5 activation and drives $CD8^+$ T cell effector function at acidic pH than at pH 7.2 typical of normal tissues. Consequently, Switch-2 is mainly uptake by CD8+ T cells localized in acidic tissues such as tumour and lymph-node, at the contrary of IL-2 that was found at a greater extent in CD8+ T cells in blood and lungs. High-dose Switch-2 therapy induces anti-tumour immunity in different tumour models (B16, MC38, and 4T1) with reduced on-target toxicity in normal tissues, whereas high-dose IL-2 therapy resulted in toxicity alone. Phenotypical and single-cell analysis on CD8+ tumour infiltrating lymphocytes (TILs) shows that Switch-2 increases cell proliferation, expansion of antigen specific cells, and cytokine expression as compared to IL-2. Therapeutic manipulation of the pH-selective activity of cytokines is a powerful approach to exploit the therapeutic efficacy of cytokines in pathological environments with reduced systemic side effects.

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P296

STRUCTURES OF THE COMPLETE EXTRACELLULAR DOMAINS OF THE INTERLEUKIN 11 SIGNALLING COMPLEX AND THE INHIBITORY MECHANISM OF A CYTOKINE VARIANT

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Abstract Content:
Introduction: Interleukin (IL) 11, a member of the IL-6 cytokine family, has roles in fibrotic diseases and solid malignancies. IL11 forms a signaling complex with the IL11 specific receptor, IL11Rα, and the shared, signal transducing, receptor glycoprotein (gp) 130. Despite the potential therapeutic significance of inhibition of IL11 signaling, the structural biology of IL11 has remained undercharacterised, hindering potential development of novel therapies.
Methods: We have solved structures of the IL11 signalling complex by cryo-electron microscopy and have conducted extensive biophysical characterisation of the complex and the mechanism of its formation using techniques including analytical ultracentrifugation, small-angle X-ray scattering, and isothermal titration calorimetry.
Results: Here, we present structures of the IL11 signalling complex, providing detail of the molecular mechanisms of complex formation and the structure and dynamics of the complete extracellular domains of gp130 within the complex. We characterise an IL11 variant, IL11-mutein, that potently inhibits IL11 signalling and describe the detailed mechanism of its action.
Conclusion: Our work presents the structural and mechanistic basis of IL11 signalling and provides high resolution understanding of the molecular mechanism of an IL11 signalling inhibitor. Together these results provide platforms for development of existing and novel therapeutics targeting IL11 signalling.

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P297

IL-27 INHIBITS IMMUNE CELL REINVIGORATION MEDIATED BY PD-(L)1 BLOCKADE AND INDUCES A TYPE 1 INTERFERON GENE EXPRESSION SIGNATURE ASSOCIATED WITH RESISTANCE TO THERAPY IN CANCER PATIENTS

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Abstract Content: Interleukin (IL)-27 is a heterodimeric cytokine expressed by macrophages and myeloid cells in several tissue microenvironments and plays a role in controlling the intensity and duration of immune system responses during infectious challenge and tumor immune surveillance. Mechanistically, IL-27 induces the expression of several immunoregulatory receptors (e.g., PD-L1, TIM-3, LAG-3, and TIGIT) and reduces inflammatory cytokine production. Loss of IL-27 function, through either genetic deficiency or pharmacologic inhibition by monoclonal antibody (SRF388), leads to tumor growth inhibition in mouse models and early clinical data have shown monotherapy activity of SRF388 in patients with cancer (NCT04374877). IL-27 induces a gene expression program characterized by an enrichment for transcripts associated with type 1 interferon (IFN) responses. This signature has also been found to be expressed in a variety of human tumors and has been associated with resistance to several therapies including chemotherapy, radiotherapy, and immune checkpoint inhibition. Here we describe the effects of IL-27 and its inhibition by SRF388 on human immune cell activation during PD-(L)1 blockade. In vitro activation of human peripheral blood mononuclear cells by anti-CD3 with either pembrolizumab (anti–PD-1) or atezolizumab (anti–PD-L1) leads to increased cytokine production compared with stimulation with anti-CD3 alone. Exogenous IL-27 counteracted the immune cell reinvigoration by anti–PD-(L)1 in this system, an effect that was also seen with type 1 IFNs, but not IFNγ. Blockade of IL-27 with SRF388 resulted in higher cytokine production (GM-CSF, TNFα, IFNγ, IL-17) when combined with PD-(L)1 than blockade of either pathway alone. These data highlight the parallels between IL-27 and type 1 IFNs and their putative roles in mechanisms that restrain immune cell activation during checkpoint inhibition.

Disclosure of Interest: J. Hill Shareholder of: Surface Oncology, Employee of: Surface Oncology, K. Golan Employee of: Surface Oncology, J. Hua Shareholder of: Novartis Corporation, Employee of: Novartis Corporation, Y. Ren Shareholder of: Surface Oncology, Employee of: Surface Oncology, Y. Yang Shareholder of: Surface Oncology, Employee of: Novartis Corporation, R. Masia Shareholder of: Surface Oncology, Employee of: Surface Oncology, D. Moodley Shareholder of: Abata Therapeutics, Employee of: Abata Therapeutics, B. Lee Shareholder of: Surface Oncology, Employee of: Surface Oncology, V. Palombella Shareholder of: Surface Oncology, Consultant for: Molecular Partners AG, Employee of: Surface Oncology
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P298

INTERFERON-ALPHA RECEPTOR ANTISENSE OLIGONUCLEOTIDES REVERSE NEUROPATHOLOGY IN A MOUSE MODEL OF TYPE I INTERFERON NEUROTOXICITY

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Abstract Content: Chronic production of the antiviral cytokine interferon-alpha (IFN-α) in the brain is neurotoxic. This is best observed in patients with genetic cerebral interferonopathies such as Aicardi-Goutières syndrome, where chronic intrathecal production of IFN-α causes debilitating disease and premature death. There is no cure for these

diseases with existing treatments largely aimed at ameliorating symptoms. Thus, a novel therapeutic strategy is urgently needed. Here, we investigated the potential of antisense oligonucleotides targeting the murine IFN-α receptor (*mIfnar1* ASOs) in a mouse model for cerebral interferonopathies. Intracerebroventricular injection of *mIfnar1* ASOs into transgenic mice with brain-targeted chronic IFN-α production resulted in a blunted cerebral interferon signature, reduced neuroinflammation, restoration of blood-brain barrier integrity, absence of tissue destruction and lessened neuronal damage. Remarkably, *mIfnar1* ASO treatment was also effective when given after onset of neuropathological changes, reversing some of the features, showing a reversible aspect of IFN-α-mediated neuroinflammation and neurotoxicity. Importantly, the results demonstrate the potential for IFN-α receptor ASOs in treating patients with cerebral interferonopathies.

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P299

NOVEL VACCINES AGAINST THE INFLAMMASOME COMPLEX COMPONENT ASC ARE SAFE IN HEALTHY MICE: A PRE-CLINICAL PILOT STUDY

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Abstract Content: The inflammasome is an innate immune complex capable of sensing a range of pathogen and damage associated molecular patterns (PAMPs and DAMPs) and initiating an inflammatory signaling cascade mediated by the maturation of interleukin-1β and interleukin-18 by caspase-1. Inflammasomes play an important role in many disease states and often contribute to a protracted cytotoxic inflammatory state in many diseases characterized by chronic inflammation, including Alzheimer's disease. While there are several different inflammasome complexes that respond to different PAMPs and DAMPs, the apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) protein is a common component of most inflammasomes and is the major component of the ASC speck, a large protein complex that serves as a signal amplification platform for enhanced inflammasome activity. We have generated a small library of virus-like particle (VLP)-based vaccines displaying peptide sequences from the ASC protein in a highly immunogenic pattern and characterized the safety profile of these vaccines in healthy C57Bl6 mice. The animals received a two-injection vaccine regimen beginning at the age of 2 months with a booster vaccination two weeks after the first injection. The vaccine conditions included three different ASC peptide sequences conjugated to a Qβ bacteriophage VLP or a non-conjugated Qβ VLP sham treatment for a total of four treatment groups with 5 mice per group. Each of the ASC-VLP groups demonstrated significantly increased IgG titers against the ASC peptides compared to the sham vaccinated mice. There were no differences in survival rates or changes in animal weights between the ASC-vaccinated groups and the sham vaccinated groups up to 4 months after vaccination. Complete blood cell counts and blood chemistry panels were also conducted for each mouse and showed no significant differences that would indicate disease or increased infections. Our findings indicate that these vaccines may be safe to use in various mouse models of disease, including Alzheimer's disease, for therapeutic intervention of inflammasome-mediated cytokine signaling in pre-clinical trials.

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P300

NOVEL TREATMENT FOR OSTEOPOROSIS CAUSED BY LIVER FIBROSIS: SUPPRESSION OF LIVER-BONE CROSSTALK MEDIATED BY FGF23

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Abstract Content: Background
Osteoporosis is a pathological condition characterized by low bone density and high bone fragility. This increases the risk of fracture, which seriously reduces quality of life; thus, the consequences of osteoporosis are dire. Osteoporosis is mainly secondary to other diseases. In particular, one in three patients with liver fibrosis develops osteoporosis. Due to a rise in unbalanced diets, the number of patients with liver fibrosis followed by osteoporosis has been rapidly increasing. Therefore, osteoporosis treatment is increasingly and socially important. However, existing treatments focusing on bone metabolism and calcium supply cannot completely suppress these pathologies.
Bone is mainly composed of calcium phosphate; thus, proper calcium and phosphorus content are important for bone maintenance. Despite the importance of phosphorus, there is no strategy targeting the phosphorus supply. Phosphorus content in the body is mainly regulated by fibroblast growth factor 23 (FGF23). FGF23 binds to FGF receptor (FGFR) on tubular cells and promotes phosphorus excretion. However, few reports have shown the relationship between FGF23-FGFR signaling and osteoporosis.
Therefore, we investigated whether regulation of FGF23-FGFR signaling aimed at phosphorus supply could be a novel treatment strategy for osteoporosis induced by liver fibrosis.

Methods
To analyze the dynamics of liver fibrosis and osteoporosis, we intraperitoneally administrated carbon tetrachloride (CCl_4) to mice for 8 weeks to induce liver fibrosis. Then, we measured the following: (1) collagen content in the liver by Masson's trichrome staining and hydroxyproline assay to confirm of liver fibrosis, (2) serum FGF23 and phosphorus content by ELISA and color-dyeing method, and (3) bone density of the femur by an animal dual-energy X-ray absorptiometry system, every 2 weeks. We also analyzed the bone microstructure of the isolated femur by a micro-CT system at 8 weeks.
Next, we made mice groups as follows: vehicle, CCl_4, and CCl_4 + FGFR antagonist (pemigatinib, daily, i.p.), and conducted the same examination to investigate the effects of pemigatinib.

Results
Masson's trichrome staining and hydroxyproline assay showed that the collagen content in the liver was upregulated in a time-dependent manner by CCl_4. In parallel with the progression of liver fibrosis, serum FGF23 levels increased and phosphorus levels decreased. Bone density also decreased with liver fibrosis. Moreover, bone microstructure indexes decreased in CCl_4-treated mice.
Next, we investigated whether pemigatinib could suppress the pathology of osteoporosis induced by liver fibrosis. The phosphorus content, bone density, and bone microstructure indexes were ameliorated by pemigatinib treatment.

Conclusion
We found the increased FGF23, decreased phosphorus content, and bone density in the liver fibrosis model. This suggested that excess FGF23 suppressed phosphorus reabsorption on the tubule, which, in turn, induced a lack of phosphorus and bone dysplasia. To support this mechanism, FGFR inhibition with pemigatinib increased the phosphorus content and ameliorated osteoporosis induced by liver fibrosis. These results indicated a novel mechanism of osteoporosis mediated by FGF23. It also revealed that controlling phosphorus levels by focusing on FGF23-FGFR signaling could be a therapeutic strategy for osteoporosis.

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P301

AN ENGINEERED IL-2 PARTIAL AGONIST PROMOTES CD8+ T CELL STEMNESS

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Abstract Content: Adoptive cell transfer of antigen-specific T cells represents a major advance in cancer immunotherapy, with robust clinical outcomes in a subset of patients. To achieve effective responses, both the number

of transferred T cells and their cell differentiation state are critical determinants. IL-2 can promote T cell proliferation, but this can also lead to T cell differentiation into effector T cells, resulting in diminished therapeutic efficacy, whereas maintenance of more stem-like properties prior to adoptive transfer is beneficial. Here, we show that H9T, an engineered IL-2 partial agonist generated on the super-IL-2 background, promoted T-cell expansion without driving terminal differentiation. This partial agonist exhibited reduced STAT5 phosphorylation and mediated distinctive downstream transcriptional, epigenetic, and metabolic programs. Interestingly, we also found that hyperactivation of STAT5 in T cells through overexpressing a constitutively active STAT5A mutant can drive the expression of T cell exhaustion-related markers, such as PD-1, TIM-3 and CTLA-4, suggesting that STAT5 is a key regulator of T cell exhaustion. We showed that H9T promoted the maintenance of a stem cell-like state through sustained TCF-1 expression and increased mitochondrial fitness. TCR transgenic and CAR-modified $CD8^+$ T cells expanded with H9T displayed robust anti-tumor activity in vivo in established mouse models of melanoma and acute lymphoblastic leukemia. Thus, tempering cytokine signaling with H9T provides a strategy for enhancing therapeutic efficacy by preserving stemness that limits T cell exhaustion. Moreover, our findings demonstrate the distinctive power of generating cytokine partial agonists with distinctive activities.

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P302

REPRODUCIBLE, MOA-REFLECTING REPORTER-BASED BIOASSAYS TO ENABLE DISCOVERY AND DEVELOPMENT OF CYTOKINE THERAPEUTICS

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Abstract Content: Cytokines and growth factors are small immunomodulatory proteins secreted by a wide variety of cells including fibroblasts, endothelial and stromal cells. Their physiological role is to regulate surrounding cells in an autocrine, paracrine or endocrine fashion. Immunocytokines represent a promising class of activators of the immune system, with the potential to be used alone or in combination with other therapeutic modalities. Many are currently FDA approved therapy agents (e.g. IFN, IL-2, and EPO), while others are targets for approved biological blocking therapies to support treatment of a variety of diseases. Examples of cytokine blocking agents include basiliximab (IL-2R), tocilizumab and sarilumabad (IL-6R), siltuximab (IL-6), ustekinumab (IL-12/IL-23 p40), secukinumab (IL-17A), bevasizumab (VEGF), and denosumab (RANKL). Many more are in development and trials as biosimilars and biobetters. IL-2 and IL-15, in particular, have emerged as clinically important cytokines as researchers look to improve potency, patient tolerance, and response by developing new molecules with sustained and targeted activities.
We have developed cell-based luciferase reporter bioassays which individually can be used for the assessment of activity for of a variety of cytokines and growth factors including IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-17, IL-22, IL-23, TNFα, TGFβ, BCMA, TPO, VEGF, and RANKL using respective mechanism of action pathways. The bioassay format is based on thaw-and-use cells, eliminating the need to establish and pre-culture cytokine responsive cell lines which provides the substantial benefits of convenience, reproducibility, and transferability.
In summary, these reporter-based bioassays provide valuable tools for the development, stability testing, and potency determination in the manufacture of cytokine therapeutics.

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P303

NOMALIZATION OF TUMOR VESSELS VIA CU06-1004 REDUCES IL-2-INDUCED VASCULAR LEAKY SYNDROME AND IMPROVES IMMUNOTHERAPY

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Abstract Content: Interleukin-2 (IL-2) is a first reproducible cancer immunotherapy with ambivalent effects on the immune system. The IL-2 therapy has been generally used for the treatment of metastatic renal cell carcinoma and metastatic melanoma. The role of IL-2 in lymphocyte regulation is suggesting a new direction for cancer immunotherapy. Additionally, the tumor-infiltrating lymphocytes increased by IL-2 administration demonstrated a significantly higher effect in patients with melanoma. However, the usage of IL-2 has been limited due to its short half-life and severe side effects on endothelial cells. These results induced drug side effects such as vascular leaky syndrome (VLS) or capillary leaky syndrome (CLS) in patients. Herein, we showed the reducing side effects with IL-2 immunotherapy through previous reported the stabilization of tumor blood vessels by endothelial dysfunctional blocker CU06-1004. Interestingly, the combination therapy of CU06-1004 and IL-2 has been confirmed the blocking of vascular leakage through intravenously Evans blue staining. These effects allowed continued administration of IL-2 by alleviating serious side effects in patients. Taken together, we suggest that CU06-1004 is a potential candidate drug that can be improved the side effects and enhanced the anti-cancer effect of IL-2 immunotherapy.

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P304

PHARMACOLOGICAL DELINEATION OF THE TNF/TAK1 SIGNALING PATHWAY FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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Abstract Content: The serine/threonine protein kinase transforming growth factor-b-activated kinase 1 (TAK1) is a key regulatory signaling node in the tumor necrosis factor (TNF) pathway, mediating proinflammatory cytokine responses in immune cells. The binding of TNF to TNFR1 complex activates TAK1 leading to downstream NF-κβ activation. Subsequent increased gene transcription of pro inflammatory cytokines associated with TAK1 led to pro inflammatory immune cell polarization. Using both genetic and pharmacological approaches, our group has elucidated the consequences of TAK1 inhibition on downstream pro-inflammatory cytokine expression. Our results indicate that inhibition of TAK1 attenuates downstream TNF, IL-6, and IL-1β signaling and may act as a novel intercellular drug target for the treatment of TNF mediated diseases. Here we show that genetic KO of TAK1 in THP-1 cells selectively impairs TNF expression following LPS. These results were further validated using the highly selective TAK1 inhibitor, EYD-001. To test the effects if TAK1 inhibition in disease relevant animal models, we next sought to test the drug efficacy of EYD-001 in the collagen induced arthritis (CIA) pre-clinical mouse model of rheumatoid arthritis (RA). In the CIA model, TAK1 inhibition led to a significant reduction in disease score, evidenced by histological reductions in edema and inflammation in the knee as well as significant reduction of TNF expression in the serum, paw and knee synovial fluid. Compared to Enbrel treated animals, TAK1 inhibition with EYD-001 performed equally or better. Based on these finding's we posit that TAK1 regulates TNF signaling and that inhibition represents a novel therapeutic axis for the treatment of TNF mediated diseases such as rheumatoid arthritis.

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P305

CONTROL OF PRIMARY TUMOR AND METASTATIC BURDEN BY HETERODIMERIC IL-15 (HETIL-15) IMPROVES THE THERAPEUTIC BENEFIT OF CHEMOTHERAPY AND SURGERY

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Abstract Content: Introduction: Metastasis is responsible for most of the cancer-related deaths (~90%). Chemotherapy pre- and/or post-surgery remains the standard of care for patients with metastatic triple negative breast cancer (TNBC). Immunotherapy has revolutionized cancer therapy with cytokines being of great interest. IL-15 stimulates the proliferation and cytolytic activity of $CD8^+$T and NK cells and has been reported to have anti-tumor and anti-metastatic activity. We have produced the native heterodimeric IL-15 (hetIL-15) which has advanced in clinical trials due to its anticancer activities. Here, we report the therapeutic effects of hetIL-15 alone and in combination with doxorubicin and surgery using the 4T1 mouse model of TNBC.
Design and methods: We studied the efficacy of the treatments on the primary tumor, survival and metastatic disease in periphery and lungs. The anti-metastatic effect was evaluated by examining the circulating tumor cells (CTCs) in blood and the metastatic foci in lungs by clonogenic assays. Metastasis in lungs was also examined by India Ink staining and histology analysis. Treatment effects in immune cells in blood, spleen, lungs and tumors were analyzed by flow cytometry and immunohistochemistry (IHC). Surgeries were performed one week after tumor cell inoculation and the treatment was given pre- and post- surgery. Cured animals were rechallenged without any treatment.
Results: Mice treated with hetIL-15 in combination with doxorubicin had significantly better survival and tumor growth delay in comparison to the other groups. hetIL-15 alone showed a modest, but significant effect on the tumor growth. H&E histological analysis in the lungs revealed substantially fewer metastatic foci after hetIL-15 monotherapy, and even fewer in the combination group. Clonogenic assays from lungs and blood, reinforced these findings showing significantly lower numbers of tumor colonies upon hetIL-15 monotherapy and combination treatment. Immune profiling of blood, spleen, lungs and tumors by flow cytometry and IHC revealed a significant systemic increase of cytotoxic effector cells ($CD8^+$T cells and NKs), combined with reduction of suppressive populations (PMN-MDSCs), especially in the combination group. hetIL-15 monotherapy or co-administration with doxorubicin, together with surgery, led to the cure of approximately 50% of the treated mice. Rechallenge of the cured animals showed better tumor growth control in the absence of any treatment.
Conclusions: Our results demonstrate that hetIL-15 reduces metastatic disease in both blood and lungs and doxorubicin enhances the anti-metastatic effect. The two agents synergize to shift the immune cell landscape towards an effector phenotype that might contribute to the anti-metastatic efficacy. After treating the animals pre- and post-surgery, the effectiveness of the treatment is maximized, and both hetIL-15 monotherapy and combination treatment cure approximately 50% of the animals. Our findings suggest exploring the use of hetIL-15 in combination with chemotherapy in clinical surgery protocols for the treatment of TNBC.

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P306

DAMPENING INFLAMMATION BY ENHANCING REGNASE-1 EXPRESSION WITH STEM-LOOP-TARGETING MORPHOLINO OLIGONUCLEOTIDES

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Abstract Content: Post-transcriptional regulations of gene expression exert controls over immune responses and have important roles in fine-tuning the inflammatory events to prevent undesired tissue damage. Previous studies have shown that RNA-binding protein Regnase-1 (also known as Zc3h12a and MCPIP1) plays essential roles in maintaining tissue homeostasis through post-transcriptional regulations of mRNA stability. In the steady state, Regnase-1 degrades pro-inflammatory mRNAs by binding to the conserved stem-loop (SL) structures within their 3'untranslated regions (UTRs). More interestingly, we found that Regnase-1 can negatively regulate its own mRNA stability by targeting the SL motifs in its 3'UTR upon TLR activation.

Here we found that disruption of the SL structural elements at Regnase-1 3'UTR specifically blocks the Regnase-1 mediated self-degradation. Introduction of antisense oligonucleotides (ASOs) targeting Regnase-1 SL structures

successfully reduced Regnase-1 binding to its mRNA, thus augmenting the expression of Regnase-1 in bone marrow-derived macrophages (BMDMs) and in human macrophages. In addition, enhancing Regnase-1 availability greatly suppressed the expressions of pro-inflammatory transcripts which are directly targeted by Regnase-1, such as *Il6* and *Il1b*, in LPS-stimulated BMDMs. Furthermore, Regnase-1 manipulation *in trans* effectively relieved the inflammatory responses in acute LPS-induced lung injury model and in experimental autoimmune encephalomyelitis (EAE) mouse model, thereby improving their clinical outcomes. Taken together, our findings present an attractive therapeutic strategy that utilizes ASOs to enhance Rengase-1 abundance, which can be beneficial for alleviating inflammation and stress responses in inflammatory diseases.

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P307

THE INTERLEUKIN-6 FAMILY OF CYTOKINES REPRESENT A THERAPEUTIC OPPORTUNITY FOR PANCREATIC CANCER

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Abstract Content:
Introduction: Pancreatic cancer has one of the worst prognoses of all malignancies, with few new drugs approved for the treatment of this cancer over the past 40 years. Pancreatic ductal adenocarcinoma accounts for the vast majority of pancreatic cancer cases and is characterised by the presence of a dense stroma that impacts therapeutic efficacy and drives pro-tumorigenic programs. The inflammatory nature of the stromal microenvironment contributes to loss of anti-tumour immunity and development of resistance to current treatments. We explored the contribution of the Interleukin (IL)-6 family of cytokines to pancreatic cancer, including their contribution to pancreatic inflammation and various roles in pancreatic cancer pathogenesis.
Methods: We undertook a systematic analysis of IL-6 family cytokine expression in the circulation, as well as within tumour tissue, in our biobank of human pancreatic cancer patients to establish correlation with treatment response and overall survival. We employed reporter mice, a genetically engineered mouse model of pancreatic cancer, and syngeneic organoid xenografts to define the specific contribution of stromal derived cytokines to cancer progression.
Results: We reveal that elevated IL-11 expression is associated with poor overall survival. We show that in both mice and humans IL-11 is produced by both cancer cells and the stromal environment and drives an oncogenic program in pancreatic cancer.
Conclusions: In recent years, there has been a large emphasis on dual targeting of cancer cells and the tumour microenvironment. Our results highlight the potential opportunities for therapeutic targeting of IL-11 as an avenue towards combating poor patient outcomes.

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P308

ENGINEERING A CYTOKINE/ANTIBODY FUSION PROTEIN THAT SELECTIVELY EXPANDS REULATORY T CELLS AND CONFERS PROTECTION AGAINST AUTOIMMUNE DISEASES

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Abstract Content: Interleukin-2 (IL-2) is a pleiotropic cytokine that mediates key functions in immune homeostasis, particularly those pertaining to the regulation of T cells. IL-2 treatment has been clinically approved to treat cancer and has also proven beneficial for autoimmune disorder therapy and regenerative medicine applications. However, high toxicity, short serum half-life, and lack of specificity limit the current clinical potential of IL-2 treatment. Recent work has shown that complexing IL-2 with anti-IL-2 antibodies can increase efficacy and reduce toxicity of the cytokine by both extending its *in vivo* half-life and selectively targeting its functions toward particular immune cell subsets. Although this approach is promising, clinical translation of these complexes is complicated by the need for optimization of dosing ratios and by the instability of the complex, as dissociation will lead to toxicity and rapid clearance of the free cytokine.
To overcome these challenges, we have developed single-chain IL-2/antibody fusion proteins (known as immunocytokines) that stably and selectively deliver IL-2 to specific immune cell populations. In particular, we leveraged insights from structural biology to engineer a fusion protein that tethers human IL-2 to an anti-IL-2 antibody known as F5111 in order to achieve selective expansion of immunosuppressive regulatory T cells (T_{Reg}s). We further applied crystallographic insights to rationally design a panel of immunocytokine variants with a range of potencies on human T_{Reg}s. *In vitro* and *in vivo* studies identified the lead molecule, which shows optimal bias toward expanding T_{Reg}s over immune effector cell populations (such as effector T cells and natural killer cells). Our lead molecule has also demonstrated therapeutic benefit in mouse models of colitis and checkpoint inhibitor-induced diabetes. Our innovative approach presents a translationally relevant and versatile therapy for selective T_{Reg} expansion, which can be used for a wide range of research and medical applications.

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P309

A PILOT STUDY OF PALLIATIVE RADIOTHERAPY AND SEQUENTIAL IMMUNOTHERAPY IN THREE PATIENTS WITH METASTATIC UVEAL MELANOMA: FEASIBILITY AND POSSIBLE BENEFITS OF COMBINATION

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Abstract Content: Background & Methods
Uveal melanoma UM is the most common primary intraocular malignancy in adults. Despite local therapy about 50% of patients go on to develop metastasis. Radiotherapy, Interleukin-2 (IL-2) and checkpoint inhibitors (ICIs) are effective treatments for metastatic melanoma. Here we report feasibility of sequential combination of all three in UM patients.
All patients received palliative radiation followed by high-dose (HD) IL-2, then nivolumab and ipilimumab every 3 weeks for 4 doses. The primary objective was to estimate objective response rate (ORR) after 12 weeks and to confirm safety and tolerability of the approach. Next generation sequencing (NGS) was conducted on formalin-fixed, paraffin-embedded metastatic biopsies. Flow cytometry (FM) analyses were performed on peripheral blood collected at various time points.
Results
Three patients with metastatic UM enrolled and have reportable results. Safety of sequential therapy was shown. No protocol defined dose limiting toxicities were observed. The best overall response was stable disease, with an 18.4% decrease in target lesion size. Two patients developed progressive disease. The ORR was not reported. One patient is alive 27 months after study entry, and overall survival (OS) was 23 months and 15 months for the other patients. After trial participation, one patient entered hospice, one received Y90 radioembolization to liver with ipilimumab, and one received nivolumab with laparoscopic microwave ablation of liver lesions. Baseline tumor NGS showed biologically relevant genomic variants in GNA11 (2), GNAQ (1), BAP1 (3), ARID1B (1), TET2 (1), microsatellite stability (3) and low tumor mutational burden (3). FM analyses showed an increase in Ki-67+ CD8+ T cells, NK cells, and regulatory T cells (T_{reg} cells) from baseline. There was a statistically significant increase in the percentage of CD8+-Ki67+ cells ($F(4, 8) = 7.466186$, $p < 0.01$) and mature NK CD56dim-Ki67+ cells ($F(4, 8) = 4.853647$, $p < 0.05$) from

baseline through day 15 pre-infusion. However, the CD56dim-CD16+ NK cell subset declined and reached a nadir on cycle 1 day 5 post treatment ($F(3, 6) = 12.28209$, $p < 0.01$). In contrast, Ki67+ NKT- cells showed a marginal statistical significant increase ($F(4, 8) = 4.961018$, $p < 0.05$). The increase in cycling T cells and NK cells was also associated with an increase in immunosuppressive T_{reg} cells ($F(4, 8) = 8.123822$, $p < 0.01$), though data suggests the effect of IL-2 on T_{reg} cells decreased over 12 weeks of dual-ICI treatment. A major limitation on reportable data in this trial was the inability to collect relevant samples at later time points of interest.

Conclusion

Preliminary results from this pilot study demonstrated radiation therapy followed by HD IL-2 and dual-ICIs was feasible in this patient cohort. While objective responses to the study treatment were not observed, OS compared favorably with historical data. Early increases in peripheral blood T cell and NK cell subsets were seen, and changes induced by the study treatment merit further investigation.

Disclosure of Interest: None Declared

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P310

SYNTHEKINES: A NOVEL COMBINATORIAL ARRAY OF BIASED SURROGATE CYTOKINE AGONISTS WITH ANTIBODY-LIKE DRUGGABILITY.

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Abstract Content: Cytokines are secreted proteins that activate signaling pathways in immune cells by bringing together two or more receptors. Their use as therapeutics is limited by the cognate receptors they can engage and by the pleiotropism that these receptors exhibit across several cell types. At Synthekine we have sought to overcome these limitations using single domain (VHH) antibodies to dimerize both native and non-native pairs of cytokine receptors. This allows us to modulate naturally occurring signals as well as create new ones aimed at specific cell types.

Here we describe three examples, in which we recapitulate the activity of Interleukin-2 and Interleukin-10 as well as create a non-natural signal derived by the hybridization of the two receptors.

VHHs specific for IL-10Rα, IL-10Rβ, IL-2Rβ and IL-2Rγ were generated by llama immunization and screening of VHH libraries prepared from peripheral blood. Seven IL-10Rα VHHs and seven IL-10Rβ VHHs were combined as expression fusions in an all-by-all matrix and in both amino/carboxy terminal orders, thus yielding 98 unique surrogate cytokines agonists (SCA). Similarly, ten IL-2Rβ and six IL-2Rγ VHHs were combined into 120 SCAs. Finally, seven IL-10Rα and six IL-2Ry VHHs yielded 84 unique SCAs. After screening on reporter cell lines, all three combinations returned a selection of SCAs able to generate activity in primary cells.

In particular, IL-2 SCAs stimulated pSTAT5 phosphorylation, proliferation and IFNγ secretion in both NK and $CD4^+$/$CD8^+$ T cells. IL-10 SCAs induced pSTAT3 phosphorylation in human Monocytes, B cells, NK cells, $CD4^+$/$CD8^+$ T cells with varying signaling strengths. They also inhibited LPS-induced secretion of IL1β and TNFα by monocytes but were less potent at inducing IFN-g and Granzyme production in T cells, thus providing an opportunity to decouple the immunosuppressive and immunostimulatory activities of IL10. Finally, the IL-10/IL-2 hybrid SCAs induced pSTAT3 signal in B cells, NK cells, $CD4^+$/$CD8^+$ T cells with little to no pSTAT3 signal in monocytes. They also induced proliferation and Granzyme production by $CD8^+$ T cell blasts generated upon CD3/CD28 activation. Consistent with the lack of STAT3 signaling in monocytes, these IL-10/IL-2 SCAs did not inhibit LPS-induced secretion of IL1β and TNFα by monocytes, suggesting selectivity and a lack of immunosuppressive activities.

We believe this platform will enable a rapid, combinatorial expansion of both existing and novel cytokine signaling solutions for specific immune cells of interest, combined with the optimal druggability of monoclonal nanobodies.

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P311

PROVIRAL EFFECTS OF ISG15 AND ANTIVIRAL POTENTIAL OF NRF2 ACTIVATORS AGAINST HUMAN CORONAVIRUS 229E INFECTION IN IPSC DERIVED VASCULAR ENDOTHELIAL CELLS

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Abstract Content: In contrast to the highly pathogenic coronavirus strains, HCoV-229E commonly circulates in the human population and is responsible for approximately 5–10% of all upper and lower respiratory tract infections. HCoV-229E evokes general upper respiratory illness in healthy individuals, but is still capable of causing more severe disease in immunocompromised and older individuals. In order to explore the role of ISG15 in the context with coronavirus infection and the emerging importance of endothelial involvement, we established hiPSC-derived vascular endothelial cells (ECs) as infection model. Contrary to murine ISG15, human ISG15 is known to have proviral effects. For instance, we have previously shown that *ISG15*$^{-/-}$ cells are less susceptible to influenza A virus infection than wild-type cells. We now show that infectivity of hCoV-229E is reduced in *ISG15*$^{-/-}$ ECs, thus adding it to the list of viruses upon which ISG15 exerts a proviral effect. We then assessed the role of the cytoprotective NRF2 signaling pathway in hiPSC-derived WT and *NRF2*$^{-/-}$ ECs. Viral replication was markedly increased in *NRF2*$^{-/-}$ cells, suggesting an antiviral role of this pathway. In order to meet the clinical demand for host-directed antiviral compounds, we tested the antiviral effect of the NRF2 activators bardoxolone-methyl, sulforaphane, and 4-octyl itaconate on HCoV-229E infection. These treatments reduced replication of HCoV-229E, but, strikingly, this antiviral effect was also seen in *NRF2*$^{-/-}$ cells. Taken together these results confirm the proviral role of human ISG15, reveal an antiviral role of NRF2 signaling in human coronavirus infection and suggest that the antiviral effects of bona fide NRF2 agonists may be mediated by as yet unidentified NRF2-independent targets. In addition, the results suggest NRF2 activators as adjunct (host-directed) treatment options for infection by coronaviruses.

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LB-33

NANOBODIES DISMANTLE POST-PYROPTOTIC ASC SPECKS AND COUNTERACT INFLAMMATION IN VIVO

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Abstract Content: Inflammasomes sense intracellular clues of infection, damage, or metabolic imbalances. Uncontrolled activation of inflammasomes and the continued release of IL-1 cytokines have been at the core of research on chronic inflammatory diseases. They have also motivated a competitive race in the pharmaceutical industry to find inhibitors of inflammasomes (e.g. NLRP3) or IL-1 signaling. Since these drugs target either a specific sensor or the IL-1 output, they result in the inhibition of both necessary host-defense and aberrant activities of the pathway.
Upon activation, inflammasome sensors polymerize the adaptor ASC to form micron-sized 'specks' that maximize the activation of caspase-1 and the processing of IL-1 cytokines. Caspase-1 also activates GSDMD leading to the formation of pores in the plasma membrane. These drive pyroptosis, a lytic form of cell death characterized by the release of intracellular content to the extracellular space. Among released material, ASC specks are found in the blood and tissues of patients in the context of chronic inflammation. However, their contribution to disease and their potential as therapeutic target mostly remained unexplored.
Here, we show that camelid-derived single-domain antibodies (VHHs) against human or mouse ASC (VHH_{ASC} or VHH_{mASC}) disassemble inflammasomes, neutralizing their extracellular prionoid and inflammatory functions. Targeting of ASC specks by VHH_{ASC} requires cytosolic access and thus occurs after pyroptosis-driven membrane perforation, which exposes ASC specks to the extracellular space. Thereby, VHH_{ASC} targets inflammasomes while preserving early IL-1β release essential to host defense. Strikingly, we show that systemically administrated $VHH_{mASC}$is highly

efficient against both acute gouty inflammation induced by intra-articular injection of monosodium urate crystals and chronic inflammation in antigen-induced arthritis (AIA). Hence, ASC-specific VHHs can effectively abrogate the inflammatory functions of ASC specks that outlive pyroptosis, revealing for the first time their crucial role in disease development. Furthermore, our data introduces the concept that ASC specks can be disassembled. These findings open an unprecedented path to specifically target post-pyroptotic inflammasomes and prevent prolonged inflammation while maintaining their essential function in host defense.

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LB-34

MITOCHONDRIA-TARGETED SULFIDE DELIVERY MOLECULES – NEW AND NOVEL PLAYERS THAT CAN SUPPRESS AND REVERSE CIGARETTE SMOKE-INDUCED INFLAMMASOME ACTIVITY

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Abstract Content: RATIONALE: Cigarette smoke (CS) is the major risk factor in development of chronic obstructive pulmonary disease (COPD). Interventions that can prevent and/or reverse disease are urgently needed. Hydrogen sulfide (H_2S) is generated in mitochondria (mt) and is crucial in maintaining mt respiration and suppressing oxidative stress/inflammation. Lung H_2S levels are reduced after CS exposure. Lung inflammation, mt damage and oxidative injury are exacerbated as a result of inhibition/silencing of H_2S enzymes, suggesting impairment of H_2S synthesis/loss of bioavailability is detrimental in COPD and negatively impacts mt health. METHODS: We have produced novel mt-targeted H_2S donors (mtH_2SD) AP39 and RT01 to investigate whether these molecules could suppress and/or reverse CS-induced inflammation and lung injury. To investigate suppression, mice were exposed to CS (or air) for 8 wks (with 1.0 mg/kg). To investigate reversal, mice were exposed to CS for 8 wks followed by either 4 wks rest or continued CS exposure, each with mtH_2SDs (1.0 mg/kg). Airway inflammation (BALF differential cell counts, IL-1β by ELISA) and lung function were assessed. RESULTS: Lung H_2S levels were reduced and inflammasome activity increased in response to CS exposure. mtH_2SD significantly suppressed CS-induced alveolar destruction, fibrosis and improved lung function. mtH_2SD treatment reversed CS-induced lung neutrophil, eosinophil and macrophage infiltration, loss of lung function, and partially reversed airway resistance in both models. CONCLUSIONS: Targeting H_2S to mitochondria may be a novel therapeutic approach to prevent and/or reverse mitochondria-driven inflammation and lung injury in COPD and related diseases.

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LB-55

ENGINEERED HUMAN IL-2/IL-2RB ORTHOGONAL PAIRS SELECTIVELY ENHANCE ANTI-GPC3 CAR T CELLS TO DRIVE COMPLETE RESPONSES IN SOLID EPITHELIAL TUMOR MODELS

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Abstract Content: CAR T cell therapy has demonstrated clinical efficacy against relapsed and refractory hematological malignancies. However, prominent barriers including poor T cell effector function, lack of proliferation, and limited CAR T cell persistence have prevented CAR T cell therapies from reaching their full curative potential, especially in solid tumors. Interleukin-2 (IL-2) is a potent stimulator of T cell proliferation, survival, and cytotoxic function, thereby making it an attractive cytokine to support CAR T cell therapy. However, therapeutic use of IL-2 is

limited by systemic toxicity due its promiscuous activation of undesired immune cell populations, including non-tumor reactive T cells and NK cells. To facilitate selective ex vivo and in vivo expansion of engineered T cells we have developed a human orthogonal (ortho) ligand/receptor system consisting of a IL-2 mutein (STK-009) that does not significantly stimulate cells expressing wild type IL-2 receptor and a mutated IL-2 Receptor Beta (orthoIL-2Rβ) that responds to STK-009 but not wild type IL-2. This system enables in vivo IL-2 signaling in engineered cells that express the orthoIL-2Rβ while avoiding stimulation of native T cells and NK cells. Previously, we demonstrated the ability of the STK-009/orthoIL-2Rb receptor pair to selectively enhance the anti-tumor efficacy of orthoIL-2Rb (hoRb) expressing CD19 CAR T cells (SYNCAR-001) in preclinical lymphoma mouse models1. We also demonstrated that STK-009 is selective for the orthoIL-2Rb expressing cells and therefore in a non-human primate model does not stimulate native T or NK cells1. Here, we demonstrate the ability of the STK-009/hoRb system to enhance the anti-tumor activity and persistence of anti-glypican 3 (GPC3) CAR T cells. GPC3 overexpression is associated with various malignancies such as hepatocellular carcinoma (HCC), pediatric sarcomas, and non small cell lung carcinoma (NSCLC). Clinical trials of GPC3 CAR T therapy are ongoing, but early data suggests a need to boost CAR T cell function and persistence to achieve significant clinical responses. We incorporated the hoRb downstream of a second generation anti-GPC3 CAR via a T2A cleavage peptide (SYNCAR-002) to allow for bicistronic expression from one lentiviral construct. In vivo, STK-009 administration enhanced the anti-tumor efficacy of SYNCAR-002 in highly aggressive subcutaneous and intraperitoneal HCC models. STK-009 treatment resulted in significant expansion of SYNCAR-002 in the peripheral blood and drove infiltration of SYNCAR-002 into tumors. STK-009 treatment also induced intratumoral granzyme B and IFN-g production by SYNCAR-002 indicating activation of effector T cell function. These findings validate that the orthogonal IL-2 platform has the potential to improve the efficacy and durability of CAR T therapy for solid tumor targets such as GPC3 by selectively expanding CAR-T cells in vivo, driving CAR-T cells into the tumor, and activating CAR-T cells in the tumor microenvironment. 1 Aspuria et al. An Orthogonal IL-2 and IL-2Rb System Drives Persistence and Activation of CAR T cells and Bulky Lymphoma. Science Translational Medicine. 13(625). Dec 2021.

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12. Other

P312

EFFICACY ASSESSMENT NATURALLY OCCURING BIOACTIVE MOLECULES AGAINST COVID-19(SARS-COV-2) USING MOLECULAR DOCKING.

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Abstract Content: The fatal virus SARS-COV-2 emerged in December, 2019 which caused global pandemic of Coronavirus disease also known as COVID-19 as millions of cases of infection confirmed by the laboratories worldwide using several tests. Identification of the natural components that can show inhibition against the proteins and enzymes of the virus SARS-COV-2 by targeting the host cells and immune system of human body is highly useful for the treatment of COVID-19. The present study is an effort to screen the assessment of naturally occurring bioactive compounds against SARS-COV-2 using docking studies*i.e.* binding affinity of the involved enzymes of the host cells to bioactive compound against SARS-COV-2. Docking studies were performed to evaluate themaximum affinity of the selected bioactive molecules along with the standards drug remdesivir and ivermectin against the important target proteins of coronavirus. As per the docking results, after analysing 23 bioactive molecules already reported for various pharmacological activities evaluated against all three important target proteins of coronavirus *i.e.* spike protein, Mpro and ACE-2 which plays a key role in viral attack as well as further multiplication to the host cell. Only curcumin and apigenin has been found effective against all proteins. There have been several reports related to curcumin but this is the first report about apigenin against all important target proteins of corona virus. Although curcumin have shown comparable response against spike protein in context of remdesivir but against MProapigenin is comparable to remdesivir and for human ACE-2 response of apigenin is more significant comparative to curcumin, remdesivir, ivermectin.The flavonoid compound apigenin because of having all significant properties on the basis of the docking parameters as well as ADME properties, it could be a possible drug candidate for curing COVID-19.

Key words: SARS-COV-2, Molecular Docking, Apigenin, Curcumin, ACE-2, Mpro

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P313

GUT MICROBIOTA REGULATES THE ANTITUMOR IMMUNE RESPONSES AGAINST GLIOBLASTOMA MULTIFORME

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Abstract Content: Glioblastoma multiforme (GBM) is a brain tumor with a very poor prognosis. Even with both radiotherapy and chemotherapy after surgical resection, the five-year survival rate is extremely low. In addition, the response rate is significantly lower for immunotherapy, which is effective in other cancers. That may be because myeloid cells dominate the environment around the glioblastoma. Thus, unlike other cancers, a novel approach is required for glioblastoma. Commensal microbiota reside in the human body and are involved in both healthy and disease states of the host. To date, research into the role of the microbiota has mainly been focused around the gastrointestinal tract. However, the role of microbes remains unknown in GBM, which occurs in tissues distant from the gastrointestinal tract. Here, we observed a distinct change in gut microbial composition and their metabolisms during GBM progression using 16S rRNA sequencing in two different GBM animal models. The amount of tryptophan was significantly reduced in the gut of GBM-bearing mice, compared to that of healthy mice. Based on these results, we devised the tryptophan supplementation. This diet improved the survival against GBM. More cDC1 and CD8 T cells were accumulated in the tumor microenvironment in tryptophan-treated mice. This improvement was commensal microbiota-dependent. Thus, we compared the gut microbiota at the species level in the gut of tumor-bearing mice after tryptophan supplementation. Surprisingly, some microbiota were restored by tryptophan treatment, comparable to gut of the normal mouse. In this point, we assumed that some homeostatic microbiota may be critical for the defense against GBM. We observed the better survival in co-housed group with normal mouse and found a species that was highly different between two groups. The colonization with this bacteria led to the increase in the survival against GBM. Collectively, we discovered the microbial species that were critical for GBM.

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P314

NITROOLEIC ACID INHIBITS MACROPHAGE ACTIVATION INDUCED BY LIPOPOLYSACCHARIDE FROM PREVOTELLA INTERMEDIA

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Abstract Content: The hypothesis of the present study was that nitro-fatty acids would suppress inflammation associated with periodontal disease. To test this hypothesis, we investigated the influence of nitrooleic acid (OA-NO_2), a prototypical nitro-fatty acid, on the inflammatory response of murine macrophages activated with lipopolysaccharide (LPS) from *Prevotella intermedia*, a pathogen associated the etiology of different types of periodontal diseases. LPS was prepared from *P. intermedia* cells employing the phenol-water protocol. Accumulation of proinflammatory mediators in the culture supernatant was assayed. The induction level of mRNA was evaluated by real-time

polymerase chain reaction analysis, and protein expression by immunoblot analysis. The secreted embryonic alkaline phosphatase reporter assay was performed to measure NF-κB activation. The transcription factor assay kit was used to evaluate DNA-binding of NF-κB subunits. The generation and mRNA expression of inducible nitric oxide (NO) synthase-derived NO, IL-1β and IL-6 induced by LPS were suppressed by OA-NO_2. OA-NO_2 encouraged the switch from proinflammatory M1 macrophages to anti-inflammatory M2 macrophage phenotype in cells activated with LPS. OA-NO_2 exerts its effect via heme oxygenase-1 induction and suppression of NF-κB signaling, independently of PPAR-γ, JNK, p38 and STAT1/3. OA-NO_2 may represent a novel class of agent as a host modulator which has therapeutic benefit in periodontal disease, though more works are needed to confirm this.

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P315

BLOOD MONOCYTE-DERIVED CD169+ MACROPHAGES CONTRIBUTE TO THE ANTITUMOR IMMUNITY AGAINST GLIOBLASTOMA MULTIFORME

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Abstract Content: Therapies against glioblastoma multiforme (GBM) have been largely ineffective due to the infiltration of immunosuppressive tumor-associated macrophages (TAMs). Recent studies demonstrated that TAMs can also be immune-activating. However, markers differentiating these heterogeneous macrophage populations have not been established. In this study, we identified a subset of macrophages expressing CD169 that promote an anti-tumoral microenvironment in GBM. Using single-cell transcriptome analysis, we found that $CD169^+$ macrophages in human and mouse gliomas produced proinflammatory chemokines, leading to the accumulation of T cells and NK cells. Depletion of $CD169^+$ macrophages shortened the survival of mice with gliomas and reduced the function of antitumor lymphocytes. We showed that IFN-γ produced by NK cells was critical for the accumulation of blood monocyte-derived $CD169^+$ macrophages into gliomas. Additionally, CD169 expression on macrophages increased the phagocytosis of apoptotic glioma cells. Our finding suggests that the $CD169^+$ subset of TAMs promotes antitumor immune responses against GBM.

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LANDSCAPE ANALYSIS OF SINGLE-CELL RNA SEQUENCING IN FCΓRIIB DEFICIENCY AGAINST BRAIN CANCER

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Abstract Content: Glioblastoma multiforme (GBM) is the most common and fatal malignant cancer in the central nerve system. Standard treatment for patients with GBM is surgical tumor resection followed by combined radio-chemotherapy. Although this standardized treatment shows improved patient survival, the prognosis for patients still remains extremely poor with 15 months of median survival. The immune checkpoint blocker (ICB) is effective on hematological tumors though the application of the ICB is still not optimistic on GBM patients. These limitations of the treatments originate from the lack of comprehensive understanding of the tumor microenvironment (TME). TME

consists of not only the heterogeneous glioblastoma stem cells but also the various types of immune cells with pro-tumoral properties. Thus, understanding of this tumor infiltrating immune cells and functional status in GBM can provide a better approach to treatment strategies. To collect the accurate and broad spectrum of the information about the tumor infiltrating immune cells, we performed single-cell RNA sequencing on immune cells of GL261 injected GBM mouse model. From the results, we identified that the GBM up-regulated inhibitory FcγR gene, *Fcgr2b*, on tumor infiltrating immune cells, and this inhibitory receptor quantitatively and qualitatively suppressed the antitumor response. FcγRIIb is commonly mediates immune homeostasis through the binding of the immune complex. Broad types of immune cells express FcγRIIb and reversely blocking or deletion of the receptor induces proinflammatory response. For the adequate understanding of the function of the FcγRIIB in GBM condition, we used the FcγRIIb-KO mouse and analyzed the landscape of tumor infiltrating immune cells. At the early time point of GBM, 10 days, tumor associated microglia are polarized from pro- to anti-tumoral states in FcγRIIb-KO mouse. The microglia enhanced the TNF-alpha signaling pathway and upregulated adaptive immune cell recruiting chemokines such as *Ccl3* and *Ccl4*. Furthermore, at a late stage of tumor progress, 20 days of GBM, total immune cell number was increased in FcγRIIb-KO mouse. Especially cytotoxic CD8 T cells increased in tumor site infiltration with enhanced cytotoxicity with enriched gene expression of *Prf1* and *Gzmb*. Based on these analyses, FcγRIIb plays critical roles in GBM infiltrating immune cells particularly microglia and cytotoxic CD8 T cells. This work is worth investigating since the understanding of the role of FcγRIIb in GBM and provides a detailed dataset for heterogeneity of GBM-associated immune cells.

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STAT3 AS AN ENABLER OF PANCREATIC CANCER

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Abstract Content: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most incurable cancers. Since mutant KRAS is the oncogenic driver in up to 90% of PDAC cases, therapeutic intervention has logically focused on the RAS/MAPK pathway. However, inhibition of effectors downstream of mutant KRAS has resulted in only modest clinical efficacy and today benefit only a minority of cancer patients. Efforts to block the action of mutant KRAS directly have led to the recent development of inhibitors that target oncogenic KRAS G12C. While these drugs represent a significant therapeutic advancement, the G12C mutation is rare in PDAC and translation into human patients showed partial responses to G12C inhibition. For these reasons efforts have focused on identifying additional cancer dependencies. Curation of human PDAC databases revealed that STAT3 expression is non-uniform and predominates in cancers that have low KRAS dependence or an enhanced resistance to KRAS inhibition. To explore the dependency of PDAC cells on STAT3, we used LentiCRISPR/CAS9 gene editing to ablate mutant KRAS and/or STAT3 in human and mouse PDAC tumor cell lines. Their combined ablation impairs tumor growth in nude mice and abrogates it in immunocompetent mice. Transcriptome profiles and immune cell tumor infiltration are expected to provide targets of intervention for rationale-based therapies.

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THE STAGE-SPECIFIC ROLE OF THE GLYCAN RECOGNITION MOLECULE GALECTIN-4 IN GASTRIC ADENOCARCINOMA

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Abstract Content: Galectin-4, a tandem repeat member of the β-galactoside-binding proteins, belongs to the family of galectin. Galectin molecules play biological roles in various diseases and can be combined with galactose-containing glycans. A 1,4-N-acetylglucosaminyltransferase (A4GnT) is responsible for O-glycans' biosynthesis. Gastric mucin, MUC6, contains unique O-glycans that carry terminal α1,4-linked N-acetylglucosamine residues (αGlcNAc), that acts an antibiotic against *Helicobacter pylori* (*H.pylori*) which is leading cause of gastric adenocarcinoma. A4GnT catalyzes the formation of αGlcNAc, but the mice deficient in *A4gnt* gene, even if they are not infected with *H.pylori*, will spontaneously display a prolonged progression to differentiated-type gastric adenocarcinoma in a hyperplasia-dysplasia-adenocarcinoma continuum. However, the exact molecular mechanism of the development of gastric cancer in *A4gnt* KO mice remains unclear. Based on the immunohistochemical screening, we selected the galectin-4 molecule from the galectin family molecules for further research to explore its role in gastric cancer. Since galectin-4 has two carbohydrate recognition domains, we considered whether galectin-4 could bind to another molecule to modulate the development of gastric cancer. Studies have also shown that in prostate cancer, galectin-4 can bind to the truncated O-glycan, Thomsen–Friedenreich antigen, catalyzed by glycosyltransferase encoded by the *C1GALT1* gene and promote the stemness characteristics of cancer cells. This study first examined whether galectin-4 binds to the glycan structures produced by C1GALT1 in gastric cancer. We found that galectin-4 is necessary for the proliferation of human gastric cancer cell lines, and the knockdown of *C1GALT1* reduced the proliferation of gastric cancer cells. Furthermore, we found that loss of galectin-4 suppressed development of gastric adenocarcinoma, but not precancerous hyperplasia/dysplasia, in *A4gnt* KO mice, and an expression of the glycan structure Galβ3GalNAc catalyzed by C1GALT1 was strongly enhanced at tumor sites during gastric cancer development. These findings suggest that galectin-4 plays stage-specific role in gastric cancer development by recognizing truncated O-glycan distinctively expressed in adenocarcinoma cells.

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ORAL ADMINISTRATION OF TRANSGENIC HUIFN-CONTAINING TOMATOES ALTERS THE CYTOKINE PROFILE AND ENSURES THE SURVIVAL OF VSV-INFECTED BALB/C MICE

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Abstract Content: We investigated the protective influence of orally administrated HuIFN from transgenic tomatoes on mice survival and cytokine profiles after infection with *Vesicular stomatitis virus* (VSV). Males of 1.5 month-old mice (BALB/c strain) were inoculated intranasal and intraperitoneally with 0.5 ml of viral dilution. The lethal dose (LD50) of VSV inoculum was 1 ml (as determined previously). HuIFN-containing tomatoes were added *ad libitum* to the experimental group of mice 3 hr prior to inoculation and given for 3 days after. Inoculated mice were examined twice daily and kept for 2 weeks before blood serum collecting. Chemokine profiles of GM-CSF, MCP-1, MCP-3, MIP-1α, MIP-1β, and RANTES were detected with Mouse Chemokine 6plex Kit FlowCytomix (BMS821FF, eBioscience, USA) by flow cytometer DxFlex (Beckman Coulter), equipped with argon laser (488 nm). Results were processed with CytExpert for DxFLEX 2.0.2.18 program. Total interferon (IFN) activity in blood serum was detected with a biological method. The statistical design was carried on by calculating median meanings.
The cytokine profile and response of IFN production in VSV-infected and uninfected mice differed. IFN activity in blood serum of uninfected intact mice was 1.25 IU/ml, while it was 5.0 IU/ml in the control group of animals treated with HuIFN-containing tomatoes. VSV infection progress in the untreated group of the infected mice led to the death of

20% of animals. The survived animals demonstrated a 25-fold increase in total IFN activity in serum (30.0 IU/ml). While IFN-activity was a third lower (22.5 IU/ml) in serum of infected mice treated with HuIFN -containing tomatoes. Thus, oral administration of transgenic IFN-containing tomatoes was accompanied by the decrease of IFN levels in serum in the late stages of VSV infection.
The same patterns were observed for proinflammatory chemokines: the levels of MCP-3 and MIP-1α increased statistically significantly and were 464 pg/ml and 131 pg/ml respectively in infected mice versus 317 pg/ml and 118 pg/ml in intact animals. In mice that consumed IFN-containing tomatoes the amount of MCP-3 and MIP-1α lowed to the values similar to intact animals and were 280 pg/ml and 117 pg/ml respectively. The amount of MCP-1 and RANTES in blood serum of intact mice was 207 pg/ml and 303 pg/ml while it increased to 313 pg/ml and 339 pg/ml respectively in VSV-infected animals. The amount of MCP-1 and RANTES rose in the group of mice treated with IFN-containing tomatoes and was 343 pg/ml and 347 pg/ml, respectively. The amount of MIP-1β and GM-CSF was below calibration in all the samples that precluded interpretation of the obtained results.
Thus, feeding BALB/c mice with HuIFN-containing tomatoes reduced lethality: all the experimental animals survived. The protective effect of treatment is associated with the increase of MCP-1 and RANTES in mice blood serum and stabilization of MCP-3, MIP-1α, and the total level of serum IFN of VSV-infected mice.

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LB-35

IL-5RA+ PLASMA CELLS IN ASPIRIN-EXACERBATED RESPIRATORY DISEASE SHOW INCREASED CELLULAR PROLIFERATION

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Abstract Content:
Background
Chronic rhinosinusitis with nasal polyps (CRSwNP) is marked by inflammatory outgrowths of paranasal sinus tissue causing nasal obstruction and anosmia. Aspirin-exacerbated respiratory disease (AERD) is a severe phenotype of CRSwNP associated with asthma and respiratory reactions to cyclooxygenase-1 inhibitors. Higher nasal polyp IgE levels are seen in AERD and correlate with disease severity.
Objective
We studied differences in the transcriptome and protein activation markers of nasal polyp plasma cells (PCs) from AERD and CRSwNP. We investigated IL-5Ra expression and function in nasal polyp PCs.
Methods
Ethmoid Sinus tissue was collected from patients with AERD and CRSwNP and digested into a single-cell suspension. For IL-5 functional studies, PCs ($CD38^+$, $CD27^+$, and $CD138^+$) were sorted and cultured *in vitro* with/without IL-5 and sent for bulk RNAseq. Single-cell suspensions were cryopreserved for batched mass cytometry (CyTOF).
Results
AERD polyp tissue contained significantly higher PCs, with increased expression of IL-5Ra compared to CRSwNP. AERD PCs expressed higher levels of B cell activation and regulatory markers (CD40, CD19, CD32, and CD38) and the proliferation marker Ki-67. AERD PCs expressed significantly higher *IL5RA*, *IGHE*, and several cell cycle and proliferation transcripts (e.g. *CCND2*, *MKI67*, *CDC25A* and *CDC25B*) compared to CRSwNP. AERD PC IL-5 stimulation led to significant transcriptomic changes inducing key cell cycle genes (*CCND2* and *PTP4A3*), whereas IL-5 stimulation in PCs from CRSwNP induced few transcriptomic changes.
Conclusion

Using CyTOF, we confirm that AERD PCs express higher levels of IL-5Ra and higher PC frequencies compared to CRSwNP. RNAseq analysis shows that nasal polyp PCs are undergoing cell cycling and proliferation predominantly in AERD compared to CRSwNP. IL-5 stimulation shows a functionally active IL-5Ra on AERD PCs.

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THE ROLE OF PROTEIN KINASE R IN THE DEVELOPMENT OF INTESTINAL INFLAMMATION AND CANCER

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Abstract Content: The protein kinase R (PKR) is an innate immune protein whose expression is induced by interferons and is activated by double-stranded RNA. PKR regulates various signaling pathways driving inflammation and is a putative target of several anti-cancer drugs, including 5-fluorouracil and doxorubicin. However, the role of PKR in modulating intestinal inflammation and tumorigenesis has remained unknown. Here, we investigated the role of PKR in intestinal inflammation and cancer. We observed in an acute model of dextran sulfate sodium (DSS)-induced colitis, littermate wild-type mice and mice lacking *Pkr* (*Pkr*$^{-/-}$ mice) developed colitis of similar severity, defined by body weight loss, diarrhea, and shortening of the colon. In addition, we did not observe any differential production of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-18, tumor necrosis factor, and chemokine KC in the colon tissue of wild-type and *Pkr*$^{-/-}$ mice. Further, both wild-type and *Pkr*$^{-/-}$ mice developed similar number of tumors in the colon following injection of azoxymethane and treatment with three rounds of DSS. We further investigated whether PKR is able to restrict spontaneous tumorigenesis by crossing *Pkr*$^{-/-}$ mice with mice harboring a heterozygous mutation in the gene encoding adenomatous polyposis coli (*Apc*$^{Min/+}$ mice). We found that the small intestine and colon from *Apc*$^{Min/+}$ *Pkr*$^{-/-}$ mice had similar tumor burden compared with *Apc*$^{Min/+}$ control mice. Overall, our results demonstrate that PKR does not mediate the development and progression of intestinal inflammation and cancer.

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CANCER CELL-DERIVED TYPE I INTERFERONS INSTRUCT TUMOR MONOCYTE FUNCTIONAL POLARIZATION

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Abstract Content: Monocytes are highly plastic immune cells that often infiltrate solid tumors and restrain antitumor immunity by being immunosuppressive. Repolarizing suppressive tumor monocytes to become immunostimulatory is a desirable strategy to boost antitumor immunity and overcome resistance to checkpoint blockade therapy. However, little is known about the specific cues that drive tumor monocyte polarization, which has made the characterization of distinct suppressive and stimulatory cancer-associated monocyte populations elusive. Using single cell transcriptomic profiling of human and mouse tumors, we identified discrete tumor monocyte subsets with unique functional properties. Additionally, using high dimensional flow cytometry profiling we define novel surface markers that faithfully distinguish immunostimulatory and immunosuppressive monocyte subsets, which are differentially enriched in syngeneic mouse tumors. Our unique framework enabled the functional characterization of distinct tumor monocyte populations and serves as a strategy to decipher tumor monocytes with robust antitumor qualities. Mechanistically, we show that cancer cell-derived type I interferons drive immunostimulatory monocyte polarization, which is

associated with efficacy of anti-PD-1 immunotherapy. Furthermore, we found suppressive monocytes can be converted into immunostimulatory monocytes *in vitro* and *in vivo* via induction of cancer cell IFN production by cGAS-STING pathway activation. Clinically, enrichment of our experimentally-defined immunostimulatory monocyte gene signature is common in immunotherapy-responsive MSI-high colorectal adenocarcinomas (COAD) and in cutaneous squamous cell carcinomas (CSCC) that responded to treatment with our anti-PD-1 antibody cemiplimab. In summary, our work establishes a framework to discriminate and polarize immunostimulatory monocytes that may enhance the antitumor immune response to checkpoint blockade therapy.

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POSTOPERATIVE STIFFNESS IS ASSOCIATED WITH A DISTINCT PERIOPERATIVE INFLAMMATORY CYTOKINE RESPONSE TO TOTAL KNEE ARTHROPLASTY

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Abstract Content: BACKGROUND and AIM: Dysregulated single-cell immune signatures have been correlated with unsatisfactory recovery in patients after joint replacement surgery, and differences in the local and circulating cytokine levels in the perioperative period have been linked to decreased range of motion and persistent pain after total knee arthroplasty (TKA). The dysregulated inflammatory and wound healing pathways in patients with osteoarthritis (OA) who develop stiffness following TKA are not well understood. In this study we aimed to identify peripheral blood gene expression signatures associated with stiffness following TKA by integrating clinical outcomes with transcriptomics analyses of differential expressed genes (DEGs) in samples from OA patients undergoing TKA.
METHODS: We identified 8 cases (stiff knees) and 10 matched controls (no stiffness) from a cohort of 179 patients with idiopathic end-stage OA scheduled for TKA, with IRB approval and patient consent. Cases and controls were identified at enrollment end. Stiffness was defined as ≤95° ROM measured by goniometer at 6 weeks (±2 weeks) after TKA. We collected heparinized venous whole blood for peripheral blood mononuclear cell (PBMC) isolation from 6 consecutive patients at day of surgery (DOS) and at 24 hours after surgery (POD1). RNA obtained from PBMCs isolated at DOS and POD1 from 6 patients was used for NanoString analyses using the human Immunology Panel. RNA-seq analyses were conducted in RNA isolated from frozen PAXgene RNA tubes using the PAXgene Blood RNA System. Globin-depleted RNA isolated from PAXgene tubes collected at DOS and POD1 from cases (n=8) and age-, sex,- race- and BMI-matched controls (n=10) were used for RNA-seq, performed using an Illumina HiSeq 4000. After sequencing the reads were processed using a dedicated RNAseq pipeline developed by bioinformaticians at the David Z. Rosensweig Genomics Research Center at HSS. A q value < 0.05 was considered significant.

RESULTS: Using RNA from PBMCs and NanoString analyses, we identified increased expression of genes associated with responses to injury, stress and inflammation at 24 hours after surgery, including increased expression of S100A9, S100A8 and SOCS3. We also observed a relative enrichment in monocyte-related genes and a decrease in T-cell-related genes at 24 hours after TKA. Comparison of gene expression on DOS vs. POD1 in cases who were found to be stiff 6 weeks after surgery (161 DEGs) vs matched controls (231 DEGs) revealed attenuated and more variable responses to TKA in stiff patients. Pathway analyses using gene expression data revealed that pathways associated with stress, inflammation, and wound healing (including IL1, ATF2, and canonical NF-kB) were differentially expressed in response to surgery in controls, but not in cases.
DISCUSSION and CONCLUSION: Together, our results show that patients with stiffness after TKA may have dysregulated responses to surgery detectable in the early post-operative period, We believe that these dysregulated blood signatures can have prognostic value, and can potentially be used to predict patients at risk of developing complications following surgery.

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CYTOKINE PROFILES IN PATIENTS WITH LIVER DISEASES: EFFECTS OF DISEASE STAGE AND THE PNPLA3I148M VARIANT ALLELE ON CIRCULATING CYTOKINES IN NAFLD AND HCC PATIENTS

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Abstract Content: Hepatocellular carcinoma (HCC) is closely linked to chronic inflammation. Non-alcoholic fatty liver disease (NAFLD) and especially steatohepatitis (NASH) increase the risk to develop HCC. Cirrhosis is the last stage of fibrosis of the liver, and is an important risk factor for developing HCC. Individuals carrying the risk variant p.I148M of the *patatin-like phospholipase domain-containing protein 3* (*PNPLA3*) have a higher susceptibility for liver diseases and HCC. This study aimed at the analysis of a selected broad panel of cytokines in the sera of patients with chronic liver diseases: NAFLD (steatosis, NASH, with or without cirrhosis), and HCC.
Multiplex sandwich-ELISA assays were used to measure the serum concentrations of a panel of 38 cytokines in a cohort of 122 individuals comprising 67 NAFLD patients without cirrhosis (57 with steatosis, 10 with NASH), 23 NAFLD patients with cirrhosis, and 21 patients with HCC (15 with cirrhosis, 6 without cirrhosis), in addition to 11 healthy controls. All patients were successfully genotyped for the *PNPLA3* variant.
HGF, IL-6 and IL-8 levels were found to be increased in sera of patients (HCC and NAFLD compared with healthy individuals, with the highest levels in patients with cirrhosis). In contrast, serum concentrations of PDGF-BB and RANTES/CCL5 were lower in patients with chronic liver diseases, with lowest levels in patients with cirrhosis. The chemokines MIF and MCP-1 were found at higher concentrations in sera from patients with NAFLD (with a maximum in NAFLD-cirrhosis) as compared to healthy controls and also HCC. No impact of the PNPLA3^{I148M} alleles on cytokine levels was observed in this cohort. High positive correlations are observed between concentrations of IL-17 and IL-4 in the total cohort (R = 0.85) or between concentrations of IFNγ and IL-1Ra (highly statistically significant in cirrhosis; R = 0.91 and 0.70 for NAFLD and HCC samples, respectively). When cytokine levels were compared to clinical parameters, positive correlations were observed between liver stiffness measurements in patients with NAFLD and their levels of circulating HGF. For HCC sera, CRP levels correlated with IL-6, IL-16, and IL-8 concentrations. Furthermore, we assessed whether cytokine levels can be used for disease prediction. Cytokines such as IL-6, IL-8 and MIF yielded high sensitivity scores for predicting non-cirrhotic NAFLD (*vs.* healthy). MIF performed best to discriminate HCC from non-HCC samples. The top pair of cytokines to predict cirrhosis in NAFLD as well as in the entire cohort was HGF + PDGF-BB.
Our study indicates that cytokines have the potential to serve as biomarkers that are particularly suited for the earlier detection of HCC in patients at risk.

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LB-40

ROLE OF T FOLLICULAR HELPER CELLS IN ANTI-TUMOR IMMUNITY

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Abstract Content: **Introduction:** Relapse and metastases are the major causes of death in melanoma. Adoptive T cell therapy using tumor-infiltrating lymphocytes rich in cytotoxic $CD8^+$ T cells (CTLs) is one of the promising approaches that helps overcome these major challenges; however, CTL cell transfer alone does not improve clinical response, suggesting a supportive role of helper $CD4^+$ T cells. There is increasing evidence on the potential contribution of tumor-infiltrated $CD4^+$ T follicular helper (Tfh) cells and intratumoral tertiary lymphoid structure formation in tumor eradication; however, to date, the role of Tfh cells in anti-tumor immunity has not been clearly elucidated.
Methods: Utilizing mouse B16-F10 and B16-Ova melanoma tumor models, we have determined the functions of Tfh cells in suppressing tumor growth, as well as their interaction with important immune components within tumor in orchestrating anti-tumor immune resposes. In addition, we have assessed the efficacy of Tfh-cell therapy by adoptive transfer of antigen-specific Tfh cells in melanoma tumor-bearing mice (therapeutic model).
Results: In our hands, both pre-clinical murine tumor studies and patient studies, show correlation between the increased number of intratumoral Tfh cells and reduced melanoma tumor growth and improved survival, suggesting the novel function of Tfh cells in promoting anti-tumor immunity against melanoma. We observed enhanced tumor growth in Tfh cell conditional knockout mice compared to wild-type mice, further indicating that Tfh cells might play an important player in anti-tumor immunity and could serve as a marker for tumor regression. Remarkably, transfer of tumor antigen-specific Tfh cells results in increased intratumoral CTL number and cytolytic function, as well as in more efficient tumor eradication, indicating the role of Tfh cells in promoting CTL expansion and responses. Moreover, we determined that Tfh cell derived cytokines interferon gamma (IFNγ) and interleukin 21 (IL-21) contributes to intratumoral CTL activity. In addition to CTLs, Tfh cells enhance the antitumor activity of natural killer (NK) and B cells. Moreover, Tfh cells contribute to anti-tumor immunity through gut microbiota.
Conclusion: Our results thus for first time indicate the therapeutic potential of Tfh cells in promoting anti-tumor immunity against melanoma and provide the basis for potential usage of these cells to improve current immunotherapy approaches.

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GENOME ENGINEERED NATURAL KILLER CELLS FOR OSTEOSARCOMA IMMUNOTHERAPY

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Abstract Content: Over the last decade, Chimeric Antigen Receptor based T cell therapy (CAR-T) has developed into an effective immunotherapy for several cancers. However, CAR-T cell therapies have several shortcomings and clinical success has primarily been limited to hematological cancers. Challenges of CAR-T cell therapy include tumor immune evasion through loss of target antigen expression by tumor cells and inhibition of CAR-T cell function by tumor expressed inhibitory molecules. Natural killer (NK) cells present an alternative to T cells that could be more effective due to their ability to perform both antigen dependent and independent killing. NK cells mediate the direct killing of transformed cells with reduced or absent MHC expression and have demonstrated antigen specific killing when engineered to express CARs. Moreover, NK cells carry out antibody dependent cell mediated cytotoxicity

(ACDD) of cells bound by antibodies via the NK cell CD16A receptor. Due to the multiple modalities for cancer cell killing, there is an increased interest in NK cells for cancer immunotherapy. As NK cells are not associated with graft versus host disease, neurotoxicity, long-term autoimmunity, nor cytokine release syndrome, they are more suited for use in allogeneic settings than T cells and have significant clinical potential for use as off-the-shelf products. However, previous publications and clinical trials have demonstrated that the use of unmanipulated NK cells to treat cancer is minimally effective, likely due to limited engraftment, little in *vivo* expansion or persistence, and suppression by the tumor microenvironment. NK cells activated and expanded with engineered feeder cells expressing membrane bound interleukin-21 (mbIL-21) and 4-1BBL have shown promising results clinically with high-risk myeloid malignancies and preclinically in several solid tumor models. *We hypothesize that activated/expanded CAR-NK cells that have been genetically edited can be used to successfully treat osteosarcoma, a disease for which patient outcome has not improved in over forty years.* Our proposed objectives are to knockout negative regulators of NK cell function (specifically, *c-CBL*, *IL-1R8*, and *SMAD3*) and evaluate the non-viral KI of several CARs, either alone or in combination, that optimally activate NK cell antigen-specific killing. Genetically engineered CAR-NK cells will be evaluated for enhanced therapeutic efficacy and safety in osteosarcoma models. Our preliminary data strongly supports the hypothesis that NK cell-based cancer immunotherapy can be fully realized using activated, genome engineered CAR-NK cells.

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05. ILC3s

P321

HIGH-FAT DIET CAUSES RAPID LOSS OF INTESTINAL GROUP 3 INNATE LYMPHOID CELLS THROUGH MICROBIOTA-DRIVEN INFLAMMATION

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Abstract Content: Innate lymphoid cells (ILCs) are increasingly appreciated to play a critical role in tissue homeostasis, immunity, and tolerance. At steady state, intestinal ILC3 are the main source of IL-22, ensuring epithelial barrier integrity, containing the microbiota, and protecting against pathogenic bacteria. In addition, ILC3 support intestinal tolerance to commensal bacteria and dietary antigens. ILC3 can also sense changes in dietary nutrients, such as vitamin A and aryl hydrocarbon receptor ligands (AHR-L) that affect their development, function and ultimately intestinal health. ILC3 have been described to be able to acquire high amounts of extracellular fat but the effect of dietary fat on ILC3 homeostasis is unknown. Here we show that ILC3 are severely depleted from the small and large intestine of obese mice fed high-fat diet (HFD) but not in leptin-deficient obese mice. Notably, consumption of HFD for only 24 hours is sufficient to trigger ILC3 cell death. Total loss of ILC3 is reached after one week of HFD without significant weight gain or impaired glucose tolerance. However, we found that this short-term consumption of HFD increases intestinal permeability and host susceptibility to *Citrobacter rodentium* infection. Unexpectedly, we found that ILC3 were maintained in germ-free (GF) mice fed HFD. However, ILC3 were depleted when HFD-fed GF mice were inoculated with either living, heat-killed bacteria or with lipopolysaccharides, which associated with intestinal permeability and inflammation. Gene expression profiling of ILC3 from short-term HFD-fed mice revealed that LPS phenocopied the differential effect of microbiota on dampening peroxisome proliferator-activated receptor (PPAR) signaling, and activating TNFα target genes involved in cell activation, exhaustion, oxidative stress and lipotoxicity. *In vitro*, lipid laden ILC3 are susceptible to TNFα induced cytotoxicity in a dose dependent manner. *In vivo*, TNFα blockade, the use of antioxidant, or TLR4 deficiency was sufficient to protect ILC3 from HFD-induced lipotoxicity. Specifically, restricted depletion of TLR4 on Cx3cr1^{+} mononuclear phagocytes curtailed their HFD-induced early expansion, TNFα production and protected from ILC3 loss. Collectively, our findings reveal differential regulation of ILC3 homeostasis by the crosstalk of dietary fat with microbiota-mediated inflammation, ranging from activation to cell death, preventing early repair of intestinal permeability, and increasing susceptibility to opportunistic pathogens independently of obesity and metabolic syndrome.

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P322

THE ROLE OF ARYL HYDROCARBON RECEPTOR IN CRYPTOPATCH 7 ISOLATED LYMPHOID FOLLICLE FORMATION

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Abstract Content: Cryptopatches are clusters of RORgt expressing Innate Lymphocyte cells 3 (ILC3s) with Dendritic cells (DCs), located in the lamina propria in proximity to intestinal crypts and form approximately two weeks after birth. Some of them accumulate B cells and transform to isolated lymphoid follicles (ILFs). Aryl Hydrocarbon Receptor (AhR) deficient mice have very few CPs/ILFs and AhR deletion in RORgt-expressing cells also causes impairment in the size and number of CP/ILFs. Although, both CCR6+ and NKp46+ ILC3 populations are affected in the absence of AhR in terms of numbers and proportions, transfer experiments of the different ILC3 subsets based on CCR6 and NKp46 surface marker expression in immunodeficient Rag2/Il2rg mice show that, only the CCR6+ RORgt+ILCs are capable to form CPs and ILFs. Our goal is to determine the role of AhR in cryptopatch formation and unravel its potential function on the different ILC3 subsets.

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P323

RIPK3- AND CASPASE 8-DEPENDENT SIGNALS CONTROL PROTECTIVE ILC3 RESPONSES IN THE GUT

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Abstract Content: Group 3 innate lymphoid cells (ILC3s) robustly colonize the mammalian intestine in early life, are responsive to microbiota-derived signals, and are critical regulators of immunity, inflammation, and tolerance. However, intestinal ILC3s are dysregulated in the context of chronic infections, colorectal cancer, and inflammatory bowel disease (IBD), ultimately resulting in the reductions of these tissue protective lymphocytes. To identify factors driving the dysregulation or depletion of ILC3s, we employed RNA sequencing to compare ILC3s isolated from inflamed versus healthy colonic tissue of IBD patients and observed a significant upregulation of RIPK3, the central regulator of necroptosis. Necroptosis is the inflammatory form of programmed cell death triggered by RIPK3 activation and subsequent membrane pore formation. In mice, the T-bet$^+$ ILC3 subset expresses higher levels of *Ripk3* and reduced levels of pro-survival genes relative to the CCR6$^+$ ILC3 subset, suggesting that T-bet$^+$ ILC3s are primed for cell death. RIPK3 is significantly upregulated in multiple ILC3 subsets in mouse models of intestinal damage, inflammation, and infection. Lineage-specific deletion of RIPK3 revealed a redundant role for this factor in ILC3 responses, which could be partly due to the blockade of RIPK3-mediated necroptosis by active caspase 8. Consistent with this, caspase 8 deletion results in a significant loss of T-bet$^+$ ILC3s in the large intestine of mice at steady state and a significant loss of all ILC3s during enteric infection or inflammation. As a result, mice lacking ILC3-specific caspase 8 exhibit enhanced epithelial damage and bacterial dissemination. The loss of ILC3s resulting from caspase 8 deficiency could be prevented by simultaneous deletion of RIPK3. Finally, we determined that activation of the caspase 8 – RIPK3 axis in ILC3s is driven by TNF or TL1A, which upregulates Fas expression on ILC3s. FasL expression is also upregulated by ILC3s in inflamed tissue, suggesting a potential mechanism of self-regulation employed by ILC3s. These results collectively define an essential role for caspase 8 and RIPK3-dependent regulation of ILC3 subsets in the healthy and inflamed intestine and potentially highlight novel approaches to prevent the dysregulation of ILC3s in chronic disease.

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P324

PREDOMINANCE OF GROUP 3 INNATE LYMPHOID CELLS IN PREDNISONE-DEPENDENT SEVERE ASTHMATICS WITH NEUTROPHILIC INFLAMMATION: EVIDENCE OF LOCAL AIRWAY INNATE LYMPHOID CELL PLASTICITY

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Abstract Content:
RATIONALE:Group 2 ILC (ILC2s) are most prevalent in the airways of prednisone-dependent severe asthmatics (SA) with uncontrolled airway eosinophilia and are considered a source of steroid-insensitive IL-5/13[1]. Although gene signatures indicate a role for IL-17A$^+$ILCs and ILC3 in type 2 low severe asthma[2], a detail phenotype of ILCs sub-groups has not been performed in these patients. In addition, alterations in the tissue cytokine milieu can orchestrate plasticity between ILC sub-groups in various chronic inflammatory conditions3,4. This cross-sectional study investigated the frequency and phenotypes of ILC subsets in various inflammatory endotypes of SA.
METHODS: We phenotyped and enumerated ILCs in SA with a pre-bronchodilator forced expiratory volume in 1s (FEV_1) <80% predicted; post-bronchodilator reversibility ≥12% and ≥200 mL in FEV_1, or a historical methacholine PC_{20} ≤8 mg/mL; receiving high-dose ICS/LABA (>880 µg/day); ≥6 months history of OCS treatment. Study participants were grouped into inflammatory subtypes based on differential sputum cell counts: eosinophilic (≥3% eosinophils), neutrophilic (≥61% neutrophils;≥15×10^6/ml total cell count [TCC]) and mixed granulocytic (≥3% eosinophilia; ≥61% neutrophilia;≥15×10^6/ml TCC)[5]. Sputum extracted cells were subject to immunofluorescent staining, and flow cytometry (LSR II and analysed by FlowJo V10) to identify ILC1, ILC2 and ILC3 with lineage specific transcription factors and intracellular cytokine expression. The pro-and anti-inflammatory cytokines in the sputum supernatants were quantified by Ella multiplex assays.
RESULTS:Sputum ILC2s were increased significantly and were inversely correlated with FEV_1 in eosinophilic SA. By comparison, total ILC3s and IL-17A$^+$ILC3s were significantly increased with an inverse correlation with FEV_1 in neutrophilic SA. Also, within the neutrophilic SA, non-conventional ILCs (IL-17A$^+$ILC2s) were detected where c-kit$^+$ILC2s expressed a significantly higher proportion of IL-17A compared to c-kit$^-$ILC2. In addition, an equivalent number of ILC3s from neutrophilic SA expressed intracellular IL-5/13 and IL-17A. The level of IL-1β, a cytokine mediating ILC2 to ILC3 plasticity and ILC3 activation was significantly higher in neutrophilic SA. The plasticity of ILC2s into ILC3 like cells *in vitro* showed that ILC2s were induced to express IL-17A and c-kit following 7-day co-culture with type 2 low cytokines.
CONCLUSIONS:This cross-sectional study data support ILC2s as the predominant ILC in eosinphilic SA. In addition, ILC3s are most abundant in neutrophilic SA as a source of IL-17A. Furthermore, detection of intermediate phenotypes between established ILC2 and ILC3s suggest that airway ILC plasticity may cause changes in chronic inflammatory endotypes of asthmatics without recruiting new peripheral ILCs. Whether changes in airway bronchitis in SA mirrors changes in ILC2/3 populations overtime requires further investigation and may provide insights into potential targets of therapy to control neutrophilic SA exacerbations.
1.Smith SG et al., JACI (2016);2. Hekking PP et al. JACI (2018);3. Van der Ploeg EK et al., Sci Immunol (2021);4. Bernink JH et al., Nat Immunol (2019);5. Svenningsen S Front Med (2017)

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P325

BI-DIRECTIONAL SIGNALLING BETWEEN THE INTESTINAL EPITHELIUM AND ILC3 REGULATES IMMUNE AND EPITHELIUM CELLS' PHENOTYPE AND FUNCTION

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Abstract Content: Group 3 Innate Lymphoid Cells (ILC3) are crucial regulators of intestinal homeostasis and integrity. ILC3 responses in intestinal homeostasis and disease are dictated by localised environmental cues, such as those derived from the intestinal epithelium. Here, we use a co-culture system of murine small intestinal epithelial (SIO) organoids with ILC3 to uncover bi-directional signalling mechanisms that underlie intestinal homeostasis.
We find significant global transcriptional changes to intestinal epithelial cells upon co-culture with ILC3, including the enrichment of secretory goblet cell signatures. In co-culture with SIO enriched for goblet cells, ILC3 upregulate expression of the Natural Cytotoxicity Receptor NKp46 and IL-22 in a contact dependent fashion. Analysis of published datasets show that Notch ligands Delta Like Canonical Notch Ligand (Dll) 1 and Dll4 are enriched in goblet cells in line with our observation of increased Dll expression in goblet enriched SIO. The knockdown of *Dll1/4* leads to decreased ILC3-derived *Il22*, demonstrating the importance of epithelial derived Dll expression for ILC3 function. In turn, in co-culture, goblet cell enriched SIO activate Notch signalling and IL-22 production specifically in T-bet+ ILC3. Upregulation of *Atoh1*, a Notch regulated transcription factor that is crucial for secretory lineage determination, in SIO co-cultured with ILC3 further highlights the importance of the Notch pathway in ILC3-intestinal epithelial interactions.
This research outlines two complimentary Notch mediated signalling modules by which tissue localized epithelial signals dictate ILC3 responses, which are relevant in intestinal homeostasis and disease.
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LB-46

MICROBIOTA-DERIVED BUTYRATE REINFORCES INTESTINAL HOST DEFENSES AFTER HYPERBARIC OXYGENATION.

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Abstract Content: Oxygen provides the greatest source of energy for animals and is crucial for sustaining large, complex life forms. It is known that hyperbaric oxygen therapy (HBO) is a useful technique for the treatment of various diseases and traumas due to the capacity of improving tissue oxygenation. However, the healthy intestinal mucosa is physiologically hypoxic and the impact of HBO is still not well understood. Here we showed that hyperbaric oxygenation affects the composition of the gut microbiota and the short-term immunity in mice, as well as the emerging increased susceptibility to intestinal *Clostridioides difficile* infection (CDI). These findings were directly related to higher blood oxygen transport and lower frequency of intestinal IL-22-producing group 3 innate lymphoid cells (ILC3). Treating mice with butyrate during HBO, a microbiota-derived short-chain fatty acid (SCFA), was able to mitigate the infection. This metabolite coordinated the chromatin accessibility by inhibiting histone deacetylase, and improved the transcriptional activity of hypoxia-inducible factor (HIF)-1 in ILC3, increasing the expression of IL-22 and its target genes in epithelial cells. Mice lacking the HIF-1α isoform in RORγt+ cells did not respond to butyrate treatment and had aggravated colitis development. In contrast, mice that overexpress HIF-1 in RORγt+ cells through conditional deficiency of VHL showed mild infection and increased production of IL-22 by ILC3. Taken together, these findings suggest that hyperbaric oxygenation dysregulates the gut microbiota and impairs the intestinal ILC3 function, increasing host susceptibility to enteric infection.

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LB-47

NOTCH SIGNALING IN ILC3 DEVELOPMENT AND FUNCTION

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Abstract Content: The intestinal mucosa of adult humans and mice is enriched for innate lymphoid cells (ILCs) that express the transcription factor RORγt (ILC3s). They serve protective roles in immune responses to infectious

organisms, are essential for the formation of lymphoid tissues, and help maintain gut homeostasis via signaling to epithelial cells through interleukin 22 (IL-22). ILC3s in the GI tract can be further categorized into three main subsets with distinct and overlapping phenotypic markers and functional roles. Signals that mediate the development and function of the various ILC3 subsets are still not completely understood. It has been shown that Notch signaling is required for the development of certain ILC3 subsets. In adult ILC3, Notch2 is known to play central role in differentiation of NCR^+ ILC3. However, whether and how other Notch receptors influence ILC3 biology is not known. Here, we examined the roles of individual Notch receptors in ILC3 development and function. Our results demonstrate that individual Notch receptors have redundant and non-redundant roles in development of individual ILC3 subsets. We find that signaling through multiple Notch receptors contributes to ILC3 phenotypes during homeostasis and maintains protective ILC3 function in the context of infection. Taken together, our results delineate differential functions of Notch receptors in ILC3 development and function.

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06. Central and Peripheral Nervous System and ILCs

P327

ILC2S LIMIT CORTICAL DAMAGE AND NEURONAL HYPEREXCITABILITY AFTER PHOTOTHROMBOTIC INJURY

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Abstract Content: Adult brain injuries such as ischemic stroke and traumatic brain injuries (TBI) are a leading cause of morbidity and mortality worldwide due to the limited capacity for adult brain regeneration, and there are no effective therapies to improve outcomes after brain damage. Injuries to the central nervous system (CNS) initiate a sterile inflammatory response that recruits peripheral immune cells, such as macrophages and neutrophils, which can in turn mediate secondary damage. In contrast, type 2 immune responses, including the cytokines Interleukin-33 (IL-33) and IL-13, are protective after CNS injury and primarily act during the resolution phase. However, the mechanism(s) for this effect, including the sources and targets of these signals, are poorly defined. Group 2 innate lymphoid cells (ILC2s) are tissue-resident innate lymphocytes that participate in wound healing and tissue remodeling processes, are abundant in the meningeal membranes that surround the brain, and have recently been shown to impact brain physiology and function. ILC2s are robustly activated by IL-33 and produce IL-13 when activated, raising the possibility that they may participate in beneficial type 2 immune responses to brain damage. Using the photothrombotic (PT) mouse model of ischemic CNS damage, we found that ILC2s in the meninges activate, expand, and limit cortical damage and corticothalamic hyperexcitability after injury, acting in a partially IL-33 dependent manner. Our working model is that IL-33 released after CNS damage activates ILC2s to produce IL-13 and regulate microglia, infiltrating myeloid cells, and neurons, thereby promoting recovery from CNS injury. Ongoing work will address the mechanism(s) through which meningeal ILC2s alter cortical outcomes after stroke, as well as the source of IL-33 that activates ILC2s in this context.

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P328

ACTIVATION OF CD81+ SKIN ILC2S BY A COLD-SENSING TRPM8 - IL-18 AXIS REGULATES SKIN BARRIER THERMAL HOMEOSTATIC REGULATION

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Abstract Content: As the outmost barrier tissue of the body, the skin harbors a large number of innate lymphoid cells (ILCs) that are suggested to help the local homeostatic maintenance in the face of changing environments. How skin-resident ILCs are regulated and function in local homeostatic maintenance is poorly understood. We herein report discovery of a cold-sensing neuron-derived pathway that activates skin group 2 ILCs (ILC2s) to help thermo-homeostatic regulation. In stearoyl-CoA desaturase 1 (SCD1)-knockout mice whose skin is defective in heat maintenance, chronic cold stress induced excessive activation of CCR10-CD81+ST2+ skin ILC2s and associated inflammation. Mechanistically, stimulation of the cold-sensing receptor TRPM8 expressed in sensory neurons of the skin led to increased production of IL-18, which in turn activated skin ILC2s to promote thermogenesis. Our findings reveal a neuron-immune link that regulates activation of skin ILC2s for thermo-homeostatic regulation while its dysregulation causes skin inflammation and disorder.

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07. ILCs and Metabolism

P329

REGULATING OBESITY VIA PD-1 EXPRESSING TYPE 2 INNATE LYMPHOID CELL IN ADIPOSE TISSUE

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Abstract Content: Obesity is not only a matter of being fat, but it also causes various metabolic disorders. Because obesity is considered a metabolic malfunction that increases insulin resistance and decreases glucose tolerance, obesity increases the chances of type 2 diabetes and cardio-metabolic disorders. Recent studies indicate that Innate lymphoid cells (ILC), especially, type 2 innate lymphoid cells(ILC2), are involved in energy metabolism homeostasis in adipose tissue. ILC2s produce Met-Enk that augments uncoupled respiration protein 1(UCP1) expression from adipose tissue. Besides, ILC2s activate and recruit eosinophils by secreting IL-5 and -13 which can regulate the energy expenditure of adipocytes. Therefore, controlling adipose tissue-resident ILC2s could be a major target in regulating obesity. By using the high-fat diet-induced obesity mice model, we found significantly increased PD-1 expressions from ILC2s along with decreased cytokine productions. In addition, obese adipose tissue showed an augmented level of osteopontin (OPN), which is associated with immune cell activations. CD44 and Integrin alpha v, well-known interacting partners of OPN, expression is significantly increased in ILC2s from obese adipose tissue. In vitro OPN treatment on ILC2 showed increased PD-1 expressions, which suggests OPN directly enhances PD-1 expressions from ILC2s. Also, PD-L1, the ligand of PD-1, was mainly expressed on adipocytes and PD-1:PD-L1 interaction inhibits ILC2s effector functions. As the PD-1 signal induces metabolic shifting from helper T cells, we measured glucose and fatty acid uptake from ILC2s to confirm whether PD-1 modulates metabolic switching from ILC2s. By treating PD-L1 recombinant protein onto isolated ILC2s, the level of fatty acid uptake was significantly decreased dose-dependently, while glucose uptake was comparable. Especially, the functions and expressions of fatty acid binding protein (FABP) 5 were negatively controlled by PD-1:PD-L1 signals. Besides, in vitro treatment of FABP5 inhibitor negatively regulate cytokine productions from. Therefore our study suggested that obesity induces PD-1 expression from adipose tissue-resident ILC2s via OPN dependent manners and interaction with PD-L1 expressing adipocytes modify energy metabolism of ILC2s and worsening obesity.

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P330

INNATE LYMPHOID CELLS TYPE 2 AND CD8+ T CELLS ARE PERTURBED IN OVERWEIGHT/OBESE INDIVIDUALS WITH ASTHMA

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Abstract Content: Obesity is a significant co-morbidity with asthma leading to significantly worse patient outcomes. Our aim was to dissect how the frequencies and phenotypes of type 2 innate lymphoid cells (ILC2s), CD4+ T helper cells and CD8+ cytotoxic T cells differed in obese and lean non-asthmatic and asthmatic individuals. A total of 88 donors were selected from the Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) cohort based on their asthma diagnosis and Body Mass Index (BMI) and % body fat (BF). The groups were defined as; overweight/obese asthma, lean asthma, overweight/obese non-asthma, and lean non-asthma. The groups were matched and for sex and sensitization. Viable frozen PBMCs samples from all individuals were thawed and stained for ILCs, CD4+ and CD8+ T cells using 18-parameter flow cytometry. Levels of IL-7 and TSLP in plasma samples were analyzed by ELISA. In vitro stimulations allowed for the assessment of steroid resistance and CD45RO induction triggered by IL-7 and TSLP. Analysis of circulatory lymphocyte populations by flow cytometry revealed that the proportion of Th2 cells were only significantly elevated in the blood of lean asthmatic individuals when compared to non-asthmatic controls. In contrast in the overweight/obese asthmatic individuals were characterized by an increased frequency of innate-like terminally differentiated effector memory (TEMRA) CD8+ T cells and CD45RO expressing ILC2. An association with BMI or % BF was found for a range of markers, including IL-7R, CD200R and CD117, in the overweight/obese groups amongst these cell types. In summary, our studies demonstrates that alterations in the CD8+ TEMRA cell and ILC2 compartments in overweight/obese asthmatics that serves to immunologically distinguish these individuals from lean asthmatics.

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LB-48

SINGLE-CELL SEQUENCING OF HUMAN WHITE ADIPOSE TISSUE IDENTIFIES MATURE AND SHARED PRECURSOR ILC SUBSETS ENRICHED IN OBESITY

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Abstract Content: White adipose tissue (WAT) is regulates energy storage and systemic metabolic homeostasis. Both immune and parenchymal cell populations contribute to regulatory networks which are necessary to maintain metabolic fitness, which becomes impaired during obesity. Using single-cell RNA sequencing and flow cytometry, we generated and validated a large-scale comprehensive cellular atlas of the stromal vascular fraction of healthy lean and obese human WAT. We found dramatic shifts in both immune and non-immune populations during obesity and identified 8 novel cell types within the human adipose tissue, including three populations of innate lymphoid cells (ILCs). We profiled four distinct lineages of human WAT ILCs, which could be differentiated from the NK cell lineage via expression of CD200R1, validating CD200R1 as a bone fide ILC marker within humans and mice. We found, for the first time, a population of WAT NKp44^{+}IL1R1^{+}CCR6^{+} ILC3, which is not present in mice, and accumulates dramatically during human obesity. Furthermore, trajectory analyses identified a restricted precursor-like cell type within the human WAT with limited differentiation potential to ILC1 and ILC3, but not ILC2, as well as the putative signals which may regulate this bifurcation. Finally, analysis of cell-cell ligand-receptor interactions and obesity-enriched signaling pathways reveal key mechanisms by which ILCs regulate adipose tissue immune cell homeostasis in the lean and obese states. We identify this ILC3 subset as a potential key regulator of adipose tissue inflammation during obesity via upregulation of TNFSF13B, TNFSF11, LIF, and MIF, directly implicating these cells in signaling pathways associated with insulin resistance in humans. Together, these results reveal a potential role for tissue-resident ILC3s in the regulation of human adipose tissue homeostasis and inflammation.

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08. ILCs and Cancer

P331

ALTERED ILC DEVELOPMENTAL PATHWAYS IN MYELODYSPLASTIC SYNDROME PATIENTS

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Abstract Content: Myelodysplastic syndromes (MDSs) are a heterogeneous group of myeloid neoplasms that primarily affect elderly people and, in the context of population aging, MDS incidence is set to increase substantially. MDSs are characterized by ineffective hematopoiesis, cytogenetic changes, molecular abnormalities and a variable risk of progression to acute myeloid leukemia (AML). Furthermore, MDSs often include the dysregulation and/or dysfunction of the immune system, with the acquisition of an immunosuppressive signature. Nowadays frontline pharmacological therapies, mainly hypomethylating agents (HMA), are not curative and patient risk stratification, based mainly on aspecific clinical/hematological parameters, ineffectively predicts patients' prognosis and response to therapies. In this regard, a deeper characterization of the immunologic landscape of MDS could allow the implementation of diagnostic/prognostic tools to better stratify MDS patients as well as to develop novel effective immunotherapies.
Innate lymphoid cells (ILCs) are emerging as important actors in generating a suppressive and tolerant environment, essential for tumor progression and aggressiveness, also in the context of hematological neoplasms. However, their role in MDS pathogenesis, severity and in the response to HMA treatment is under-investigated.
To this end, we explored through extensive flow cytometry the phenotype and the function of ILCs in bone marrow and in peripheral blood of MDS patients stratified in low-risk (LR, n=44) and high-risk (HR, n=23) of progression towards AML based on blast counts. Our data demonstrated that the distribution of helper ILC subsets changes among according to MDS severity. Indeed, the ILC1 $CD56^{neg}$ frequency increases with the worsening of the disease at the expense of ILCp and ILC1-like $CD56^{pos}$ in HR pre-HMA patients. In addition, the expanded ILC1 $CD56^{neg}$ subset is characterized by an impaired production of type 1 cytokines in HR patients.
Our data also demonstrated that the cytotoxic subset of ILCs, namely Natural Killer (NK) cells, is reduced in frequency and show an impaired cytotoxicity in HR patients, regardless HMA therapy. Focusing on the phenotype of these cells, we observed a reduction of the more differentiated and cytotoxic NK cell subset counterbalanced by a higher frequency of less differentiated NK cells the expansion of $lineage^{neg}CD45RA^{pos}CD34^{pos}$ ILC precursors in HR patients in HR pre-HMA patients. The data obtained also show that the treatment with HMA restores the distribution of ILC subset and precursor frequency in HR patients. Moreover, we observed the expansion of a peculiar NK cell subset characterized by the expression of the maturity markers NKG2C and CD57 in HR post-HMA.
Taken together these results suggest a possible block in the differentiation of NK cells in HR patients, that leads to a preferential differentiation towards ILC1 $CD56^{neg}$, which could be partially restored by HMA treatment.

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P332

INVESTIGATING INNATE LYMPHOID CELLS IN OVARIAN CARCINOMA

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Abstract Content: Ovarian carcinoma (OC) is one of the most commonly occurring cancers in women worldwide, with an estimated 22,240 newly diagnosed patients in 2018 in the US. Majority of patients with OC do not respond to immune checkpoint inhibitors, prompting the need to further investigate anti-tumour and immune regulatory pathways in the ovarian tumour microenvironment (TME). Innate lymphoid cells (ILCs) represent a heterogenous population of cells that mostly reside within non-lymphoid peripheral tissues. The tissue-resident properties of ILCs position them well to respond rapidly to tissue-specific stimuli, including cancer progression, and orchestrate adaptive immunity. Our group previously identified an immunosuppressive $CD56^{+}$ ILC population from that suppressed T cells in *ex vivo*

tumour-infiltrating lymphocyte (TIL) cultures. This suggests a potential regulatory role of ILCs in ovarian tumours. The objective of this study was to provide an in-depth characterization of ILC subsets in primary tumour and ascites of patients with OC (n=30) using single-cell RNA-sequencing (scRNA-seq) and multi-parametric flow cytometry. We identified subsets of ILC1s, ILC2s, and ILC3s within lineage (Lin)$^-$CD127$^+$ population using CD117 and CRTH2. Moreover, we found that CD56$^+$ ILCs were the predominant source of intratumoural ILCs. Early studies have uniformly labeled CD56$^+$ ILCs as natural killer cells, but it is now recognized that they are composed of a heterogenous population of cells (i.e., ILC1s, ILC3s, and regulatory ILCs). In ovarian tumours, we found a population of CD56$^+$GZMB$^-$ CD49e$^-$ ILCs that exhibited tissue-resident properties similar to intraepithelial ILC1s (ieILC1s). Interestingly, these ieILC1-like cells were associated with poor progression free survival (PFS) and altered TIL phenotype. Moreover, ieILC1-like cells can be induced from peripheral blood CD56$^+$ cells using supernatant from ascites and/or a combination of cytokines. These findings suggests that ieILC1-like CD56$^+$ cells may play a unique role in modulating the tumour microenvironment. Further investigation into the biology of ILCs in human tumours may provide therapeutic targets for patients with ovarian carcinoma.

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P333

PPARɣ DRIVES IL-33-DEPENDENT ILC2 PRO-TUMORAL FUNCTIONS

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Abstract Content: Group 2 innate lymphoid cells (ILC2s) play a critical role in protection against helminths and in diverse inflammatory diseases by responding to soluble factors such as the alarmin IL-33, that is often overexpressed in cancer. Nonetheless, regulatory factors that dictate ILC2 functions remain poorly studied. Here, we show that peroxisome proliferator-activated receptor gamma (PPARγ) is selectively expressed in ILC2s in humans and in mice, acting as a central functional regulator. Pharmacologic inhibition or genetic deletion of PPARγ in ILC2s significantly impair IL-33-induced Type-2 cytokine production and mitochondrial fitness. Further, PPARγ blockade in ILC2s disrupts their pro-tumoral effect induced by IL-33-secreting cancer cells. Lastly, genetic ablation of PPARγ in ILC2s significantly suppresses tumor growth in vivo. Our findings highlight a crucial role for PPARγ in supporting the IL-33 dependent protumorigenic role of ILC2s and suggest that PPARγ can be considered as a druggable pathway in ILC2s to inhibit their effector functions. Hence, PPARγ targeting might be exploited in cancer immunotherapy and in other ILC2-driven mediated disorders, such as asthma and allergy.

Group 2 innate lymphoid cells (ILC2s) play a critical role in protection against helminths andin diverse inflammatory diseases by responding to soluble factors such as the alarmin IL-33,that is often overexpressed in cancer. Nonetheless, regulatory factors that dictate ILC2functions remain poorly studied. Here, we show that peroxisome proliferator-activatedreceptor gamma (PPARγ) is selectively expressed in ILC2s in humans and in mice, acting as acentral functional regulator. Pharmacologic inhibition or genetic deletion of PPARγ in ILC2ssignificantly impair IL-33-induced Type-2 cytokine production and mitochondrial fitness.Further, PPARγ blockade in ILC2s disrupts their pro-tumoral effect induced by IL-33-secretingcancer cells. Lastly, genetic ablation of PPARγ in ILC2s significantly suppresses tumor growthin vivo. Our findings highlight a crucial role for PPARγ in supporting the IL-33 dependent protumorigenicrole of ILC2s and suggest that PPARγ can be considered as a druggable pathwayin ILC2s to inhibit their effector functions. Hence, PPARγ targeting might be exploited incancer immunotherapy and in other ILC2-driven mediated disorders, such as asthma andallergy.

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P334

NOVEL NEURO-IMMUNE REGULATOR AXIS IN BLADDER TYPE 2 INNATE LYMPHOID CELLS

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Abstract Content: Type 2 innate lymphoid cells (ILC2s) are key regulators of tissue homeostasis and play important roles in inflammatory diseases as well as in mediating tumor responses. We previously demonstrated that ILC2s act as pro-tumoral players in bladder cancer patients by modulating the MDSC-T cell ratio in an IL-13 dependant manner. Accordingly, we found that mice lacking ILC2s show a better survival compared to wild-type animals after intravesical instillation of the murine MB49 bladder tumor cell line. Nevertheless, the upstream molecular triggers of ILC2s in the bladder remained unknown so far.
By screening for putative ILC2 activators, we measured elevated levels of a neural mediator in the urine of bladder cancer patients and its increased expression correlated with poor patients' survival in the The Cancer Genome Atlas. We identified the specific expression of its receptor in human and mouse ILC2s, both a molecular and protein level. Exposure of ILC2s to this neural mediator resulted in potent triggering of ILC2 functions, including type 2 cytokine secretion and cell migration, but not proliferation, in a mTOR-dependant signalling pathway. The effect was reverted when the interactions was blocked by a selective inhibitor or when the ligand-receptor binding was abrogated by CRISPR/Cas9-based deletion of the receptor on ILC2s.
Overall, we identified a novel neuro-immune axis shaping ILC2 functions. Further, our results suggest a neuro-immune interplay between ILC2s, the bladder environment and bladder cancer cells in maintaining tissue homeostasis and sustaining malignancy, respectively.

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P336

GITR-INDUCED EPIGENETIC REMODELING OF IL9 LOCUS IN GROUP 2 INNATE LYMPHOID CELLS EXERT ANTI-TUMOR EFFECTS

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Abstract Content: Group 2 innate lymphoid cells (ILC2) are strongly associated with maintenance homeostasis and exertion inflammation in lung environments. Glucocorticoid-induced tumor necrosis factor receptor (TNFR)-related protein (GITR) is highly expressed on ILC2s rather than other TNFRSF molecules. Interestingly, engagement of GITR via agonistic antibody (DTA-1) enhances ILC2s to produce IL-9 specifically. RNA-sequencing analysis reveals that GITR signaling promotes DNA binding transcription factor activity compared to IgG2b control. Notably, Activating transcription factor 3 (ATF3) is significantly up-regulated by GITR signaling. Tanshinone IIA, a ATF3 inducer, also up-regulate IL-9 expression in ILC2s. Furthermore, assay for transposase-accessible chromatin (ATAC)-sequencing reveals that short promoters relatively are opened compared to IgG2b control in ILC2s. These results suggest the probability of GITR-induced ATF3 and chromatin remodeling of IL9 locus to exhibit enhanced IL-9 secretion in ILC2s GITR signaling exert anti-tumor activity in various tumor model. In lung metastasis tumor model, the degree of metastasis is inhibited by DTA-1 administration and this anti-tumor effects are impaired by neutralization of IL-9 in $Rag1^{-/-}$ mice. Moreover, administration of DTA-1 increases ILC2 number and IL-5 and IL-13 production. Interestingly, eosinophils are also increased by injection of DTA-1. Notably, recombinant mouse IL-9 administration also increases ILC2s/eosinophils and shows anti-tumor activity. IL-9 receptor (IL-9R) is predominantly expressed in ILC2 compared to eosinophils. Indeed IL-9 directly up-regulates IL-5 and IL-13 production in ILC2s and neutralization of IL-9 in ILC2

culture supernatant restricts the production of these cytokines. In lung cancer patients, ILC2s have high GITR expression in tumor lesions compared to normal lesions. In melanoma, lung squamous cell carcinoma and lung adenocarcinoma patients TCGA database, ILC2 signature (GITR, GATA3, IL-5, IL-13, IL-9 etc.) eosinophils signature (Siglec8, CCR3, ECP etc.) and IL-9 signature (IL-9, IL-9R, JAKs, STATs etc.) show positive correlation each signature gene set. Our ongoing research suggests the possibility of GITR induced chromatin remodeling of *IL9* locus enhances IL-9 production in ILC2s and demonstrates that GITR-ILC2-IL-9 axis exhibits impaired tumor growth by autocrine manner of IL-9 in ILC2.

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P337

INTRATUMORAL INNATE AND ADAPTATIVE LYMPHOCYTES IN METASTATIC COLORECTAL CANCER WITH PERITONEAL CARCINOMATOSIS TREATED WITH HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY (HIPEC)

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Abstract Content: Colorectal cancer (CRC) is the third most deadly cancer worldwide and 1.85 million cases are diagnosed every year. About 5- 10% of them will have peritoneal carcinomatosis (PC) at the time of diagnosis, and about 5% will later develop PC. Previously, patients with metastatic CRC with PC qualified for palliative treatment, but lately a new treatment option has evolved: hyperthermic intraperitoneal chemotherapy (HIPEC). However, there is still a need of prognostic markers identifying patients who will benefit from the HIPEC therapy as it only offers a cure in certain patients. While T cells play an undisputed role in CRC, our group and others have described dysregulated innate lymphoid cell (ILC) function in primary CRC but their role in metastatic CRC with PC remains unknown. Here set out to characterize intratumoral stromal cells as well as the innate (ILCs and NK cells) and adaptive (T and B cells) lymphocyte landscape of primary CRC tumors and CRC-derived peritoneal metastases with the aim to identify mechanisms that regulate tumor immunity for improved diagnosis (biomarkers) and treatment (drug targets).
Our flow cytometry data show that similar to primary CRC tumors, PC metastases are infiltrated by lymphocytes, in particular T and B cells but also distinguishable subsets of NK cells, helper ILCs and atypical ILCs lacking CD127. However, while B cell infiltration is lower, T cell infiltration is even more pronounced in PC metastases than in primary CRC tumors. Single-cell RNA-sequencing of lymphocytes of PC metastases (n=5), primary CRC tumors and paired healthy colons (n=3) have preliminary revealed distinct subsets of helper ILCs, atypical ILCs, NK, T-, B- and stromal cells, some which are unique to the healthy colon, primary CRC tumors or PC tumors. These data suggest distinct immune microenvironments in these disparate tissues. In addition to acquisition of more data, current analysis is focused on more elaborative analysis including potential lymphocyte-stromal cell crosstalk in the tumor microenvironment. Interesting findings will be validated in a separate patient cohort where our findings can be prospectively related to HIPEC treatment outcome. Collectively, these studies will reveal the innate and adaptive lymphocyte landscape of CRC-PC. Additionally, we will unveil stromal-cell derived signals regulating intratumoral innate and adaptive lymphocytes. Overall, these studies will advance our understanding of tumor immunity in CRC-PC.

Disclosure of Interest: None Declared

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THE PULMONARY ENDOTHELIUM ARMS NATURAL KILLER CELLS FOR ANTI-METASTATIC RESPONSES

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Abstract Content: The lung is a common site for metastasis for many patients suffering from different tumor types. The success of metastatic growth is determined by the continuous interactions tumor cells establish with a wide range of immune cells. Natural Killer (NK) cells play a key role in inhibiting lung metastasis. It is however not clear when, where and how NK cells control metastatic tumor cells extravasating to the lung tissue. In this study, we found that the elimination of metastasizing tumor cells by NK cells in the lung is a very rapid process that takes place intravascularly. As such, NK cells interact with the pulmonary endothelium through the integrins Lymphocyte Function-associated Antigen 1 (LFA-1) and Very Late Antigen (VLA-4), which bind to endothelial ICAM-1 and VCAM-1, respectively. Targeted disruption of the integrin-mediated NK cell interaction with the pulmonary endothelium leads to increased apoptosis and a decrease of mature lung NK cells. Our findings suggest that the crosstalk between NK cells and endothelium may enable NK cells to survive and mature, so they can perform the necessary functions for tumor elimination. Modulating this interaction may be key for the development of lung-specific antimetastatic therapies.

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LB-49

A NOVEL GENE SIGNATURE IN ILC1-LIKE TISSUE-RESIDENT NK CELLS PREDICTS SURVIVAL IN PATIENTS WITH LUNG ADENOCARCINOMA

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Abstract Content: Tissue-resident natural killer (trNK) cells were recently identified in the human lung, and show similarity to tissue-resident memory T (TRM) cells. TRM cells have been widely studied in cancers and are associated with improved survival outcomes and better responses to immune checkpoint blockade. In this study we analyse the immune infiltrate of human lung tumours and matched non-cancerous lung margin (n = 35). We show that tumours have an increased abundance of trNK cells when compared with non-cancerous lung margin. We find that the number of trNK cells correlates with the number of TRM cells, and that trNK cells, like TRM, are associated with better survival outcomes in our patient cohort.
We also perform bulk RNA-sequencing of trNK cells and conventional NK cells isolated from both tissue sites (n = 5), and *ex vivo* stimulations with IL-12 and IL-18. This transcriptional, phenotypic and functional analysis revealed no overt tumour-specific trNK cell dysfunction, with these cells instead showing strong similarity between tumours and non-cancerous lung regions. We find that inhibitory molecules such as CD200R are expressed by trNK cells, as well as co-stimulatory molecules such as ICOS, indicating that trNK cells may represent a new cell of interest to target for lung cancer therapies. Using our transcriptional data we generate a gene signature for trNK cells, which we use to stratify survival outcomes for a larger lung cancer patient cohort from The Cancer Genome Atlas and again find an association with improved survival outcomes. However, this was specific to lung adenocarcinomas. We highlight differences between lung adenocarcinomas and lung squamous cell carcinomas in terms of the prognostic significance of trNK cells and TRM cells, and the factors associated with their development.
Studies of trNK cells in mice now commonly refer to these cells as type I innate lymphoid cells (ILC1s). We also find phenotypic similarities between our population of trNK cells in the lung and studies that describe ILC1-like cells in other human tissues. Comparisons of our lung trNK cell transcriptional data with recent single-cell RNA-sequencing studies reveal strong similarities to intraepithelial ILC1s in head and neck cancer, and cytotoxic ILC1s in renal cancer. These ILC1-like cells were also associated with anti-tumour activity and improved survival outcomes. Moreover, as in our study, IL-15 was shown to have a key role in the programming of these ILC1-like cells. The most common genes that were highly expressed in trNK cells and ILC1-like cells were *ITGA1*, *GZMK*, *CAPG* and *CXCR6*, with common downregulation of the conventional NK cell-associated genes *CX3CR1*, *PRSS23*, *FCGR3A* and *S1PR5*. We highlight the need for a universal nomenclature for this cell type.

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LB-50
HSP70 AND BC12 EXPRESSION AS DIAGNOSTIC AND PROGNOSTIC TOOL IN ORAL PRECANCER AND CANCEROUS LESIONS
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Abstract Content: Early dysregulation at molecular level could not be correlated with clinical presentations because of complex interactions at molecular levels. HSP70 (Heat shock Protein 70) and BCL2 (B cell lymphoma 2) are known molecular players in oncogenesis. However, their interaction is not known till date. Expression analyses of BCL2 and HSP70 was done through immunohistochemistry of BCL2 and HSP70 in biopsy samples obtained from 150 OSCC, 90 Oral Potentially Premalignant Disorders (OPMD) and 10 controls.
Our result showed that expression of both BCL2 and HSP70 is increased in same set of samples of oral pre-cancer cases which points out the proposed interaction between them. Decreased expression of HSP70 at the expense of increased BCL2 expression in OSCC cases is a novel finding of our study. Our study relevance lies in statement that targeting BCL2 together with HSP70 can be very effective strategy. Identifying such OPMDs where co expression of both is detected can have better prognostic results.

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09. ILCs and Inflammatory Diseases
P339
DISTINCT SUBSETS OF INNATE LYMPHOID CELLS IN NASAL POLYP
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Abstract Content: **Background:** Group 2 innate lymphoid cells (ILC2s) contribute to the pathogenesis of eosinophilic chronic rhinosinusitis with nasal polyps (CRSwNPs). However, the role of other subsets of ILCs and the differentiation of ILCs in CRSwNPs is not well understood. This study aimed to characterize the ILC subsets and evaluate the differentiation of ILCs from ILC precursors (ILCPs) in NP tissue.
Methods: ILC subsets and ILCPs were evaluated by flow cytometry in fresh sinonasal mucosa from patients with CRSwNPs and control subjects. Subsets were compared based on clinical variables and immunological features of the patients. Sorted ILCPs ($Lin^-CD127^+CD117^+CD45RA^+IL1R1^+$) were cultured with cytokines.
Results: The frequency of ILC1s and IFN-γ-producing ILC1s increased in non-eosinophilic NPs, whereas that of ILC2s and IL-5-producing ILC2s increased in eosinophilic NPs, particularly in patients with comorbid asthma. The frequency of ILC1s and IFN-γ-producing ILC1s, and frequency of ILC2s and IL-5-producing ILC2s positively correlated with that of neutrophils and eosinophils, respectively. The proportion of IFN-γ-producing ILC1s positively correlated with clinical severity and levels of IFN-γ and IL-8. The proportion of IL-5-producing ILC2s positively correlated with levels of IL-5, CCL24, and total IgE. ILCPs were identified in NP tissue and differentiated into IFN-γ-producing or IL-5-producing ILCs in response to increased IL-12 and IL-18 or IL-25 and IL-33 in non-eosinophilic NPs and eosinophilic NPs, respectively.
Conclusions: ILC1s and ILC2s contributed to inflammation (neutrophilic and eosinophilic, respectively) in CRSwNPs. In addition, ILCPs located in the sinus mucosa could differentiate into IFN-γ- or IL-5-producing cells in response to local cytokine stimuli.

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INNATE LYMPHOID CELLS ARE ACTIVATED AND THEIR LEVELS CORRELATE WITH VIRAL LOAD IN PATIENTS WITH PUUMALA HANTAVIRUS CAUSED HEMORRHAGIC FEVER WITH RENAL SYNDROME.

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Abstract Content: Innate lymphoid cells (ILCs) are involved in immunity and homeostasis but, except for Natural Killer (NK) cells, their role in human viral infections is not well known. Puumala virus (PUUV) is a hantavirus that causes hemorrhagic fever with renal syndrome (HFRS), an acute zoonotic respiratory disease. Humans are infected via inhalation of hantavirus-contaminated rodent excreta, leading to strong systemic inflammation. NK cells have been shown to be highly activated in HFRS, suggesting that also other ILCs might be responding during infection.

To investigate this, peripheral blood samples were obtained from PUUV-infected HFRS patients in the acute and convalescent phases of disease, as well as from healthy controls. Peripheral blood mononuclear cells were isolated and ILCs were phenotypically characterized by flow cytometry. Additionally, levels of cytokines, chemokines, clinical markers, and viral load were measured in the plasma fraction.

Overall, the frequencies of NK cells and naïve ILCs were reduced while ILC2 were increased in frequency during acute HFRS as compared to the convalescent phase of HFRS and to healthy controls. Specifically, the recently described ILC2-lineage committed c-Kit^{lo} ILC2 subset was increased. Phenotypically, ILCs displayed an activated profile with increased proliferation, and the expression of the homing markers α4β7, chemokine receptor (CCR)6, and CCR10 was altered in ILCs during acute HFRS. When analyzing for potential mechanisms behind the observed findings of activated and proliferating ILCs, we found that plasma levels of inflammatory proteins, including the ILC-associated cytokines interleukin (IL)-13, IL-23, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), were elevated during acute HFRS. Further, we found a negative correlation between viral load and frequencies of both NK cells and non-NK ILCs in acute HFRS.

In conclusion, we show that peripheral ILCs are activated and proliferating, and that several ILC-associated plasma proteins are elevated in HFRS patients, indicating general involvement of ILCs in the antiviral immune response. Further, the findings suggest a link between viral load and ILCs and a possible relocation of ILCs to tissue in HFRS. Altogether, our findings constitute the first comprehensive study of ILCs in a hantavirus-caused disease, aiding in further understanding the role of these cells in HFRS pathogenesis and in human viral infections in general.

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ILC3S IN HIV INFECTED LYMPH NODES UPREGULATE INFLAMMATORY PATHWAYS LINKED TO TISSUE FIBROSIS

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Abstract Content: People living with HIV (PLWH) develop extensive fibrosis and collagen deposition throughout their lymphoid tissues not reversed by antiretroviral therapy (ART). Innate lymphoid cells (ILCs) play essential roles in

lymphoid tissue damage and repair. However, no studies exist on ILCs in lymph nodes (LNs) during HIV infection. We hypothesized that ILCs are modulated by HIV infection and contribute to LN fibrosis in PLWH. We obtained fresh mesenteric, celiac and hepatic lymph nodes immediately after gastrointestinal (GI) surgery from 64 patients recruited from areas in South Africa, home to the highest HIV prevalence in the world. LNs from PLWH receiving ART exhibited extensive collagen deposition and CD4 T-cell depletion compared to uninfected controls characteristic of HIV LN pathology. We found no correlation between CD4 T-cell levels and ILC subsets in LNs, but reduced CD4 levels in both blood and LNs of HIV suppressed PLWH. Strikingly, we found no depletion of any of the ILC subsets in LNs, except a slight reduction of the dominant ILC3s located outside the germinal centers and close to HIV-infected cells. In contrast, circulating ILC3s were severely depleted in PLWH and consistent with our previous work. Single-cell transcriptional profiling revealed activation of the dominant ILC3 subset during HIV infection, suggesting ILC3s are directly involved in the HIV response. HIV-infected LNs expressed more heterogenous ILC3 subsets, including NK-like and 'ex-ILC3s', with increased FOS expression involved in cellular differentiation, suggesting that HIV infection induces trans-differentiation away from conventional ILC3 subsets towards cytotoxic type I responses. Moreover, we consistently found elevated levels of TGF-beta producing ILC3s. This subset was enriched in inflammatory pathways in PLWH, suggesting that these cells may play a central role in fibrosis formation through fibroblast-induced collagen deposition. Transcriptional profiling of the myeloid populations from matched LNs identified macrophages as the dominant source of IL1-B production and, therefore, may serve as innate sensors and drivers of ILC3 activation and differentiation in HIV-infected LNs. Here, we performed the first single-cell analysis of ILCs in HIV-infected LNs and identified ILC3s as potential contributors to LN fibrosis, a major pathological consequence of HIV infection that warrants further investigation.

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P342

THE ESOPHAGEAL ILC2S-EPITHELIAL STEM CELLS AXIS EXACERBATES EOSINOPHILIC ESOPHAGITIS FROM THE IL-33 MEDITATED INFLAMMATION

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Abstract Content: The Esophagus, besides the hollow shape tube that transports food from the oral cavity to the stomach, has a distinct mucosal Immune system. In the context of continuous exposure to harmful stimulants, Esophageal mucosa undergoes aggressive Immune responses that are mediated by Esophagus resident immune cells.

In the last decade, Eosinophilic Esophagitis (EoE) has become one of the most common Esophageal dysfunctions, which represents robust type 2 inflammation characterized by esophageal eosinophilia. As a dysregulated type 2 Inflammation breaks down the balance between Epidermal stem cells (aka Basal cells) proliferation and differentiation, resulting in abnormal remodeling in the Esophageal epithelium like Basal cells Hyperplasia and Dilated epithelium. It has derived Clinical symptoms such as Esophageal dysfunction, food impaction, and dysphagia. However, the underlying EoE, the Basal cell regulatory signaling network, remains incompletely understood until now.

Like other allergic disorders, Interleukin-33 (IL-33) was extensively upregulated in Esophagus biopsies with EoE, ki-67+ proliferating epithelial basal cells. IL-33 acts as a prominent activator of group 2 Innate Lymphoid Cells (ILC2s) that prompt local expansion and produce Type 2 cytokines IL-5,13 and Amphiregulin (Areg). Above all Areg acts as one of the low-affinity ligands of Epidermal Growth factor receptors (EGFR), which induces epithelial stem cell proliferation. Despite the potency, neither the presence nor role of Esophagus resident ILC2s get poorly understood.

So, we hypothesized that Esophagus resident ILC2s play a critical role in the Esophageal mucosa demonstrated ILC2s have contributed to the Pathogenesis of EoE, destroyed the balance of Basal cells proliferation and differentiation.

For this study, Firstly, we characterized the Esophageal resident ILC2s in both Active EoE patients' biopsy and C57BL/6 mice and revealed the pathogenic role of the IL-33 induced EoE mice model. Following, IL-5+ ILC2s accumulated in Esophagus mucosa by IL-33 treatment and Activated ILC2s mediated abnormal Epithelium remodeling dependent on the Areg-Egfr signaling cascade developed EoE phenotypes. Interestingly, Unlike Egf, Areg produced by ILC2s gets to harbor Areg-Egfr signaling cascade by suppressing Egfr downregulation, which in Human Esophagus Epithelium cell lines. In doing so, treatment of anti-Areg attenuates Basal Cells Hyperplasia and reduces Eosinophils infiltration and Inflammation in the IL-33 induced EoE mice model. As a result, modifying the 'IL-33-ILC2s-Areg-Egfr' axis can be a Therapeutic target of EoE.

Keyword: Eosinophilic Esophagitis (EoE), ILC2s, IL-33, Amphiregulin (Areg), EpdSCs

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INNATE LYMPHOID CELL (ILC1-ILC3) AXIS IS DYSREGULATED IN POST-MENOPAUSAL OSTEOPOROTIC MICE MODEL

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Abstract Content: Introduction: Osteoporosis is an inflammatory bone disorder that increases a person's risk of fracture due to lower bone mineral density (BMD), and decreased bone strength. Postmenopausal osteoporosis develops after menopause when estrogen levels drop precipitously thereby enhancing osteoclastogenesis without a corresponding increase in osteoblastogenesis leading to a net loss of bone mass. The role of various immune cells in osteoporosis has been termed by our group as ***"Immunoporosis: The Immunology of Osteoporosis"***. But till now only the role of very few lymphocytes has been established in the field of Immunoporosis. The role of innate lymphoid cells (ILCs) which are the innate counterparts of T cells and secrete the same cytokines as their adaptive counterparts is still unexplored. Thus, in the present study, we evaluated the role of the ILCs population in osteoporosis.
Aims and Objective: We were interested in investigating the role of ILCs on bone health since ILCs are mirror images of T cells and have similar functions as their T cell counterparts. We thus hypothesized to dissect the role of ILCs population in ovx induced osteoporotic mice model.
Materials and Methods: Female C57BL/6 mice were divided into two groups viz. sham and ovx (6 mice per group). After 45 days mice were sacrificed and the ILCs (ILC1, ILC2 and ILC3) population in the bone marrow (prime site of osteoclastogenesis) was analyzed in both the groups through Flow cytometry. To determine the extent of bone loss/osteoporosis in the collected bone samples (femur, tibia, and lumbar vertebrae) micro-CT (μCT) analysis was done.
Results: Ovariectomized condition led to bone loss in female mice as confirmed by the μCT. Excitingly, we observed that the percentage of inflammatory ILC1 ($Lin^-CD45^+CD127^+Tbet^+$) and ILC3 ($Lin^-CD45^+CD127^+RORγT^+$) was significantly enhanced in the ovx mice as compared to the sham mice. However, we observed no significant changes in the ILC2 ($Lin^-CD45^+CD127^+GATA3^+$) population between the sham and ovx mice gorups indicating that ILC1-ILC3 axis may have an important role in the regulation of bone health in ovx mice.
Conclusion: Taken together our results for the first time establish that the ILC1-ILC3 axis gets dysregulated during the post-menopausal conditions. These results thereby open a novel front to manipulate ILCs as a therapeutic target in inhibiting bone loss under inflammatory post-menopausal osteoporotic conditions.

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LB-51

RORA CONTROLS INNATE LYMPHOID CELL RESPONSES IN MUCOSAL INFLAMMATORY DISEASES

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Abstract Content: Innate lymphoid cells (ILCs) are frontline immune-modulatory cells involved in the early stages of host defense and maintenance of tissue homeostasis, particularly at mucosal surfaces such as the small intestine, lung, and skin. The ILC family is classified into five major groups: NK, ILC1s, ILC2s, ILC3s, and LTi, based on their developmental trajectories and functional characteristics. Their significance in specific immune responses and diseases is only beginning to be understood. ILCs express RORα, a key transcription factor important in their development and function. However, the role of RORα-dependent ILCs in mucosal inflammatory conditions still remains elusive and requires further investigation. Previously, our lab showed that RORα-deficient ($RORα^{sg/sg}$) mice lacked ILC2s and contained dysfunctional ILC3s. Single cell RNA-seq of inflamed cecal ILCs demonstrates that RORα-deficiency reshapes the transcriptional spectrum of ILCs, altering their metabolic program and function. To pinpoint the functional role of RORα+ ILC2 and ILC3 populations in vivo, we have now developed $IL17Rb^{eGFP\text{-}CreERT2}R26R^{RFP}RORα^{fl/fl}$ and $RORc^{cre}RORα^{fl/fl}$ transgenic mice that selectively lack RORα in ILC2s or ILC3s, respectively. In the lungs of naïve mice, ILC3s are extremely rare (<5% of total ILCs). Strikingly, we observe an increased proportion of ILC3s in the lungs of $RORc^{cre}RORα^{fl/fl}$ mice suggesting RORα-negative ILC3s may expand to compensate for RORα loss or that RORα normally restrains ILC3 expansion. Our preliminary results suggest a critical role for RORα in two subsets of ILCs and we will use our novel mouse models to explore how targeting RORα expression or activity may be of therapeutic benefit in a variety of mucosal inflammatory diseases.

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LB-52

IL-23-DEPENDENT TYPE 3 INNATE LYMPHOID CELLS (ILC3) LINK CRYSTAL-INDUCED INTRARENAL INFLAMMASOME ACTIVATION WITH KIDNEY FIBROSIS

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Abstract Content: Chronic inflammasome activation promotes fibrosis in various tissues, including the kidney. The cellular and molecular links between the inflammasome and kidney fibrosis are unclear. We fed mice lacking various immunological mediators an adenine-enriched diet, which leads to crystal precipitation in renal tubules, crystal-induced inflammasome activation and renal fibrosis.
Kidney fibrosis depended on an intrarenal inflammasome-dependent type 3 immune response driven by its signature transcription factor RORc, and was mainly carried out by type 3 innate lymphoid cells (ILC3). Depletion of these cells or genetic deficiency of RORc attenuated kidney fibrosis. Of the two inflammasome-derived cytokines, only IL-1β, but not IL-18 expanded ILC3, and only inhibition of IL-1β attenuated fibrosis. Inhibition of the type 3 signature cytokine, IL-17, was only partially protective, whereas deficiency of the type 3 maintenance cytokine, IL-23 was even more protective than IL-1β inhibition. This may be explained by the downregulation of the IL-1R by ILC3 early in the disease, whereas IL-23R expression was maintained. Mechanistically, ILC3s co-localized with renal mononuclear phagocytes in vivo, resulting in the production of TGF-ßthat stimulated fibroblasts to produce collagen.
ILC3s are a previously unrecognized pro-inflamamtory immune cell type in the kidney. These cells are regulated by IL-1β and IL-23 and link inflammasome activation in mononuclear phagocytes with renal fibrosis.

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10. Human ILCs

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PHENOTYPIC, FUNCTIONAL, AND TRANSCRIPTIONAL CHARACTERIZATION OF HUMAN CORD BLOOD ILC1 INDICATE CLOSE DEVELOPMENTAL RELATIONSHIPS WITH T CELLS

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Abstract Content: Innate lymphoid cells (ILCs) have been described to be vital for innate immunity due to maintenance of tissue homeostasis and barrier functions. Despite the well described role of ILCs within tissues, the function of circulating ILCs is presently incompletely understood. In order to deepen our understanding of neonatal innate immunity but also to determine similarities and differences of circulating ILCs with NK and T cells, transcriptomes and effector functions of ILC1, ILC2, and ILC3 were defined in cord blood (CB). Whereas ILC3 clustered closer to NK cells, ILC1 were more similar to T cells. Interestingly, CB ILCs showed a unique *ID3/ID2* ratio >1, which was not seen in ILCs from PB or tonsils but resembled the *ID3/ID2* pattern in CB $CD4^+$ T cells[1]. Notably, ILC1 showed the highest *ID3/ID2* of all ILC subsets and also showed close T cell resemblance by expressing the molecules CD5, CD6, CD4, CD8, CD28, and CD2. Furthermore, CB ILC1 expressed *TRAV* and *TRBV* transcripts as well as CD3▫▫ and CD3▫▫ but this was not accompanied by TCR expression on the cell surface[2]. Further subset analysis based on CD5/CD161 expression revealed that T cell-associated expression characteristics were preferentially found in the presumably more immature $CD5^+CD161^-$ subset whereas $CD5^-CD161^+$ ILC1 represented a more differentiated subset with IFNg-mediated effector functions. Surprisingly, when conducting single cell cloning experiments, all four CB ILC1 populations could be efficiently differentiated into mature KIR^+NKG2A^- NK cells with highly diversified KIR repertoires[2]. Our data suggest that CB-derived ILC1 on the one hand share close developmental relationships with T cells but on the other hand also contain developmental potential to differentiate into mature NK cells.

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CD45RA+CD62L- ILCS IN HUMAN TISSUES REPRESENT A QUIESCENT LOCAL RESERVOIR FOR THE GENERATION OF DIFFERENTIATED ILCS

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Abstract Content: Innate lymphoid cells (ILCs) are highly plastic and predominantly mucosal tissue-resident cells that contribute to both homeostasis and inflammation depending on the microenvironment. The discovery of naive-like ILCs suggests an ILC differentiation process that is akin to naive T cell differentiation. Delineating the mechanisms that underlie ILC differentiation in tissues is crucial for understanding ILC biology in health and disease. Here, we showed that tonsillar ILCs expressing CD45RA lacked proliferative activity, indicative of cellular quiescence. CD62L distinguished two subsets of CD45RA+ ILCs. CD45RA+CD62L+ ILCs (CD62L+ ILCs) resembled circulating naive ILCs because they lacked the transcriptional, metabolic, epigenetic, and cytokine production signatures of differentiated ILCs. CD45RA+CD62L− ILCs (CD62L− ILCs) were epigenetically similar to CD62L+ ILCs but showed a transcriptional, metabolic, and cytokine production signature that was more akin to differentiated ILCs. CD62L+ and CD62L− ILCs contained uni- and multipotent precursors of ILC1s/NK cells and ILC3s. Differentiation of CD62L+ and CD62L− ILCs led to metabolic reprogramming including up-regulation of genes associated with glycolysis, which was needed for their effector functions after differentiation. CD62L− ILCs with preferential differentiation capacity toward IL-22–producing ILC3s accumulated in the inflamed mucosa of patients with inflammatory bowel disease. These data suggested distinct differentiation potential of CD62L+ and CD62L− ILCs between tissue microenvironments and identified that manipulation of these cells is a possible approach to restore tissue-immune homeostasis.

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CONFRONTING THE KILLERS: MAPPING NATURAL KILLER CELLS IN HEALTHY HUMAN KIDNEY AND ALLOGRAFT REJECTION AT SINGLE-CELL RESOLUTION

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Abstract Content: Tissue-resident innate lymphoid cells (ILCs) promote organ-adapted immunity and maintain homeostasis. Little is known about ILCs within the human kidney immune niche at homeostasis, or in the context of transplantation and graft rejection. The major driver of kidney transplant failure is antibody-mediated rejection (AMR), which occurs when donor-specific antibodies (DSA) recognize donor human leukocyte antigens (HLA) and initiate immune-mediated attack of the graft. Natural Killer (NK) cell presence is implicated in AMR pathology and associated with poorer clinical outcomes, suggesting that targeted inhibition of NK cells may limit AMR. Further, many cases of AMR occur despite maintenance immunosuppressive medications, indicating a need for more effective prevention and treatment for AMR. Here, we characterize ILCs in healthy human kidney and identify changes in ILC populations that occur in AMR. Our sex-balanced single-cell RNA sequencing map of healthy kidney from 19 living kidney donors revealed that $CD56^{+}CD16^{+}$ NK cells constitute a major immune population within healthy human kidney, with ILC2s present in very low abundance. Kidney-resident NK cells are marked by elevated expression of *AREG* and *CD69* compared to circulating NK cells, and may be recruited by myeloid cells through a CXCL16-CXCR6 axis. Alongside other lymphocytes, NK cells express prostaglandin synthesis and receptor genes, indicating potential niche regulation via prostaglandins. In post-transplant samples of patients with AMR, ILC1s appear to be the

dominant helper ILC population in addition to a high proportion of NK cells. NK cells in AMR grafts exhibit altered transcriptional profiles compared to NK cells in healthy kidney, including upregulating genes associated with activation and degranulation and downregulating expression of tissue-reparative factor *AREG.* Cell-cell interaction inference predicted increased interactions between NK cells and endothelial cells in AMR, supporting an active role for NK cells in AMR pathology. Ongoing work is characterizing how NK cell responses are impacted by conventional immunosuppressive medications, focusing on features of NK cell populations that escape immunosuppression, and working to identify novel ways of targeting NK cells in AMR. Collectively these studies are advancing the knowledge of ILC function and phenotype in human kidney at homeostasis and in the context of transplant rejection.

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MULTIPLEXED HISTOLOGY ANALYSES FOR THE PHENOTYPIC AND SPATIAL CHARACTERIZATION OF HUMAN INNATE LYMPHOID CELLS

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Abstract Content: ILCs are mainly tissue resident cells, abundant in barrier sites and act as tissue sensors. Therefore, ILCs are heterogeneous, plastic and contribute to both tissue homeostasis and inflammation.
In line with this, their microanatomical localization within and across tissues is of particular interest. However, the lack of a unique ILC marker demands for high parametric analyses to unambiguously identify these cells. Conventional immunofluorescence microscopy can only resolve 5 parameters and, thus, while reporter mouse strains enable *in situ* analysis of murine ILCs, little is known about the precise localization of their human counterparts and their interactions with neighboring cells.
To address this challenge, we have applied highly multiplexed immunofluorescence to several human tissues, allowing us to stain more than 50 markers in the same tissue section. We combined such image acquisition system with a computational analysis pipeline to extract single-cell, spatially-resolved data. Thereby, we could identify Lin^- $CD127^+$ ILCs *in situ*, and characterize these cells and their microenvironments. We pinpointed novel markers for human ILC characterization, such as IRF4 and CD138, and identified stromal landmarks for ILC localization. We found these landmarks to be conserved across chronically inflamed tissues, pointing to the existence of a fibrovascular niche for human ILCs, in line to what has been described in mice. We found that ILCs share tissue niches with tissue resident plasma cells in the tonsil and, interestingly, despite their rare abundance, ILCs tended to localize in close-contact groups of 2 to 4 cells. Based on the principle that spatial relationships play an important role in the orchestration of local immune responses, our work also serves as a resource for future computational and multiparametric histological analysis of ILCs that should improve our understanding of ILC heterogeneity and plasticity, what we expect to be closely linked to their spatially discrete niches within tissues.

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DISTINCT DEVELOPMENTAL AND TRANSCRIPTIONAL PATTERNS OF HUMAN INNATE LYMPHOID CELLS ACROSS FETAL TISSUES

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Abstract Content: Fetal immune cells emerge in early gestation and likely play crucial roles in fetal development and maternal-fetal tolerance. Increased understanding of early immune development is needed as it not only lays the foundation for the post-natal immune system but may also be used for future *in utero* therapies. Innate lymphoid cells (ILCs) are some of the earliest immune cells to arise in the human fetus with a distinct distribution of subsets depending on tissue and age. While in adults it has been shown that ILCs have tissue specific imprints, this is less established in the human fetus. Furthermore, it is largely unknown where different ILCs develop in the fetus, and at what point in differentiation ILCs seed various tissues. To investigate this, we used multiple paired tissues from human fetuses for high throughput single cell analyses of mature ILCs and their progenitors. RNA sequencing and flow cytometry revealed that ILCs and $CD34^+$ progenitors have distinct transcriptional and protein expression patterns between tissues and identified putative ILC progenitors in fetal intestine. As this suggests tissue dependent ILC development, we are currently using in vivo lineage tracing approaches with the aim to decipher developmental pathways of fetal ILCs across tissues at single cell level.

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SINGLE CELL RNA SEQUENCING PERFORMED ON FRESHLY PREPARED OR FROZEN AND THAWED INNATE LYMPHOID CELLS REVEALS A GENE SIGNATURE OF FREEZING AND THAWING THAT OVERLAPS WITH CELLULAR MARKERS OF TISSUE RESIDENCY

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Abstract Content: Single cell RNA sequencing performed by drop sequencing, utilizing platforms such as 10x genomics and others, is an increasingly common technique that can rapidly produce large amounts of data for both hypothesis generation and testing. As with any high throughput method of data generation, the smallest changes in preparation and experimental procedure can have profound impacts on the final product. An outstanding question of relevance is the difference between sequencing cells from freshly prepared tissue-cell suspensions in comparison to those that have been frozen and subsequently thawed. To investigate any effects of freezing on innate lymphoid cells, natural killer cells, and T cells we isolated lymphocytes from a human tonsil and immediately proceeded to sorting and sequencing for part of the sample while freezing the rest of the cells in FCS/10% DMSO. One week later a sample of these cells were thawed, sorted and sequenced following the same protocol as the fresh cells. We found that while freezing had no major impact upon library quality the cells in general did have higher mitochondrial and ribosomal gene content per cell, likely reflecting freezing and thawing-induced cellular stress. We also found that all cell types were equally represented in both fresh and frozen data sets, confirming that they were all robust to freezing and thawing. We did however find that, whereas freezing and thawing had little impact on the pattern of gene expression, it did affect the magnitude of expression of some genes. More specifically, freezing caused significant up- and downregulations of genes, some which were unique to specific cell types and others which impacted all cell populations. Using this latter set of genes we were then able to identify a genetic signature of freezing and thawing. Many of the most upregulated genes in the signature were the same as genes frequently used as markers of tissue residency including *CD69*, *CXCR4*, *FOS*, and *KLF6* amongst others. These findings will inform future studies that

utilize single-cell sequencing techniques by offering a guide map of genes up- and downregulated by freezing and thawing and will help to better elucidate the overlap of genetic markers of tissue residency and cellular stress.

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GLIADIN-REACTIVE VITAMIN D-SENSITIVE PROINFLAMMATORY ILCPS ARE ENRICHED IN CELIAC PATIENTS

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Abstract Content: Celiac disease (CD) is a multisystem disease in which different organs may be affected. It is well accepted that innate lymphoid cells (ILCs) play a crucial role in intestinal immunity of CD, however their role in the periphery remains uncertain. We investigated whether circulating ILCs contribute to the CD peripheral inflammatory status. We found that the CD cytokine profile was characterized by high concentrations of IL-12p40, IL-18 and IFN-γ paralleled by an expansion of ILC precursors (ILCPs). In the presence of the gliadin peptides p31-43 and pα-9, ILCPs from CD patients increased transglutaminase 2 (TG2) expression, produced IL-18 and IFN-γ and stimulate $CD4^+$ T lymphocytes. IFN-γ was also produced upon stimulation with IL-12p40 and IL-18 and inhibited by the addition of vitamin D. Low levels of blood vitamin D correlated with high IFN-γ and ILCP presence and marked the CD population mostly affected by extraintestinal symptoms. Dietary vitamin D supplementation appears to be an interesting therapeutic approach to dampen ILCP-mediated IFN-γ production.

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LB-53

ALTERATION IN INNATE LYMPHOID CELL SUBSETS DURING PARTUITION AT THE MATERNAL-FETAL INTERFACE

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Abstract Content: **Introduction**: Innate lymphoid cells (ILCs) are an immune cell type which lack expression of antigen receptors but rather sense environmental signals such as soluble cytokines, prostaglandins and metabolites. In other tissues, ILCs act as inflammatory amplifiers or suppressors depending on the stimuli, and they have been hypothesized to perform a similar role in pregnancy, which requires immune adaptation throughout gestation, including during partuition. Recent studies consistently demonstrate that ILCs are present at the maternal-fetal interface (MFI) during pregnancy, however, the distribution of ILC subsets throughout the MFI in term pregnancy during partuition is currently unclear.
Methods: Maternal blood, cord blood, and placenta (n=9 each) specimens were obtained from women with uncomplicated pregnancies at the time of scheduled Cesarean delivery (unlabored) or after vaginal delivery (labored) at term (37-41 weeks). Mononuclear cells were isolated from blood samples, and placenta samples were digested with collagenase to isolate immune cells. High-parameter flow cytometry was performed using a panel of 22 cell surface markers to identify total ILCs (CD127+, lineage negative), ILC1 (CD117/CRTH2 neg), ILC2 (CD117neg/CRTH2+), and ILC3 (CD117+/CRTH2neg) subsets. Unpaired t-test statistical testing was performed to determine significant differences in ILCs between unlabored and labored samples.
Results: Among the tissues at the MFI, cord blood contains the greatest median concentration of ILCs (0.42% of total lymphocytes) compared to placenta (0.17%), and maternal blood (0.12%) in unlabored subjects. There was a

significant increase in total ILCs in cord blood (p=0.001) and placenta (p=0.02) in subjects who underwent labor compared to unlabored subjects. Maternal blood ILCs are primarily of the ILC2 subset, while placental ILCs consist predominantly of ILC3, and cord blood ILCs are equally distributed between subsets in unlabored subjects. There was significant increase in ILC1 (p=0.01 and 0.02) and decrease in ILC3 (p=0.04 and 0.03) subsets in maternal blood and placenta in labored compared to unlabored subjects.
Conclusions: There are significantly increased ILCs in the MFI during partuition, primarily due to increased frequency of pro-inflammatory ILC1 subset. Partuition is also associated with decreased frequency of ILC2 and ILC3 subsets, which are considered anti-inflammatory. Due to plasticity in ILC subsets, it is unclear whether this change in ILC subsets is associated with enhanced recruitment of ILCs to the MFI or alterations in existing ILC populations. Further studies are necessary to demonstrate ILC gene expression and metabolic signals which affect ILC activity at the MFI during partuition as well as pregnancy complications such as preterm labor to identify potential therapeutic targets.

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11. ILCs as Therapeutics

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ADOPTIVE TRANSFER OF GROUP 2 AND GROUP 3 INNATE LYMPHOID CELLS PREVENTS REJECTION IN HUMANIZED MODELS OF ISLET TRANSPLANT

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Abstract Content: Type-1 diabetes (T1D) is characterized by loss of self-tolerance and T cell-mediated attack of pancreatic islets resulting in the destruction of insulin-producing β-cells. Islet transplantation restores insulin production for individuals with T1D, however it does not eliminate the underlying autoimmunity, requiring life-long immunosuppression to prevent immune-mediated rejection of transplanted islets. In murine islet transplant models, group 2 ILCs (ILC2s) and group 3 ILCs (ILC3s) limit rejection and promote β-cell function. To assess the potential of harnessing human ILCs to improve islet transplantation, we examined the ability of human ILCs to *i)* support engraftment of allogeneic human islets and, *ii)* regulate harmful T cell subsets associated with pathology of T1D. We found no cytotoxic effects of allogeneic ILC2s or ILC3s towards islets, and adoptive transfer of ILC2s enhanced islet engraftment and function *in vivo*. To assess whether adoptive transfer of ILC2s or ILC3s could prevent immune-mediated rejection of islets, we employed an antigen-specific model of transplant where HLA-A2$^+$ human islets are transplanted into NOD-*scid*IL2Rgamma^{null} (NSG) mice. Following islet engraftment, HLA-A2-specific CAR T cells are transferred intravenously to induce antigen-specific rejection. Both ILC2s and ILC3s inhibited CAR T cell proliferation and cytokine production *in vivo*, resulting in complete inhibition of CAR T cell-mediated rejection of islets. *In vitro* studies revealed ILC2s suppressed CD4$^+$ and CD8$^+$ T cell IFN-γ production and expression of the apoptosis-inducing molecule FasL by CD8$^+$ T cells. ILC3s also directly inhibited T cells, albeit not as efficiently as ILC2s, together supporting a direct role for ILC2s and ILC3s in inhibiting T cell-mediated islet rejection. For therapeutic translation, whether ILCs are ideally sourced from donor or recipient is an important consideration. Further, we assessed whether ILCs had the potential to regulate autologous and allogeneic CD4$^+$ T helper cell and CD8$^+$ T cell subsets. We observed differential impacts of ILC2s and ILC3s on autologous versus allogeneic T cells and found ILC2s demonstrate greater ability to regulate both CD4$^+$ and CD8$^+$ T cells subsets than ILC3s. Ongoing efforts are delineating the mechanism by which ILC2s and ILC3s limit T cell rejection of islets. Collectively, these findings support human ILC2s and ILC3s may have applications in adoptive cell therapies aimed at promoting tolerance in transplantation.

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GENERATING HUMAN TYPE 3 INNATE LYMPHOID CELLS TO PREVENT GRAFT-VERSUS-HOST DISEASE VIA ADOPTIVE TRANSFER

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Abstract Content: Allogeneic hematopoietic cell transplantation (allo-HCT) is often applied to cure patients with hematological malignancies, but can be fatal when graft-versus-host disease (GvHD) occurs. GvHD is characterized by mucosal inflammation and disruption of epithelial barriers. Type 3 innate lymphoid cells (ILC3) are mostly tissue-resident innate immune cells that regulate local inflammatory responses, suppress T cell alloreactivity and are important in maintenance of epithelial barriers via interleukin (IL)-22 production. Conditioning of allo-HCT recipients (i.e. chemotherapy and radiation) depletes all types of ILC from the blood and patients with delayed ILC3 recovery in blood before and after allo-HCT are more prone to develop mucositis and GvHD. Moreover, allo-HCT grafts with lower than median numbers of ILC3 predisposed patients to the development of acute GvHD. Together these data suggest that ILC3 can protect against GvHD. We hypothesize that enrichment of allo-HCT grafts with ILC3 will enhance ILC3 recovery and decrease the risk to develop GvHD. The aim of this study is to generate adequate numbers of bona fide ILC3 *ex vivo* for adoptive transfer to prevent GvHD.

We cultured human cord blood-derived CD34+ hematopoietic stem and progenitor cells (HSPC) *ex vivo* in the presence of successive cytokine mixes for a total of five weeks (HSPC-ILC) and performed flow cytometry to analyze expression of ILC-related surface and intracellular markers. Furthermore, we stimulated HSPC-ILC for 5 or 24 hours with phorbol myristate acetate (PMA), ionomycin and ILC3 stimulatory cytokines, to measure IL-22 production by flow cytometry (5 hours) and ELISA (24 hours).

We found that culturing human cord blood-derived HSPC in the presence of 50 ng/ml recombinant human IL-15 from day 9 led to a mean 425x expansion of cells that included ILC3, NK cells and myeloid cells. 13% of these cells were bona fide CD94- CD117+ NKp44+ ILC3 that produced IL-22. Using this HSPC-ILC culture protocol, we would require ~$1x10^5$ CD34+ HSPC to generate ~$10x10^6$ ILC3 which we anticipate to be sufficient for adoptive transfer of ILC in a 1:100 ratio with T cells into a transplant recipient of 70 kg. This ratio is similar to proportions *in vivo* in human blood. Preliminary data suggests that the HSPC-ILC culture protocol can be made more efficient using the EZH1/2 inhibitor UNC1999. Adding UNC1999 increased the percentage of ILC3 from 10% (control) to 67% at day 28 without a decrease in expansion and viability. IL-22 production at day 35 was also increased in the presence of UNC1999. Experiments to compare different HSPC sources, including cord blood, mobilized blood, bone marrow and fetal liver to generate human ILC3 *in vitro* and to test them in a MISTRG mouse model are underway.

Together, our data indicate that (1) functional ILC3 can be generated from human cord blood-derived CD34+ HSPC, with (2) the ILC3 yield optimized by the addition of UNC1999 to the HSPC-ILC protocol, to generate human ILC for adoptive transfer to prevent GvHD after allo-HCT.

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