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Structure-Function & Systems Biology of Cytokines

Abstract Book

Contents

Plenary 1: Cytokines and COVID19

O01

DISTINCT SYSTEMIC AND MUCOSAL IMMUNE RESPONSES TO SARS-COV2

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Abstract Content: Coronavirus disease 2019 (Covid-19) is characterized by distinct patterns of disease progression implying diverse host immune responses. We performed an integrated immune analysis on a cohort of fifty Covid19 patients with various disease severity, in both plasma and the nasopharynx, which reflects the initial site of infection. Using state-of-the-art technologies including ultra-sensitive digital Elisa (Simoa) to measure low levels of interferons (IFN), mass cytometry (CyTOF), ultrasensitive droplet based digital PCR (ddPCR), Nanostring nCounter technology and an ultrasensitive innovative antibody detection technic (S-Flow), we identified a unique phenotype in severe and critical patients. It consisted of a profoundly impaired type I IFN response characterized by low IFN-α production and activity, associated with a persistent blood viral load, an exacerbated inflammatory response, and a strong SARS-CoV2-specific antibody response. Inflammation was partially driven by the transcriptional factor NFκB and characterized by increased tumor necrosis factor (TNF)-α and interleukin (IL)-6 production and signaling. These inflammatory cytokines in all patient groups were significantly correlated with circulating viral levels, which was not associated with systemic type I interferon responses. Furthermore, we determine that while plasma inflammatory cytokine levels were a hallmark of COVID-19 regardless of disease severity, cytokines present in the nasopharynx allowed a better categorization of disease severity. In addition to expected inflammatory markers this also included locally regulated growth factors and chemokines suggesting viral induced mucosal tissue dysregulation. We propose that type-I IFN deficiency in the blood could be a hallmark of severe Covid-19 and provide a rationale for combined therapeutic approaches which may be further optimized with a better understanding of infected tissue immune responses.

Disclosure of Interest: None Declared

Plenary 2: Cytokines in bacterial diseases

O02

HUMAN ENTERIC VIRUSES SHAPE DISEASE PHENOTYPE THROUGH DIVERGENT IMMUNOMODULATION

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Abstract Content: Environmental signals determine phenotype of disease in the context of genetics. It is well established that perturbations in enteric bacteria combined with genetic defects in the host immune responses to them determine phenotypes of inflammatory bowel disease (IBD). However, the contributions of gut-resident eukaryotic and prokaryotic viruses (collectively, the virome) that also fluctuate in IBD to host immunity and disease outcomes are unknown. Here we show that viromes derived from healthy, ulcerative colitis (UC) or Crohn's disease (CD) patient intestine regulate host immunity differently and can independently modify disease state. Viromes from non-IBD individuals directly elicited anti-inflammatory programs in macrophages and intestinal epithelial cells. Conversely, viromes from both UC and CD patients promoted an inflammatory response. Notably, healthy viromes successfully dampened the IBD-virome inflammatory response. Furthermore, mice with a "humanized" healthy virome had attenuated intestinal inflammation *in vivo* while "humanized" IBD virome mice exhibited intestinal inflammation at steady state and exacerbated induction of colitis. Finally, IBD-associated genetic variationin *IFIH1* encoding the virus receptor MDA5 abrogated responses to the healthy enteric virome and disrupted intestinal epithelial integrity. Together, these studies demonstrate that changes in the human virome, and host immune responses to it, autonomously drive divergent disease phenotypes. Manipulation of the human enteric virome may be beneficial in IBD.

Disclosure of Interest: None Declared

O03

STAT2 FACILITATES INTESTINAL INFLAMMATION BY INTERFERING WITH ANTIMICROBIAL IL-22 SIGNALING

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Abstract Content: Ulcerative colitis (UC) is one of two major forms of inflammatory bowel disease. This debilitating chronic condition causes inflammation in the colon and heightens the risk of colorectal cancer. What causes UC and its pathogenesis remains largely unclear. Type I and type III interferons (IFN) are cytokines known to protect against colitis. The transcription factor STAT2 is a key component of the ISGF3 complex that drives downstream the transcriptional responses of both cytokines. The aim of our study was to determine the role of STAT2 in the development of intestinal inflammation by employing the murine dextran sodium sulfate (DSS) model of human UC-like colitis. We found that STAT2 deficiency caused less body weight loss and mild intestinal inflammation when compared to wild type mice. Pathology scoring of *Stat2KO* colon sections showed reduced damage to the epithelial gut barrier and attenuated immune cell infiltration. Co-housing of wild type with *Stat2KO* mice did not transfer colitis susceptibility. Furthermore, colons from DSS-treated *Stat2KO* mice showed delayed and reduced IL6 induction accompanied by early and progressive increased induction of the antimicrobial cytokine IL-22; known to be protective against colitis. IL-22 treatment of intestinal cells with STAT2 knockdown revealed increased STAT3 activation. Thus, our findings reveal that STAT2 is damaging and may promote

colitis by impairing IL-22 signaling by an as yet unknown mechanism that we are currently investigating. Collectively, this bring to the forefront an unrecognized and damaging feature of STAT2 in the pathogenesis of UC, that when deregulated may progress CRC.
Disclosure of Interest: None Declared

Symposium 1: Cytokines in microbiome dynamics

O04

COMMENSAL BACTERIA LIKELY CONTRIBUTE TO OCULAR MANIFESTATIONS AND EXCESSIVE IL-1 BETA RESPONSE IN AUTOINFLAMMATORY DISEASES DUE TO NLRP3 GENE MUTATIONS

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Abstract Content: We reported previously (PMID: 28709803) that ocular surface of healthy wild-type mice is colonized by a commensal shared between mice and humans, *Corynebacterium mastitidis* (*C. mast*). On the murine ocular surface, *C. mast* elicits IL-17 from γδ T cells and promotes local host defense. Based on this we hypothesized that ocular inflammation in Autoinflammatory disease patients, with *NLRP3*-associated mutations, may be triggered by an overactive response to their own ocular surface bacteria. We found that, unlike wild-type mice, knock-in mice bearing an *NLRP3* gene mutation found in patients, developed conjunctivitis, conjunctival neutrophilia, and increased IL-1β and IL-17 responses following ocular exposure to *C. mast*. scRNA-seq of murine conjunctival cells confirmed that γδ T cells were a major immune cell population and a source of *Il-17*, *Ccr2* and *S100a8* transcripts, possibly explaining the neutrophilia. Importantly, patient PBMCs stimulated with*C. mast* lysate overproduced IL-17A compared to healthy controls by ELISA. However, in contrast to mice, scRNA-seq of patient conjunctival cells and PBMCs showed upregulation of Th1 and Th17 signaling moleculesin ▫▫β T cells rather than in γδ T cells. This suggests that response to *C. mast* in patients may be mediated by different immune cells, and/or that other commensals may drive their ocular phenotype. Our study shows that commensals can elicit an exaggerated immune response in humans and mice with *NLRP3*-related autoinflammatory disease conditions. If indeed conjunctivitis in these patients reflects a response to their own commensals, adjunct antimicrobial therapy could be an option.
Disclosure of Interest: None Declared

Symposium 2: Inflammation

O05

CYTOSOLIC DNTP CATABOLISM PREVENTS NLRP3 INFLAMMASOME OVERACTIVATION

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Abstract Content: Aberrant activation of NLRP3 inflammasome drives the development of many diseases, including atherosclerosis, gout, osteoarthritis, Alzheimer's disease, macular degeneration, type 2 diabetes and cancer. However, how the NLRP3 inflammasome activity is regulated at the molecular level remains incompletely understood. Our previous work demonstrated that new mitochondrial DNA (mtDNA) synthesis, induced at the inflammasome priming step, is a prerequisite for producing oxidized mtDNA (ox-mtDNA)—an activating ligand of NLRP3. Here we show that SAMHD1, a cytosolic dNTP hydrolase, functions as a macrophage-intrinsic gatekeeper that prevents NLRP3 inflammasome overactivation. Mechanistically, inflammasome priming activates SAMHD1 to inhibit cytosolic dNTP buildup, thereby preventing their transport to mitochondria. This in turn protects mitochondria from uncontrolled new mtDNA synthesis and ox-mtDNA production, resulting in the attenuation of NLRP3 inflammasome activation. Consistent with these in vitro observations, relative to their wild-type counterparts, SAMHD1-deficient mice produced substantially more IL-1b in vivo and were more sensitive to developing NLRP3 inflammasome-dependent immunopathology. Together, these results not only identify SAMHD1 as a novel cell-intrinsic inhibitor of NLRP3 inflammasome, but also demonstrate that cytosolic dNTP metabolism plays an essential role in orchestrating inflammation.
Disclosure of Interest: None Declared

Symposium 3: Interferon stimulated genes

O06

IMMUNITY-RELATED GTPASE IRGM1 GUARDS AGAINST SPONTANEOUS INTERFERON-DRIVEN AUTOIMMUNITY THROUGH MITOCHONDRIAL MAINTENANCE

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Abstract Content: Type I interferon (IFN) plays a central role in the pathogenesis of common autoimmune disorders. Recently, we reported that mice deficient in the interferon-γ-inducible immunity-related GTPase - Irgm1, which regulates autophagy and also localizes to mitochondria, have an autoimmune exocrinopathy accompanied by increased autoantibodies and spontaneous induction of IFN-stimulated genes in several organs. We hypothesized that the pathogenic IFN response observed with Irgm1 deficiency is

driven by inappropriate sensing of cleared mitochondrial DNA (mtDNA). Here, we show that genetic deletion of the type I interferon receptor rescues the autoimmune tissue pathology and excess autoantibody levels in *Irgm1*$^{-/-}$ animals, demonstrating the pathogenicity of type I interferon in these animals. Abnormal induction of type I interferon in *Irgm1*$^{-/-}$ fibroblasts is associated with simultaneous dysfunction in mitochondria and lysosomes, resulting in soiling of the cytosol with mtDNA. The interferon response is normalized upon depletion of cellular mtDNA and partially corrected by restoration of lysosomal function. Further, we show that mtDNA activates the double stranded DNA sensor – cGAMP synthase (cGAS) and its adaptor, stimulator of IFN genes (STING), to drive type I interferon production in fibroblasts. In vivo, we found that the cGAS-STING pathway underlies the pathology of salivary glands, autoantibodies as well as inflammatory cytokine production in Irgm1-null mice. Interestingly, the type I interferon response in *Irgm1*$^{-/-}$ macrophages is dependent on endo-lysosomal signaling and is associated with increased delivery of damaged mitochondria to functional lysosomal compartments. Taken together, our findings support a model wherein Irgm1-mediated mitochondrial maintenance and turnover represses inappropriate mtDNA-dependent activation of autoinflammatory interferon responses, possible in a tissue-dependent manner. We propose that this fundamental mechanism may play a key role in suppression of type I interferon-dependent autoimmune syndromes.
Disclosure of Interest: None Declared

O07
A WEST NILE VIRUS RESISTANCE SNP GENERATES A MEMBRANE-TARGETED ISOFORM OF OAS1 WITH ENHANCED ANTIVIRAL ACTIVITY
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Abstract Content: The antiviral activity of interferons, at least in part, is mediated by the upregulation of interferon stimulated genes (ISGs) whose protein products interfere with virus replication. Collectively, these ISGs confer at least some protection against virtually all human viruses. However, mechanistic explanations for the antiviral activity and specificity of many ISGs is lacking. This is further complicated by the fact that many antiviral ISGs are alternatively spliced, which could allow for fine tuning of the antiviral specificity or activity of an ISG. In this study, we show a membrane-targeted isoform of oligoadenylate synthetase 1 (OAS1) has enhanced antiviral activity against Flaviviruses and Picornaviruses. OAS proteins are an antiviral system critical for restricting many different viruses. Upon sensing viral RNA (vRNA), the catalytically active OAS proteins generate the second messenger 2-5A, which activates the latent ribonuclease RNase L. Activated RNase L potently restricts viral replication by degrading cellular RNAs to block host translation and induce apoptosis. Human OAS1 is C-terminally spliced into several isoforms, and previous genetic studies have implicated a splice acceptor site SNP (A/G, rs10774671) in the OAS1 gene with West Nile virus resistance. The susceptibility allele (A) controls p42 expression and the resistance allele (G) controls p46 expression, which suggests these isoforms have distinct antiviral activity. We tested the antiviral activity of the p42 and p46 isoforms against encephalomyocarditis virus (EMCV), Coxsackie virus B (CVB), and West Nile Virus (WNV) and found p46, compared with p42, displays significantly stronger antiviral activity against EMCV, CVB3, and WNV. Although both OAS1 isoforms require catalytic activity and RNase L for antiviral activity, we discovered a C-terminal CaaX motif in p46. Proteins containing CaaX motifs at their C-termini are prenylated and targeted to membranes. Using confocal microscopy, we found p46 localized to the Golgi while p42 is cytosolic. Since EMCV, CVB3, and WNV are positive-strand RNA viruses and replicate their vRNA on intracellular organelle membranes, we hypothesized membrane-targeting positions p46 in proximity to vRNA replicating on organelle surfaces. In support of this hypothesis, EMCV vRNA was immunoprecipitated with p46, but not p42, and membrane-targeting was required for p46 to access v RNA. This was also confirmed by confocal microscopy, as p46, but not p42, localizes to sites of viral RNA accumulation during infection. These data support a model in which membrane-targeting of OAS1 p46 enhances its antiviral activity by placing this sensor in proximity to sites of positive-strand RNA virus replication at organelle surfaces. Collectively, our study shows how OAS1 specificity can be diversified through alternative splicing and suggests differential subcellular targeting of OAS proteins may be important for the broad antiviral specificity of this pathway.
Disclosure of Interest: None Declared

Symposium 4: Cytokines in cancer

O08
THE ACTIVATING STAT5BN642H DRIVER MUTATION DISRUPTS T-CELL DEVELOPMENT PROGRESSING TO T-CELL NEOPLASIA
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Abstract Content: The activating STAT5B^{N642H} mutation was found in over 120 patients suffering from various T-cell neoplasia subtypes. Studies have revealed that up to 8% of T-cell acute lymphoblastic (T-ALL) patients have STAT5B^{N642H}, correlating with poor prognosis and a higher risk of relapse. Confirming the transforming capacity of this mutation, we used a STAT5B^{N642H} *vav*-transgenic mouse model, which rapidly develops CD8^{+} T-cell lymphoma that is transplantable and aggressively infiltrates into non-

hematopoietic organs. These transformed T-cells are mature and display a cytotoxic phenotype. Therefore, we questioned biological and transforming consequences governed by STAT5B^{N642H} during T-cell development. Comparing thymocytes of STAT5B^{N642H} to WT mice, we found that cells double negative stage 1 (DN1) were highly enriched, whereas cells in DN2-4 were decreased. Importantly, double positive (DP) cell percentages were drastically reduced and CD8^{+} and CD4^{+} single positive (SP8/SP4) cells were expanded in STAT5B^{N642H} thymi, suggesting that STAT5 promotes T-cell maturation. This disruption in T-cell development caused by the mutation was accompanied by an expansion of the thymic medulla, where more mature T-cells reside. Comparing transcriptomes of DN, DP and SP8 cells by RNA-sequencing (seq), we found that STAT5B^{N642H} triggers a highly activated and cytokine-releasing and -responsive T-cell signature. To investigate whether STAT5B^{N642H} can still exert this function independently of the T-cell receptor (TCR), we crossed our transgenic mice to a RAG2$^{-/-}$ background. Strikingly, these mice developed thymic neoplasia that retained immature T-cell features with high TdT and reduced surface CD3 expression, resembling a T-ALL-like phenotype. Furthermore, high expression of TdT and oncogenes PIM1 and BCL-2 in diseased T-cells mirrors the situation in STAT5B^{N642H}-positive T-ALL patients. The mutation sufficed to overcome RAG2$^{-/-}$-mediated DN stage arrest as most STAT5B^{N642H} RAG2$^{-/-}$ thymocytes were DP and SP8 T-cells. Comparing STAT5B^{N642H} RAG2$^{-/-}$ to RAG2$^{-/-}$ thymocytes by RNA-seq showed upregulation of several TCR signaling genes despite TCR deficiency, and enrichment of MYC targets and mTOR signaling in these cells. We conclude that STAT5 hyperactivation induces an activated T-cell phenotype and thus drives proliferation in aggressive T-cell neoplasia. Indeed, human T-ALL cell lines with highly activated STAT5, caused by STAT5B^{N642H} or IL-7R mutations, were sensitive to a specific STAT5 inhibitor, that decreases proliferation of these cells, whereas cell lines with low STAT5 activation respond less. In summary, hyperactive STAT5B disrupts thymic development, pushing T-cells into the CD8^{+} cytotoxic lineage and driving cytokine releasing and responsive factors. A RAG2$^{-/-}$ background demonstrated the mutation's potency to overcome DN stage arrest and drive immature T-cell neoplasia, which can be explained by a partially restored TCR activation signature. STAT5B is a clear target in many T-cell neoplasias and a beast in patients carrying the STAT5B^{N642H} driver mutation.
Disclosure of Interest: None Declared

O09

MECHANISMS OF IMMUNE MODULATION IN THE TUMOR MICROENVIRONMENT

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Abstract Content: The ability to modulate the immune system forms the basis of immunotherapy, which promises new interventions for myriad clinical conditions including cancer. We focus on the modulation of the innate immune system, which constitutes the first line of defense to keep infection at bay while mobilizing the adaptive immune response. Dendritic cells (DCs) and macrophages are key innate immune cell types that secret a cytokine milieu to establish a favorable immune environment for the recruitment and activation of other immune cells. Within the tumor microenvironment (TME) of various cancers, however, DCs and macrophages have been found to exert immunosuppressive effects. How the TME facilitates tolerance or immune evasion has not been fully defined. We dissected the specific mechanisms by which the hepatocellular carcinoma TME modulates DC activity. We also investigated the role of tumor-associated macrophages in promoting metastasis in breast cancer. Mechanistic insights from these studies will inform the design of novel tumor vaccine strategies. We are currently developing a vaccine platform that utilizes extracellular vesicles to load tumor antigens into DCs, which are then delivered to patients transdermally by microneedle patches. Successful implementation of this strategy will allow efficient delivery of DC vaccines that can overcome the immunosuppressive TME to orchestrate an effective anti-tumor immune response.
Disclosure of Interest: None Declared

Symposium 5: Innate immunity

O10

TYPE I AND III INTERFERONS DISRUPT LUNG EPITHELIAL REPAIR DURING RECOVERY FROM VIRAL INFECTION.

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Abstract Content: Excessive cytokine signaling frequently exacerbates lung tissue damage during respiratory viral infection. Disease severity is linked to lung epithelial destruction, due to both cytopathic viral effects and immune-mediated damage. Type I (IFN-α/β) and III (IFN-λ) interferons are host-produced antiviral cytokines. Due to widespread expression of the type I IFN receptor (IFNAR) in immune cells, IFN-α/β responses can result in immunopathology during viral infections. The IFN-λ receptor (IFNLR) is mainly expressed at epithelial barriers, and responses are therefore often characterized by their ability to confer localized antiviral protection at the site of infection, without driving damaging proinflammatory responses associated with IFN-α/β.
In our study, we show that IFN signaling interferes with lung repair during influenza recovery (*1*). Treatment with exogenous type I or III IFN late during infection reduces lung epithelial proliferation. Using IFNAR and IFNLR deficient mice, we show host-produced IFN-λ predominantly drives lung pathology. Additionally, comparison of IFN subtypes at equal biological potency in primary airway epithelial cell cultures revealed IFN-λ most potently induced antiproliferative pathways.
By studying specific effects in the respiratory epithelium, we identify a mechanism by which IFN exacerbates respiratory

virus disease, independent of immunomodulation. IFN-induced p53 directly reduces lung epithelial proliferation and differentiation during recovery from influenza virus infection, therefore increasing disease severity, and susceptibility to bacterial superinfections. Thus, excessive or prolonged IFN-production aggravates viral infection by impairing lung epithelial regeneration. Our data indicate the need for effective regulation of host IFN responses, and the importance of timing and duration when considering IFNs as therapeutic strategies to treat respiratory virus infections, including influenza and 2019 coronavirus disease (COVID-19).

(*1*) J. Major *et al*., Type I and III interferons disrupt lung epithelial repair during recovery from viral infection. *Science* 10.1126/science.abc2061 (2020)
Disclosure of Interest: None Declared

O11
REACTIVE OXYGEN SPECIES OXIDIZE STING AND SUPPRESS INTERFERON PRODUCTION
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Abstract Content: Reactive oxygen species are by-products of cellular respiration that can promote oxidative stress and damage cellular proteins and lipids. One canonical role of ROS is to defend the cell against invading bacterial and viral pathogens. Curiously, some viruses, including herpesviruses, thrive despite the induction of reactive oxygen species, suggesting that reactive oxygen species are beneficial for the virus. However, the underlying mechanisms remain unclear. Here, we found that reactive oxygen species impaired interferon response during herpesvirus infection and the inhibition occurred downstream of cytoplasmic DNA sensing. We further demonstrated that reactive oxygen species suppressed the type I interferon response by oxidizing Cysteine 147 on murine stimulator of interferon genes (STING), an ER-associated protein that mediates interferon response after cytoplasmic DNA sensing. This inhibited STING polymerization and activation of downstream signaling events. These data indicate that redox regulation of Cysteine 147 of mouse STING, which is equivalent to Cysteine 148 of human STING, controls interferon production. Together, our findings reveal that reactive oxygen species orchestrate anti-viral immune responses, which can be exploited by viruses to evade cellular defenses.
Disclosure of Interest: None Declared

Symposium 6: Cytokine gene regulation and non-coding RNA

O12
RNA M6A METHYLATION GUIDES IL-17-DRIVEN AUTOIMMUNITY THROUGH THE RNA-BINDING PROTEIN IMP2
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Abstract Content: IL-17 is the founding member of a unique and poorly understood cytokine subclass. Dysregulated activity of IL-17 and Th17 cells underlies many autoimmune conditions, but the molecular mechanisms by which IL-17 mediates pathogenic inflammation remain poorly understood. IL-17 regulates pathogenic inflammatory genes by two key transcription factor classes, NF-KB and CCAAT/Enhancer Binding (C/EBP) proteins, specifically C/EBPδ and C/EBPβ. Compared to activation of NF-KB, surprisingly little is known about mechanisms that activate C/EBPs, either by IL-17 or indeed most other inflammatory stimuli. In seeking to understand how IL-17 upregulates C/EBPs, we found that IL-17 signaling enhanced *Cebpd* mRNA stability, concomitant with increased levels of C/EBPδ translation. In contrast, IL-17 had only a marginal inductive effect on *Cebpb* mRNA, yet C/EBPβ protein was nonetheless strongly upregulated. Examination of *Cebpb* and *Cebpd* noncoding sequences identified consensus sites for N6-methyladenosine (m^6A) modification, an epitranscriptomic mark that influences mRNA fate. The m^6A pathway is still very understudied in the immune system, so to determine if this pathway was indeed involved in IL-17 signaling, we knocked down the m^6A 'writer' METTL3 and the 'eraser' demethylase FTO. Indeed, METTL3 enhanced C/EBP expression, which was reversed by FTO. Moreover, we found that loss of an unusual m^6A 'reader' IGF2BP2 (IMP2), an RNA binding protein known to control mRNA stability and translation, impaired IL-17 induction of C/EBPs and consequently C/EBP-dependent genes. IMP2 bound directly to *Cebpb* and *Cebpd* transcripts, leading to enhanced *Cebpd* half-life and enhanced translation of both C/EBPδ and C/EBPβ. Mutation of putative IMP2 binding sites within the *Cebpd* 3' UTR impaired IMP2 binding. Transcriptomic analysis revealed that IMP2 regulates C/EBP-dependent genes, including IL-6 and Lcn2 (Lipocalin 2). Consistent with this, *Imp2*$^{-/-}$ mice were refractory to experimental autoimmune encephalomyelitis (EAE, driven by IL-6) and autoantibody-induced glomerulonephritis (AGN, driven by Lipocalin 2). Thus, IL-17-induced autoimmunity is mediated through m^6A-dependent post-transcriptional regulation of C/EBP transcription factors, illustrating cross-talk between transcriptional and post-transcriptional programs driven by IL-17. This is one of the few studies demonstrating that the m^6A pathways is operative in cytokine signaling, and the first to show that this pathway regulates C/EPBs and autoimmune pathogenesis.

Disclosure of Interest: None Declared

O13

HOST CXCL10 IS REPRESSED BY THE MALARIA PARASITE AND SERVES AS A DECISION MAKING FACILITATOR FOR PARASITIC GROWTH AND SEXUAL DEVELOPMENT

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Abstract Content: The pathogenesis of infectious diseases is dominated by dynamic interactions between pathogen and host, as in the devastating parasitic disease malaria caused by *Plasmodium falciparum*.These interactions rely on a network of secreted proteins, including chemokine CXCL10, which is found at high levels in cerebral malaria patients, but is reduced in non-complicated patients. We showed that *Plasmodium falciparum* actively inhibits the synthesis of CXCL10 in monocytes using a sophisticated mechanism involving blocking the association of the immune host's ribosomes to the CXCL10 RNA transcript. The underlying inhibition cascade involves delivery of RNA cargo via *Plasmodium falciparum* derived EVs into host monocytes. The EV-derived RNA triggers host RIG-I, which then leads to the binding of HUR1 to an AU-rich domain in the 3'UTR of the *CXCL10* transcript. Remarkably, in response to the presence of CXCL10, the parasite starts accelerating growth and stimulating its sexual development. Thus, we identified a novel decision-sensing mechanism the deadly malaria parasite *Plasmodium falciparum* employs during the most severe form of malaria infection, cerebral malaria. We reveal a 'decision sensing system': high CXCL10 expression serves as an 'alert facilitator', prompting *Plasmodium falciparum* to shift tactics and initiate escape actions.
Disclosure of Interest: None Declared

Symposium 7: Inflammation and barrier integrity

O14

THE PROSTAGLANDIN D2 RECEPTOR CRTH2 SUPPRESSES EPITHELIAL RESPONSES DURING INTESTINAL HELMINTH INFECTION

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Abstract Content: Type 2 inflammation that is required for tissue repair and expulsion of helminth parasites must be regulated to prevent chronic inflammation. While previous studies show that cytokines play key roles in promoting and resolving Type 2 inflammation, the role of bioactive lipid mediators such as prostaglandin D_2 in regulating these processes is less clear. Here we show that PGD_2 is produced during helminth induced type 2 inflammation and that PGD_2 and its receptor CRTH2, known for their pro-inflammatory role during chronic Type 2 inflammation in the lung, have a previously unappreciated anti-inflammatory role during helminth-induced Type 2 intestinal inflammation. CRTH2 deficient mice infected with *Nippostrongylus brasiliensis* cleared their worms more efficiently and had increased intestinal mucin responses compared to wild type mice. Single cell RNA sequencing revealed an enrichment for secretory lineage epithelial cells in the small intestine of CRTH2 deficient compared to wild type mice. Critically, small intestinal epithelial cells and specifically goblet and tuft cells expressed the gene that encodes for CRTH2, *Gpr44*, and murine small intestinal organoids stimulated with Type 2 cytokines downregulated expression of goblet cell- and tuft cell-associated genes following culture with PGD_2. Finally, bone marrow chimeric mice that had CRTH2 deficiency isolated to the non-hematopoietic system cleared worms more efficiently and had elevated goblet cell responses compared to mice with a wild type non-hematopoietic compartment, confirming a role for CRTH2 in regulating epithelial responses and resulting worm clearance *in vivo*. These findings highlight a novel regulatory effect of the PGD_2-CRTH2 pathway on helminth-induced Type 2 intestinal inflammation and may inform the development and use of therapies for treatment of Type 2 inflammatory diseases.
Disclosure of Interest: None Declared

O15

CYTOKINES AND SKIN BARRIER INTEGRITY

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Abstract Content: When it comes to the skin functioning as a protective coat, the two essential components have traditionally been considered as the epidermal keratinocytes producing a physical barrier, complemented by neuro-immunocytic cells generating innate and adaptive immune reactions to maintain homeostasis in an ever-changing ecosystem. As skin is our outermost organ, its accessibility to biopsies and visual assessments of clinical changes has made it an ideal target for translational studies and precision medicine yielding important lessons and insights into many inflammatory reactions beyond the integument. Almost 30 years ago, the myth that keratinocytes were only passive targets of immune responses, was dispelled by evidence indicating these cells were active producers of cytokines, as well as adhesion molecules capable of initiating cutaneous inflammation (Lancet 337:211, 1991). Indeed, we now recognize that barrier perturbation in skin activates cytokine production, as well as the reciprocal ability of cytokines to alter barrier function (both tight junctions and filaggrin levels). It also has now evident that both bacterial and fungal commensal microbials contribute to cutaneous immunobiology across the epidermal surface. Greater insights into the immunopathogenesis of two common chronic inflammatory skin diseases characterized by altered barrier function-psoriasis and atopic dermatitis have revealed complex, highly choreographed interactions between cytokines and resident skin cells leading to

significant therapeutic progress for these immune-mediated disorders. In this presentation we will review the importance of cytokines contributing to skin barrier integrity, and roles for commensal organisms and dysregulated cytokine networks perturbing barrier function and contributing to chronic skin inflammation. Clearly there has been meaningful progress in our appreciation of the role for cytokines and interferons in skin health and disease far beyond the initial discovery that recombinant IFNg could induce MHC class II HLA-DR expression by human epidermal keratinocytes in 1984 (J Invest Dermatol 83:84, 1984).

Disclosure of Interest: B. Nickoloff Employee of: Eli Lilly & Co.

O16

THYMIC STROMAL LYMPHOPOIETIN (TSLP) PRIMARILY DRIVES TYPE 2 INFLAMMATION THROUGH IL-4RA-DEPENDENT SIGNALING

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Abstract Content: Dupilumab, a fully human monoclonal antibody that binds IL-4Ra and inhibits signaling of both IL-4 and IL-13, is approved for the treatment of multiple Type 2 (T2) inflammatory diseases, including asthma, atopic dermatitis (AD) and chronic sinusitis with nasal polyps, and has shown efficacy in eosinophilic esophagitis. Recently studies with tezepelumab, a TSLP-neutralizing antibody, have also demonstrated positive effects in both eosinophilic and non-eosinophilic asthma, but interestingly not in AD. Given these observations, these studies highlight the need for a mechanistic comparison of these two pathways on T2 inflammation to better understand the relative contribution of each pathway to T2 immunity.

Here, we employ cytokine overexpression studies (intranasal cytokine administration and hydrodynamic DNA delivery) and multiple preclinical models of lung inflammation to evaluate the functional contributions of the TSLP and the IL-4/IL-13 signaling pathways to T2 airway inflammation. We demonstrate that induction of T2 immunity by TSLP requires IL-4Ra signaling. Several features of T2 airway inflammation induced by TSLP overexpression, including goblet cell metaplasia (GCM), lung tissue eosinophilia and serum IgE are abrogated in *Il4ra* KO mice. TSLP overexpression also dramatically alters the lung myeloid landscape, by inducing changes in alveolar macrophages and dendritic cell subsets consistent with coordinating a T2 immune response, and requires intact IL-4Ra signaling to mediate these changes. Conversely, IL-4 or IL-13 overexpression promotes several of these T2 hallmarks independently of TSLP signaling. Next, using antibody-mediated pathway blockade in two distinct models of airway inflammation (the experimental ova-alum model and the clinically relevant house dust mite model), we show that blunting IL-4Ra signaling is significantly more effective than blocking TSLP at ameliorating key measures of T2 inflammation, including GCM, lung tissue eosinophilia and associated chemokines. Thus, collectively across numerous endpoints in multiple models, we demonstrate that TSLP promotes T2 pulmonary inflammation indirectly via IL-4Ra signaling, but that IL-4Ra signaling can drive T2 inflammation in the lung independently of TSLP. Consequently, these data re-iterate that IL4Ra signaling is the central cytokine node in promoting and maintaining T2 inflammation.

Research sponsored by Regeneron Pharmaceuticals, Inc.
Disclosure of Interest: None Declared

Symposium 8: Regulators of interferons - Philip I. Marcus Symposium

O17

THE UNIQUE TYPE-I INTERFERON-EPSILON (IFNE) CONSTITUTIVELY PROTECTS THE FEMALE REPRODUCTIVE TRACT FROM ZIKA VIRUS INFECTION.

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Abstract Content: Zika virus (ZIKV) can be transmitted sexually with infection of the female reproductive tract (FRT) and the developing foetus *in utero*. Like many RNA viruses ZIKV abrogates type-I and type-III IFN antiviral responses. This immune evasion is mediated by the actions of multiple viral non-structural (NS) proteins. However, the unique type-I IFN-epsilon (IFNε), is constitutively expressed by the mucosal epithelium of the FRT. Therefore, we hypothesised IFNε circumvents ZIKV mediated IFN evasion by constitutively protecting the FRT from infection.

To explore the role of IFNε *in vivo* we intravaginally inoculated IFNε KO (IFNε $^{-/-}$), type-I IFN receptor KO (IFNAR1$^{-/-}$) and wildtype (WT) mice with ZIKV (PRVABC59). IFNε$^{-/-}$ mice had increased viral burden in the uterus and ovary (qRT-PCR) and greater levels of ZIKV in vaginal washes when compared to WT mice (plaque assay). Next, we intravaginally administered 4 µg of recombinant IFNε to IFNε$^{-/-}$ mice that resulted in the restoration of antiviral activity in the FRT compared to buffer treated controls. Furthermore, application of 100 µg IFNε neutralising antibody to WT mice increased their susceptibility to infection compared to isotype controls.

Next, we demonstrated that in primary transformed FRT cell lines IFNε induced the expression of hundreds of interferon-stimulated genes similar to the antiviral profile induced by IFNλ-III, not IFNα as expected being a type-I IFN (NextSeq550 V2.5, qRT-PCR). Interestingly, IFNε did not induce expression of IRF1 and displayed limited induction of pro-inflammatory cytokines compared to IFNα and IFNλ-III. Pre-treatment with IFNε in FRT cell lines inhibited ZIKV replication like IFNα and IFNλ-III (qRT-PCR, plaque assay). However, post-infection these antiviral responses were blocked via NS5 mediated STAT2 degradation. Conversely,

the expression of IFNε was impervious to ZIKV NS4A, NS1 and NS5 mediated inhibition downstream of activated RIG-I that delayed the production of other type-I and III IFNs (qRT-PCR). Furthermore, we demonstrated by immunoblot that IFNε signals constitutively despite IFN induced receptor desensitisation that limits IFNα mediated signal transduction.

Collectively these findings highlight the unique biological role of constitutively expressed IFNε in prevention of ZIKV infections in the FRT. Importantly, this shifts our understanding of FRT innate immunity from reliance on reactionary responses to pre-emptive protection against viral infections. Combined with continued studies of IFNε expression in humans, this knowledge will help to inform sexual health recommendations for ZIKV and other infections of the FRT.

Disclosure of Interest: None Declared

O18

MATERNAL INTERFERON LAMBDA SIGNALING LIMITS TRANSPLACENTAL TRANSMISSION AND MEDIATES FETAL PATHOLOGY IN A GESTATIONAL STAGE-DEPENDENT MANNER DURING CONGENITAL ZIKA VIRUS INFECTION IN MICE

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Abstract Content: Interferons have a key role in the development of the placenta and surrounding tissues throughout pregnancy, and are regulated carefully to ensure fetal development as well as protection from maternal pathogens. Unlike most viruses that cause congenital infections in humans, Zika virus (ZIKV) pathogenesis can be modeled in mice. Mouse models of congenital ZIKV infection provide a genetically tractable tool to distinguish maternal and fetal immune responses to viral infection, and their distinct impacts on fetal outcomes. IFN-λ is secreted constitutively from human placental trophoblasts, and limits ZIKV transplacental transmission in mouse congenital infection models, but it has yet to be determined if IFN-λ is secreted constitutively from the mouse placenta and what cell types respond to IFN-λ to restrict ZIKV congenital infection.

To determine the patterns of IFN-λ secretion from the mouse placenta, we evaluated IFN-λ activity in infected and uninfected dams at varied time points in gestation. In uninfected dams, IFN-λ activity was present in placentas from laboring dams, but was undetectable in mid-gestation placentas. However mid-gestation placentas from ZIKV-infected dams had the highest levels of IFN-λ, indicating that it is secreted in the mouse placenta under inflammatory conditions such as infection or labor, but not at baseline mid-gestation.

To define the tissues that respond to IFN-λ at the maternal-fetal interface, we assessed ZIKV transplacental transmission in mid-gestation (E9-E15) mouse pregnancies in which either fetal or maternal tissues lacked the IFN-λ receptor *(Ifnlr1$^{-/-}$)*. Unexpectedly, the antiviral effects of IFN-λ resulted exclusively from signaling in maternal tissues, as fetal viral loads depended only on maternal Ifnlr1 genotype, not the fetal/placental genotype. Furthermore, we observed that there was no IFN-λ antiviral effect early in gestation (infection at E7 compared to E9), indicating that IFN-λ antiviral activity is dependent on gestational stage. Using mice also lacking the type I IFN receptor (*Ifnlr1$^{-/-}$ Ifnar1$^{-/-}$*), we studied the effect of IFN-λ signaling in pregnancies with enhanced ZIKV replication and again found an antiviral effect of maternal IFN-λ signaling. Interestingly, early-gestation pregnancies (infection at E7) with maternal IFN-λ signaling also exhibited enhanced fetal pathology, suggesting that IFN-λ signaling can contribute to fetal pathology in high-viremia models. Together these data show that IFN-λ signals to maternal tissues and has discrete functions at different gestational stages.

Disclosure of Interest: None Declared

Symposium 9: Host response to SARS-CoV-2

O19

LY6E IMPAIRS CORONAVIRUS FUSION AND CONFERS IMMUNE CONTROL OF VIRAL DISEASE

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Abstract Content: Zoonotic coronaviruses (CoVs) are significant threats to global health, as exemplified by the emergence of two severe acute respiratory syndrome CoVs (SARS-CoV and SARS-CoV-2) and Middle East respiratory syndrome CoV (MERS-CoV) within two decades. Host immune responses to CoVs are complex and regulated in part through antiviral interferons. However, interferon-stimulated gene products that inhibit CoVs are not well characterized. Here, we show that lymphocyte antigen 6 complex, locus E (LY6E) potently restricts infection by multiple CoVs, including SARS-CoV, SARS-CoV-2, and MERS-CoV. Mechanistic studies revealed that LY6E inhibits CoV entry into cells by interfering with spike protein-mediated membrane fusion. Importantly, mice lacking Ly6e in hematopoietic cells were highly susceptible to a murine CoV, mouse hepatitis virus. Exacerbated viral pathogenesis in Ly6e knockout mice was accompanied by loss of hepatic immune cells, higher splenic viral burden, and reduction in global antiviral gene pathways. Accordingly, we found that constitutive Ly6e directly protects primary B cells from murine CoV infection. Our results demonstrate that LY6E is a critical antiviral immune effector that controls CoV infection and pathogenesis. These findings advance our understanding of immune-mediated control of CoV *in vitro* and *in vivo*, knowledge that could help inform strategies to combat infection by emerging CoVs.

Disclosure of Interest: None Declared

Symposium 10: Cytokines in neurobiology

O20

THE ROAD TO EPITHELIAL BARRIER HEALTH: TARGETING THE IL-36R PATHWAY

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Abstract Content: The Road to Epithelial Barrier Health: Targeting the IL-36R Pathway

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Increasing evidence points to an important role for the interleukin (IL)-36 pathway in mediating a variety of inflammatory responses. The three IL-36 agonist cytokines (IL-36a, IL-36b and IL36g) are expressed by epithelial and immune cells and mediate signal transduction on epithelial cells, immune cells and fibroblasts by binding to a common receptor composed of IL-36R and IL1RAcP, in a manner analogous to other IL-1/R family members. IL-36Ra is a naturally occurring antagonist that binds to IL-36R but is unable to recruit IL1RAcP and induce downstream signaling. Hyperactivation of the IL-36 pathway due to the absence of IL36Ra in generalized pustular psoriasis (GPP) suggests its fundamental role in the pathogenesis of pustular skin diseases.

In this presentation, we will describe novel IL-36 pathway biology suggesting key regulatory roles in a) the loss of epithelial barrier integrity in the inflamed skin and intestine, and b) driving intestinal aberrant tissue remodeling associated with Crohn's disease and ulcerative colitis. Furthermore, we will discuss the rapid and sustained clinical improvement achieved by selective IL-36R blockade with spesolimab in GPP patients presenting with an acute moderate to severe disease flare. Evidence for specific cellular and molecular changes associated with treatment response will be described.

Based on accumulating preclinical and clinical data, spesolimab combines potent anti-inflammatory and anti-fibrotic effects, demonstrates differentiation from standard of care treatments, and may offer significant therapeutic benefits in a range of inflammatory and fibrotic disorders.

Disclosure of Interest: None Declared

O21

CD28 AND B7 RECEPTOR HOMODIMER INTERFACES CONTROL FORMATION OF THE B7/CD28 COSTIMULATORY AXIS AND LETHAL PRO-INFLAMMATORY SIGNALING

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Abstract Content: The inflammatory response is indispensable for protective immunity, yet pathogens, including coronavirus, often elicit a vastly excessive response harmful to the host. Full T-cell activation requires interaction between costimulatory receptors B7 and CD28. In the extracellular domains of the CD28 as well as the B7 receptors, the self-adhesive homodimer interfaces are remote from the coligand binding sites. Yet, our finding is that short mimetic peptides derived from distinct regions within the composite CD28 homodimer interface attenuate B7-2(CD86)/CD28 engagement as well as the tighter B7-1(CD80)/CD28 interaction, and thereby attenuate signaling through the human B7/CD28 costimulatory axis, to dampen induction of inflammatory cytokines. Conversely, short peptide mimetics derived from distinct regions within either the composite B7-1 or B7-2 dimer interfaces selectively attenuate engagement of CD28 by the cognate B7 coreceptor, thereby attenuating B7/CD28 signaling and dampening the induction of inflammatory cytokines. Even when administered at exceedingly low concentrations, such peptides are capable of protecting mice from lethal bacterial and viral infections. Our results reveal that the CD28 and B7 homodimer interfaces each control formation of the B7/CD28 costimulatory axis and highlight the protective potential against severe infections of attenuating pro-inflammatory signaling through these receptor domains. Our approach, development of host-oriented therapeutics that target the human inflammatory response to control and prevent the cytokine storm, is unlikely to be circumvented by pathogen mutations.

Disclosure of Interest: None Declared

Symposium 11: Cytokines in neuroimmunology

O22

INTERFERON SIGNALLING IN ENDOTHELIAL CELLS MEDIATES PATHOGENESIS IN CEREBRAL TYPE I INTERFERONOPATHIES

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Abstract Content: Cerebral type I interferonopathies are a group of diseases that stem from chronic and elevated type I interferon (IFN-I) signalling, which is usually associated with antiviral and immunoregulatory functions. These diseases include genetic disorders like Aicardi-Goutières syndrome, autoimmune diseases such as CNS-lupus and chronic and congenital viral infections. IFN-Is induce pathology, but the contribution of different cell types is not well understood. A hallmark of these diseases centres around abnormal blood vessels in the brain. We thus hypothesised that endothelial cells play a critical role in mediating interferonopathic brain disease. To address our hypothesis, we used transgenic mice with CNS-targeted chronic production of IFN-α, termed GIFN mice. GIFN mice are the only mouse model that recapitulates key clinical symptoms and pathologies of patients with cerebral type I interferonopathies including a pronounced vasculopathy with aneurysms, vessel stenoses and perivascular calcification. We analysed the transcriptome of single cells from the CNS of GIFN mice to explore endothelial cell responsiveness to IFN-I *in vivo*. We also genetically ablated IFN-I signalling in endothelial cells in GIFN mice (termed GIT mice) to gauge the contribution of endothelial cells to disease. In the CNS, endothelial cells had the highest expression of the IFN-I receptor genes and in response to IFN-I showed a major change in their transcriptome that reflected disease-relevant processes including endothelial dysfunction. Loss of IFN-I in endothelial cells in the presence of elevated IFN-α in GIT mice resulted in a significant improvement of many disease features. This included reduced mortality and motor impairment, the absence of calcification, aneurysms and neurodegeneration and minimal leukocyte infiltration and glial cell reactivity. Importantly, vascular morphology and blood brain barrier integrity was rescued. These findings demonstrate that vasculopathy is a major contributing factor to cerebral type I interferonopathies. Moreover, our findings indicate that targeting the blood vessels offer a highly promising and novel approach to treat patients as in contrast to the brain parenchyma the brain's blood vessels are more easily accessible for therapeutic interventions.
Disclosure of Interest: None Declared

O23
ZIKA VIRUS INFECTION IS ASSOCIATED WITH ALTERATIONS IN MYELINATION DURING PRENATAL DEVELOPMENT
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Abstract Content: Zika virus (ZIKV) infection during pregnancy causes congenital Zika syndrome (CZS) characterized by fetal abnormalities at birth and neurological defects that manifest into early childhood. At present, there is no approved vaccine or therapeutic for clinical use to prevent ZIKV vertical transmission, making ZIKV an enduring worldwide public health concern for pregnant women. Nonhuman primate (NHP) models of ZIKV infection in pregnancy have demonstrated vertical transmission and fetal neuropathology; however, the impact of ZIKV infection on fetal brain development is underexplored. We performed a comprehensive characterization of NHP fetal brain following ZIKV exposure *in utero*. Healthy pregnant pigtail macaques (PTM) received subcutaneous inoculations of ZIKV (*n*=5) or media (*n*=3) in the forearms between 60-119 days gestation (first-second trimesters). At birth, the PTM fetal brain was serially sectioned and samples collected for histologic, transmission electron microscopy (TEM) and RNA-seq analyses. In ZIKV-infected fetal brains, quantitative IHC analysis revealed decreased immunoreactivity of myelin basic protein (MBP) compared to control brains. MBP alterations were not associated with significant changes in the percent positive staining for Olig2+ nuclei in the white matter. Decreased MBP protein was also observed in NHP fetal brainstem by Western blot analysis and confirmed using a human oligodendrocytic M03.13 cell culture model. H&E analysis of NHP fetal brain found no obvious signs of immune cell infiltration or inflammation in post-ZIKV fetal brain, while EM analysis indicated enhanced multifocal decompaction to myelin sheaths. Differentially expressed (DE) genes related to myelination were upregulated in most post-ZIKV fetal brains and associated with gliogenesis genes in the recovery of ZIKV infection. Our data sets and analyses define fetal neurological profiles linked with ZIKV-induced brain injury patterns. We hypothesize loss of MBP leads to instability of myelin sheaths. ZIKV may directly target MBP for degradation through an NS5-mediated mechanism that is similar to ZIKV interference of type I interferon signaling. The impact of ZIKV infection on the initiation of myelination during prenatal development may have long term consequences into early childhood neurodevelopment.
Disclosure of Interest: None Declared

Symposium 12: Immune cell fate and function

O24
STRUCTURAL CELLS ARE KEY REGULATORS OF ORGAN-SPECIFIC IMMUNE RESPONSES
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Abstract Content:
BACKGROUND: The mammalian immune system implements a remarkably effective set of mechanisms for fighting pathogens. Its main components are haematopoietic immune cells, including myeloid cells that control innate immunity and lymphoid cells that constitute

adaptive immunity. However, immune functions are not unique to haematopoietic cells, and many other cell types display basic mechanisms of pathogen defence.
METHODS: To advance our understanding of immunology outside the haematopoietic system, we systematically investigate the regulation of immune genes in the three major types of structural cells: epithelium, endothelium and fibroblasts. We performed multi-omics profiling of these cell types across twelve mouse organs - brain, caecum, heart, kidney, large intestine, liver, lung, lymph node, skin, small intestine, spleen and thymus. Single-cell suspensions were analysed by flow cytometry, and sort-purified cell populations were profiled with three genome-wide assays: (i) gene expression profiling by low-input RNA-seq; (ii) chromatin accessibility profiling by ATAC-seq; and (iii) epigenome profiling by ChIPmentation with an antibody against promoter/enhancer-linked histone H3K4me2.
RESULTS: We observed widespread expression of immune regulators and cytokine/chemokine signalling molecules in structural cells, organ-specific adaptation to the tissue environment, and unexpectedly diverse capabilities for interacting with hematopoietic cells. These cell-type-specific and organ-specific differences in immune gene activity were reflected by characteristic patterns of chromatin regulation. Most notably, we found evidence of an epigenetically encoded immune potential under homeostatic conditions, and the affected genes were preferentially upregulated in response to an immunological challenge induced by systemic viral infection. We validated and functionally dissected this epigenetic potential of structural cells by further in vivo experiments with recombinant cytokines.
CONCLUSION: Our study highlights the prevalence and organ-specific complexity of immune gene activity in non-haematopoietic structural cells, and it provides a high-resolution, multi-omics atlas of the epigenetic and transcriptional networks that regulate structural cells in the mouse.
Disclosure of Interest: None Declared

O25

ARGINASE 2 IS ESSENTIAL FOR IL-10 METABOLIC REPROGRAMMING OF INFLAMMATORY MACROPHAGES AT THE MITOCHONDRIA

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Abstract Content: Recent evidence highlights the importance of mitochondrial bioenergetics and dynamics in regulating macrophage polarisation. We demonstrate that Arginase-2 (Arg2), the mitochondrial isoform of arginase, is an interleukin-10 (IL-10) regulated gene in inflammatory macrophages. Using approaches such as siRNA-induced knockdown, overexpression plasmids, site-directed mutagenesis, and genetic knockout mice in metabolic flux assays, we aimed to understand the impact of Arg2 on IL-10's role as a metabolic rheostat in macrophages. We demonstrate that IL-10 specifically enhances Arg2 localization at the mitochondria in inflammatory macrophages, where it radically promotes a state of 'fusion', an effect that is dependent on both Arg2 catalytic activity and its physical presence at the mitochondria. We demonstrate that Arg2 is critical for IL-10 induced oxidative respiration and ATP production in these cells. Mechanistically, we illustrate that Arg2 distinctly influences complex II (also known as succinate dehydrogenase (SDH)), a bi-functional enzyme that links the mitochondrial electron transport chain (ETC) and the Tricarboxylic acid (TCA) cycle. Via its regulation of SDH, Arg2 is essential for IL-10 mediated downregulation of inflammatory mediators mitochondrial reactive oxygen species (mtROS) and interleukin 1β (IL-1β). Moreover, in an acute model of inflammation when IL-10 expression is compromised, Arg2 expression is suppressed, suggesting that Arg2 may be critical for IL-10 mediated protection and homeostasis. Altogether, these findings demonstrate a novel IL-10 polarization model where macrophages leverage their metabolic enzymes as a mechanism to maintain an anti-inflammatory state.
Disclosure of Interest: None Declared

O26

THE IL-10-STAT3 AXIS IS A CRITICALLY IMPORTANT LUNG-SPECIFIC PATHWAY DRIVING BLIMP-1 MEDIATED TH2 CELL DIFFERENTIATION TO PROMOTE ALLERGIC ASTHMA

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Abstract Content: Allergic asthma is a chronic inflammatory disease of the lung that is growing worldwide and has limited new therapeutic options. Lung inflammation is mediated by T cells responding to allergens that secrete type 2 cytokines such as IL-4, IL-5 and IL-13 that drive airway hyperresponsiveness, increased mucus, and recruit inflammatory cells such as eosinophils to the lung tissue causing widespread damage. Th2 cell differentiation classically requires signaling via the IL-4/STAT6 pathway, which activates the master transcription factor GATA3. However, evidence of IL-4-independent Th2 cell differentiation has demonstrated roles of IL-2/STAT5 and STAT3 pathways. Blimp-1 is a transcriptional repressor pleiotropically expressed by most effector T cells that constrains T cell-mediated autoimmunity. Using a house dust mite induced murine model of allergic asthma, we have revealed that Blimp-1 is required for Th2 cell development

in the lung to inhaled allergens. T cell specific deficiency of Blimp-1 intrinsically led to a significant decrease of Th2 cells in the lung and a subsequent reduction in lung inflammation and pulmonary function. In contrast, IgE and T_{FH} cells were intact, suggesting Blimp-1 molecularly dissects Th2 and T_{FH} differentiation to allergens. We found STAT3 was required to drive Blimp-1 expression in CD4 T cells and for Th2 cell development in response to allergens. Importantly, we show that Blimp-1 is required for Th2 cells responding to inhaled but not skin-injected allergens, suggesting there are lung-specific pathways driving Th2 cells to allergens. Here, we sought to determine the cytokines responsible for expression of Blimp-1 through STAT3 to promote Th2 cells in allergen induced lung inflammation. We found than neither IL-6 nor IL-21 was responsible for Blimp-1 expression in Th2 cells in the lung. Counterintuitively, we found that the anti-inflammatory cytokine IL-10 was critical for Blimp-1 expression in Th2 cells and subsequent Th2 cell development in response to allergens. Mechanistically, we found that Blimp-1 acts through Bcl6, which is necessary to drive GATA3 expression and subsequent Th2 formation in the lung in response to inhaled allergens. These data have uncovered a previously unrecognized IL-10-STAT3-Blimp-1 axis that is critically important for lung-specific allergen-induced Th2 cell differentiation driving allergic asthma.
Disclosure of Interest: None Declared

O27

EXCESS IL-18 AND PERFORIN DEFICIENCY DISTINCTLY AND SYNERGISTICALLY PROMOTE PATHOLOGIC CD8 T-CELL ACTIVATION AND EXPERIMENTAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Abstract Content: CD8 T-cell activation is necessary for host defense, but overactivation can also drive life-threatening immunopathology as seen in Hemophagocytic lymphohistiocytosis (HLH). HLH is associated with genetic impairment of cytotoxic function (e.g. perforin deficiency), and $Prf1^{-/-}$ mice succumb to typically-mild LCMV infection via CD8 T-cell/IFNγ-mediated immunopathology. Macrophage Activation Syndrome (MAS) is clinically similar to HLH, but occurs in certain rheumatic/autoinflammatory diseases and has been associated with extraordinarily and chronically elevated serum IL-18. Both HLH and MAS are life-threatening cytokine storm disorders, and an improved understanding of their pathogenesis could significantly alter patient screening, diagnosis, and management.
Using mice with excess IL-18 (Il18tg) to model the serum findings in MAS, we found a baseline increase in PD-1+ CD8 T-cells over WT littermates. Il18tg mice develop enhanced immunopathology upon repeated systemic challenge with a TLR9-agonist, whereas TLR9-induced immunopathology was comparable between $Prf1^{-/-}$ and WT mice. Additionally, Il18tg mice developed features of MAS-like immunopathology upon infection with LCMV (Armstrong), which was largely mediated through CD8 T cells and IFNγ. As with LCMV-infected Prf1-/- mice, CD8 T cells from LCMV-infected Il18tg mice showed increased activation and cytokine production, but did not exhibit cytotoxic impairment or persistent antigen presentation. They retained KLRG1+ terminal effector differentiation and their transcriptional program was more comparable to WT than $Prf1^{-/-}$ mice in their absence of an exhaustion signature.
Mounting evidence suggests heterozygous mutations in cytotoxicity-related genes like PRF1 may promote hyperinflammatory responses in MAS patients, who also have highly elevated IL-18 levels. Though neither excess IL-18 nor perforin-deficiency individually cause immunopathology without inflammatory challenge, we observed lethal spontaneous hyperinflammation in $Il18tg;Prf1^{-/-}$ mice. These mice showed expansion of a splenic PD-1+, TIGIT+, and Tim-3+ CD8 T-cell population, yet show increased IFNg production. Additionally, spontaneous immunopathology was partially abrogated by CD8 depletion or IFNg neutralization. Together, these data suggest that IL-18 and cytotoxicity can independently and synergistically drive pathologic CD8 T-cell activation and life-threatening immunopathology in HLH and MAS.
Disclosure of Interest: None Declared

Symposium 13: Cytokines in autoimmunity

O28

EARLY-ONSET AUTOINFLAMMATORY DISEASE CAUSED BY ETS FACTOR MUTATION IN HUMANS AND MICE

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Abstract Content: Unbiased disease gene discovery in monogenic inflammatory disorders illuminates molecules essential for controlling the destructive potential of the immune system directly in humans. Here, we describe three unrelated male patients with early-onset mucosal inflammation, oral ulcers, and fever caused by loss-of-function variants in the gene encoding the ELF4 ETS family transcription factor. The mutations disrupt transcriptional activity, and genetically engineered mice with patient-derived mutations similarly develop more inflammatory responses. Deficiency in *ELF4* results in elevated serum IL-12p40, IL-18, and CXCL1, hyperinflammatory myeloid cells, altered interferon-stimulated gene (ISG) induction, and exaggerated IL-17 responses from lymphocytes. Kinetic transcriptomic analysis revealed that mutant cells have relatively normal early responses (0-4 hrs) but fail to sustain expression of anti-inflammatory genes, including IL-1R antagonist (IL-1Ra), over time (16-24 hrs). Moreover, clinical treatment with recombinant IL-1Ra or neutralizing anti-IL-12p40 reversed features of disease. Thus, we identify a novel human autoinflammatory disease and define ELF4 as a disease-relevant transcription factor with specialized function in orchestrating anti-inflammatory gene programs.
Disclosure of Interest: None Declared

Symposium 14: Japanese Interferon Society symposium

O29

SYSTEMATIC DISSECTION OF CYTOKINE RESPONSES IN LYMPH NODES AT SINGLE-CELL RESOLUTION

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Abstract Content: Cytokines mediate a highly complex intercellular signaling network in health and diseases. While many individual cytokines have been studied in-depth, we do not have a systems-level view of cell-type-specific responses to each cytokine and do not yet understand how cytokines orchestrate cell-cell communication networks in a complex immune response. To address these two knowledge gaps in cytokine biology, we performed single-cell RNA sequencing to measure gene expression profiles of over 17 mouse lymph node cell populations including lymphocytes, myeloid cells, and stromal cells in response to over 80 cytokines *in vivo*. Using these data, we derived a compendium of cell-type-specific cytokine signatures, with the goal of using this compendium to systematically compare cytokine responses across cell types and to deconvolve complex immune responses. We first investigated how distinct cell types respond to the same cytokine, and found that while some pathways are activated across multiple cell types (e.g., anti-viral programs in response to interferons), many cytokines induced cell-type-specific gene expression signatures. Next, we performed a global analysis to compare across cytokines and found that cytokines induced both unique and shared biological processes. For example, we found that IL-2/7/12/15/27/33/36a, IFNa1/b/g/e/k, LIF, cardiotrophin-1, and neuropoietin each induced a cytolytic program in NK cells, but also cytokine-specific gene expression programs involved in antigen presentation, NF-kB activation, and mRNA splicing. Finally, we used our compendium of cytokine signatures to study the dynamic cytokine networks that become active in mouse lymph nodes after immunization with vaccine adjuvants. By deconvolving single-cell RNA-sequencing data from lymph nodes of immunized mice, we predict that more than 10 cytokines, including type I interferons and IL-15, act on and potentially activate cross-presenting dendritic cells after immunization with a STING ligand-based adjuvant. Our study provides a global view of cell-type-specific cytokine responses *in vivo*, providing new hypotheses for cytokine functions. Furthermore, the framework can be readily applied to other transcriptomic datasets for assessing the roles of cytokines and cell-cell communication networks in any immune response.

Disclosure of Interest: None Declared

O30

TRANSCRIPTOMIC RESPONSES TO CYCMV/SIV VACCINATION IN MAURITIAN CYNOMOLGUS MACAQUES

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Abstract Content: A pre-clinical HIV vaccine based on rhesus cytomegalovirus (RhCMV) vector, strain 68-1, directs protective immunity against SIV infection in the rhesus macaque (RM) model. This vaccine elicits a population of CD8+ T cells that are restricted by MHC-E presentation. As RMs show extensive MHC genetic diversity, we explored vaccine efficacy in Mauritian cynomolgus macaques (MCM), which exhibit limited MHC-E genetic diversity that is similar to humans. Vaccination with a MCM derived CMV vector (CyCMV) provided 50% protection from SIV infection, similar to protection levels in the RM model. To uncover the molecular mechanisms underlying protection following CyCMV vaccination, we performed longitudinal whole blood transcriptional profiling of protected and non-protected MCMs across 10 vaccination time points prior to challenge. Bioinformatic analyses and computational modeling revealed a robust transcriptional response to vaccination among the protected animals. This protection signature was largely driven by the induction of genes involved in Rig-I-Like Receptor Signaling, Death Receptor Signaling, and Interferon Signaling in protected relative to non-protected animals. To further evaluate this outcome, we compared the CyCMV transcriptomic protection signature to the RhCMV transcriptomic signature linked with 68-1 vaccine protection against SIV in RM. We found that nearly 400 genes are shared between the two signatures and that the shared genes are enriched for innate antiviral immunity pathways. Interestingly, the CyCMV protection signature also showed substantial overlap with the interleukin (IL)-15 signaling component of the RhCMV protection signature. Since IL-15 signaling is integral to SIV protection in RMs, we treated an independent cohort of MCMs with IL-15 and performed longitudinal whole blood transcriptional profiling to further interrogate the contribution of IL-15 to SIV vaccine protection. Our analyses identify many genes and specific functional pathways shared between the RM and MCM vaccine protection, each underscored by an IL-15 signature linked with vaccine protection. Thus, conservation of whole blood signatures of immune programming and protection link IL-15 response and innate immune programs with CMV vector-mediated SIV vaccine protection. Further, our studies reveal specific genes as blood biomarkers of vaccine protection that will inform human studies of CMV-based vaccine vectors for protection against HIV infection.

Disclosure of Interest: None Declared

Symposium 15: Late breaking talks in innate immunity, infection and cancer

O31

SARS-COV-2 ORF6 HIJACKS NUP98 TO BLOCK STAT NUCLEAR IMPORT AND ANTAGONIZE INTERFERON SIGNALING

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Abstract Content: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic that is a serious global health problem. Evasion of interferon-mediated antiviral signaling is a common defense strategy that pathogenic viruses use to replicate and propagate in their host. In this study, we show that SARS-CoV-2 is able to efficiently block STAT1 and STAT2 nuclear translocation in order to impair transcriptional induction of interferon (IFN) stimulated genes (ISGs). Our results demonstrate that the viral accessory protein Orf6 exerts this anti-IFN activity. We found that SARS-CoV-2 Orf6 localizes at the nuclear pore complex (NPC) and directly interacts with Nup98-Rae1 via its C-terminal domain to impair docking of cargo-receptor (karyopherin/importin) complex and disrupt nuclear import. In addition, we show that a methionine-to-arginine substitution at residue 58 impairs Orf6 binding to the Nup98-Rae1 complex and abolishes its IFN antagonistic function. All together our data unravel a new mechanism of viral antagonism in which a virus hijacks the Nup98-Rae1 complex to overcome the antiviral action of IFN.

Disclosure of Interest: None Declared

O31b

DIVERSE VIRAL PROTEASES ACTIVATE THE NLRP1 INFLAMMASOME

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Abstract Content: The NLRP1 inflammasome is a multiprotein complex that is a potent activator of inflammation. Mouse NLRP1B can be activated through proteolytic cleavage by the bacterial Lethal Toxin (LeTx) protease, resulting in degradation of the N-terminal domains of NLRP1B and liberation of the bioactive C-terminal domain, which includes the caspase activation and recruitment domain (CARD). However, a natural pathogen-derived effector that can activate human NLRP1 remains unknown. Here, we use evolution-guided approach to identify a sequence within human NLRP1 that mimics the consensus protease cleavage site of proteases from human enteroviruses. Proteolytic cleavage at this primate-specific sequence is necessary and sufficient to activate pro-inflammatory cytokine release from cells in which the NLRP1 inflammasome has been reconstituted. Moreover, we find that proteases from several other human picornaviruses cleave NLRP1 within a rapidly evolving region of the protein, leading to host-specific and virus-specific activation of the NLRP1 inflammasome. Our work demonstrates that NLRP1 acts as a “tripwire” to recognize the enzymatic function of a wide range of viral proteases, and suggests that host mimicry of viral polyprotein cleavage sites can be an effective evolutionary strategy to activate a robust inflammatory immune response in the ongoing arms races between hosts and their viruses.

Disclosure of Interest: None Declared

O32

DIETARY PALMITIC ACID INDUCES LPS TOLERANCE AND INCREASED SEPSIS-ASSOCIATED IMMUNOPARALYSIS AND MORTALITY

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Abstract Content: Sepsis is a deleterious immune response to infection that leads to organ failure and is the 11th leading cause of death worldwide. During sepsis, detection of microbial ligands by monocytes and macrophages leads to an early acute inflammatory immune response followed by a robust anti-inflammatory immune response. The early acute inflammatory response is orchestrated by activated circulating monocytes and tissue-resident macrophages that produce pro-inflammatory cytokines (ex: IL-6 and TNF). Inhibition of this inflammatory response is termed sepsis-associated “immunoparalysis” and is correlated with increased systemic IL-10 and higher mortality rates in septic patients. Our recent publication defined a role of Western Diet (WD), a diet high in SFA and sugar, in driving decreased activated circulating monocytes, increased immunoparalysis, and increased mortality during lipopolysaccharide (LPS)-induced sepsis in mice, compared to mice fed a Standard low-fat low-sugar Diet (SC). We found that this phenotype was independent of microbiome and diet-associated obesity, suggesting the nutritional components within the WD were regulating immunoparalysis. Here we use this same model of sepsis and find that a diet enriched only in SFAs and not sugar (Ketogenic Diet; KD), leads to the same induction of immunoparalysis and increased mortality, compared to mice SC-fed mice. These data suggest the enriched SFAs within the diet enhance immunoparalysis and worsen disease outcomes. Next, we wanted to identify specific dietary SFAs responsible for enhanced immunoparalysis and worsened disease outcome. It is well documented that circulating SFAs are dependent on the SFAs consumed in the diet;

thus, we applied liquid chromatography with tandem mass spectrometry to serum of SC- and KD-fed mice and found the SFA palmitic acid (PA) was significantly higher in the blood of KD-fed mice, compared to SC-fed mice. PA has been historically described as an inflammatory lipid and, like LPS, induces expression of pro-inflammatory cytokines in a TLR4-dependent manner in monocytes and macrophages. Importantly, prolonged exposure to LPS results in a decreased TLR4-dependent pro-inflammatory response in monocytes and macrophages, termed LPS tolerance. Here we found that, similarly to prolonged exposure to LPS, 12-24h exposure of a physiologically relevant level of the PA induces LPS tolerance in macrophages by inhibiting expression of *il-1β*, and not *tnf* or *il-6,* compared to untreated macrophages. We show this induction of *il-1β*-specific LPS tolerance was unique to PA, and not other enriched SFAs found in the KD. This is the first observation of dietary SFAs inducing LPS tolerance. Further, we found that monocytes from KD-fed mice showed increased expression of anti-inflammatory cytokine IL-10 when exposed to LPS, compared to SC-fed monocytes, suggesting KD-associated PA is driving LPS tolerance *in vivo*. Together, these data implicate dietary SFAs as inducers of sepsis-associated immunoparalysis and describe PA as a regulator of LPS tolerance in monocytes and macrophages. Importantly, diet-induced alterations to monocytes and macrophages may be critical to consider in the treatment of septic patients, and dietary manipulation may serve as an essential therapeutic tool, especially for those that consume excessive amounts of SFAs.

Disclosure of Interest: None Declared

O33

ESTROGEN-DRIVES EXPRESSION OF A UNIQUE TYPE I INTERFERON, IFN-EPSILON, AND PROMOTES NEISSERIA GONORRHOEAE INFECTION

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Abstract Content: We explored the role of type I IFNs, particularly IFN-e, in controlling *Neisseria gonorrhoeae* (*Ng*) infections of the genital tract. In previous studies, we established that type I IFNs significantly enhance *Ng* replication in Mø and PMNs. Epithelial cells (EC) in the human as well as mouse genital tract produce a unique type I IFN, IFN-epsilon (IFN-e), in response to estrogen. Clinical studies indicate that estrogen play an important role in susceptibility of women to *Ng* infection. In mice, estrogen is essential for *Ng* infection in the female genital tract while progesterone inhibits *Ng* infection.

Our studies demonstrate that Type I IFN and IFN-e in particular promote intravaginal *Ng* infection in estrogen treated mice. Thus, estrogen-treated WT mice were susceptible to *Ng* infection with prolonged colonization (7-12 days p.i.). In contrast, estrogen-treated IFNAR KO mice remained *Ng* infected for only 2-3 days. Similarly, WT mice treated systemically with anti-IFNAR Ab cleared intravaginal *Ng* infection at the same rate as IFNAR KOs. We discovered that IFN-e was critical for intravaginal *Ng* infection. IFN-e KOs phenocopy the IFNAR KOs with attenuated *Ng* infection compared to WT mice. The rapid clearance of *Ng* from the mouse genital tract of IFN-e KOs was not due to neutrophils. In fact, *Ng* clearance was not impeded when neutrophils, T lymphocytes or NK cells (or all three cell types together) were systemically and locally eliminated using depleting mAbs. Importantly, recombinant IFN-e delivered topically into the vaginal tract was sufficient to entirely restore susceptibility to *Ng* infection in IFN-e KO. (Interestingly, IFN-e is suppressed by progesterone and progesterone treatment also prevents *Ng* infection in mice.)

We suggest that estrogen-dependent enhancement of *Ng* infection is due to estrogen-induced IFN-e expression in the vaginal tract and that IFN-e engagement of IFNAR promotes *Ng* infection both within the genital tract and within myeloid cells. Thus, estrogen, via the IFN-e/IFNAR axis, provides a permissive niche for *Ng*. The cellular and molecular mechanisms of IFN-e/IFNAR enhanced *Ng* infection are currently under investigation

Disclosure of Interest: None Declared

O34

TDP-43 TRIGGERS MITOCHONDRIAL DNA RELEASE TO ACTIVATE CGAS/STING IN ALS

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Abstract Content:

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Background: Cytoplasmic neuronal accumulation of the normally nuclear protein TDP-43 is a disease hallmark for many cases of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD), with some familial cases caused by mutant forms of the protein. Both diseases are associated with a neuroinflammatory cytokine profile related to upregulation of NF-kB and type I IFN pathways.

Objectives: In this project we sought to identify the innate immune sensor responsible for generating neuroinflammation in response to TDP-43.
Methods: We employed in vitro models with overexpression of TDP-43 in cell lines, and ALS patient derived iPSC cells differentiated into motor neurons. These were subjected to CRIPSR mediated deletion or pharmacological inhibition of various innate immune pathways. For in vivo studies a mouse model with human TDP-43(A315T) expressed under the prion promoter was used, crossed to mice that were genetically deficient for STING.
Results: Here we show that the cytoplasmic DNA sensor, cyclic GMP-AMP synthase (cGAS), is activated by an accumulation of wild-type TDP-43 and further exacerbated by mutant TDP-43. Mislocalized TDP-43 invades mitochondria via TIM22 and causes the mitochondrial Permeability Transition Pore (mPTP) to release mitochondrial DNA (mtDNA) into the cytoplasm, which directly activates cGAS. Pharmacological inhibition or genetic deletion of cGAS and its downstream signalling partner STING, prevents upregulation of NF-kB and type I IFN induced by TDP-43 in human cell lines. Furthermore, genetic deletion of one allele of *Sting* in ALS-mutant TDP-43 transgenic mice is sufficient to increase lifespan, improve motor control and prevent neuron loss, demonstrating that partial inhibition of the pathway is sufficient to impact on disease. Finally, we document elevated levels of the specific cGAS signalling metabolite cGAMP in iPSC-derived motor neurons and post-mortem spinal cord samples of patients with ALS.
Discussion and Conclusions: Our results identify mtDNA release and cGAS/STING activation as critical determinants of TDP-43 driven neurodegeneration and demonstrate the potential for targeting this pathway in neurodegenerative disease.
Disclosure of Interest: None Declared

O35

A CO-OPTED BINDING MECHANISM NORMALLY RESERVED FOR DE-ISGYLATION IS ESSENTIAL FOR THE CONTROL OF TYPE I IFN SIGNALLING

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Abstract Content: The innate immune response is underpinned by the conserved type I interferon (IFN-I) system and is of fundamental importance for our response to infection, cancer and immunotherapy. Importantly, IFN signalling must be moderated to prevent autoinflammatory disease and tissue damage. The ubiquitin-like protein ISG15 is strongly induced by IFN and is critical for regulating how cells respond to IFN. As part of the antiviral response, ISG15 can modify proteins (incl. viral proteins) in a process known as ISGylation, where the C-terminal di-glycine motif of ISG15 covalently attaches to lysine residues of target proteins. As a reversible process, the ISG15-specific isopeptidase USP18 deconjugates ISG15 and this requires engagement of USP18's catalytic triad with the ISG15 di-Gly motif. However, independently of its protease activity, USP18 is critical for down-regulating IFN-I signalling. Likewise, it was recently shown that ISG15 is critical for negative regulation of IFN-I signalling; in IFN-treated ISG15-deficient cells, USP18 protein is rapidly degraded leading to a model suggesting that ISG15's essential role is to stabilise USP18, thus allowing regulated signalling.
To investigate this further, we generated lentiviral vectors that express wild-type (wt) or mutant forms of ISG15 under the control of the endogenous, IFN-regulated, ISG15 promoter and reconstituted ISG15-deficient cells. Reconstituted wt ISG15 retained its ability to bind USP18, whereas mutants could either bind with intermediate affinity (C-term ΔGG) or were unable to bind USP18 (C-term di-Ala or ISG15-AA). Importantly, we show that following IFN treatment the capacity of ISG15 mutants to regulate IFN signalling faithfully mirrored their ability to bind USP18. For example, the expression of ISGs in IFN-treated cells expressing ISG15-AA were significantly higher than controls and similar to that in ISG15-KO cells. Expression was, to some extent, regulated in ISG15-ΔGG-expressing cells with restoration of normal, regulated expression in wt ISG15-expessing cells. As expected, these data were corroborated when we investigated the levels of phospho-STAT1 (a marker of activated signalling) and following viral infection of IFN-treated cells: ISG15-deficient and ISG15-AA-expressing cells were resistant to infection, ISG15-ΔGG were partially permissive while wt ISG15-expressing cells were fully permissive to infection. To stabilise the ISG15:USP18 interaction, USP18 interacts with a hydrophobic patch in ISG15 (coordinated by Trp123 or W123) that leads to a structural rearrangement of USP18 and access of ISG15's di-Gly motif to the catalytic groove in USP18. Importantly, expression of mutant ISG15-W123R (with di-Gly motif) unable to bind USP18 did not restore regulated IFN-I signalling and phenocopied ISG15-deficient and ISG15-AA-expressing cells. Significantly, all ISG15 mutants were able to stabilise USP18 showing that, in contract to existing models, the ISG15-dependent stabilisation of USP18 is necessary but not sufficient for regulated IFN-I signalling.
Therefore, we hypothesise that non-covalent binding of ISG15 and USP18 is necessary to facilitate USP18's inhibitory function. Intriguingly, this is achieved through co-option of a binding mechanism normally reserved for de-ISGylation.
Disclosure of Interest: None Declared

O36

TUMOR-SPECIFIC CD8 T CELL-MEDIATED ANTITUMOR ACTIVITY INTRODUCED BY INTRATUMORALLY INJECTED ANTI-CD40 ANTIBODY TOGETHER WITH IL-15 IS CRITICAL IN TRAMP-C2 TUMORS IN MICE

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Abstract Content: Intratumoral immunotherapy that aims at generating a potent priming of antitumor immunity, uses the tumor as its own vaccine, for a systemic and durable clinical

benefit. Recently, Dr. Jeffrey Ravetch's Group found that use of the optimized anti-CD40 monoclonal antibody (2141-V11) not only increases its antitumor efficacy but also reduces its side effect through intratumoral injection (i.t.) (Cancer Cell 2016, 29(6), 820-831). Previously, we demonstrated that the combination regimen of the agonistic anti-CD40 monoclonal antibody (FGK4.5) with IL-15 showed synergistic therapeutic efficacy when compared with either monotherapy alone in mouse tumor models (J Immunol 2012, 188(12): 6156-64). Furthermore, IL-15 alone or agonistic CD40 did not yield tetramer positive tumor specific CD8 T-cells in the TRAMP-C2 model, whereas the combination showed an at least 10-fold increase in the number of such tumor specific CD8 T-cells.
Here, we investigated the combination of IL-15 with the intratumoral anti-CD40 therapy using a dosing schedule based on our previous study in the TRAMP-C2 model. We demonstrated that given intratumorally of anti-CD40 mAb had the abscopal efficacy and shrunk the left, untreated site of tumor size. Also, the combination of IL-15 and anti-CD40 mAb robust increased the numbers of the tumor specific tetramer+CD8+ T cells, which associate the boost of the efficacy compare to the single treatment. These findings support the view that anti-CD40 given intratumorally has abscopal efficacy and IL-15 combined administration increases this effect in Tramp-C2 tumor-bearing mouse model.
Disclosure of Interest: None Declared

O37
NEUROIMMUNE CELLULAR AND CYTOKINE PROFILE IN HIV-1 ACUTELY INFECTED PATIENTS
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Abstract Content: Neurological conditions associated with HIV-1 remain a significant cause of morbidity and mortality globally with manifestations occurring throughout the central nervous system (CNS). Despite effective treatment with antiretroviral therapy, HIV-1 and chronic neuroinflammation can persist in the brain. The extent of viral replication in the CNS and origin of neuropathology associated with HIV-1 are poorly understood. In this study, we assessed the cellular and innate immune profiles in both the CNS and peripheral compartments of patients (n = 6) during early acute HIV-1 infection, when viral replication is maximal. Cell-associated viral RNA and associated host cytokine profiles were assessed in matched peripheral blood (PB) mononuclear cell and cerebral spinal fluid (CSF) samples and compared to uninfected donors (n = 3). PB and CSF CD4+ and CD8+ T cells and macrophages were sorted and phenotyped for activation markers by flow cytometry directly *ex vivo*, followed by cytokine, chemokine and viral RNA gene expression profiling using highly multiplexed targeted RT-qPCR (Fluidigm Biomark). We observed robust CD4+ T cell infection in the CSF during Fiebig III-IV HIV-1 infection, with 0.04-0.84% (median 0.14%) expressing spiced viral RNA indicative of productive infection. A similar frequency was present in PBMC. As expected, markers of cellular activation were increased, including CD38 on CD4+ and CD8+ T cells, and CD38 and CD169 on monocytes in both PB and CSF ($p<0.05$). Parallel studies in a SHIV rhesus macaque model indicated upregulation of *CXCL10* in CSF CD4+ T cells and monocytes compared to uninfected controls, indicating a CNS inflammatory response during acute infection. These studies provide direct evidence of viral replication in CD4+ T cells and broad immune activation in both the periphery and CNS during acute HIV-1 infection, likely contributing to early CSF inflammation and neuropathology.
Disclosure of Interest: None Declared

Lightning Talk Session 1: Adaptive Immunity

LT001
SINGLE-CHAIN SOLUBLE RECEPTOR FUSION PROTEINS AS VERSATILE CYTOKINE INHIBITORS
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Abstract Content: Cytokines are small secreted proteins that among many functions also play key roles in the orchestration of inflammation in host defence and disease. Over the past years, a large number of biologics have been developed to target cytokines in disease, amongst which soluble receptor fusion proteins have shown some promise in pre-clinical studies.
We have previously reported the development of a new biologic, termed IL-33trap, comprising the ectodomains of the cognate receptor ST2 and the co-receptor IL-1RAcP fused into a single-chain recombinant fusion protein. We showed that IL-33trap is able to neutralize IL-33 released in response to a fungal aeroallergen *Alternaria alternata* and to reduce lung inflammatory responses in a murine model of airway inflammation. Here we extend the biophysical and biological characterisation of IL-33trap variants, and we show that our design allows the formation of a single-chain fusion protein with high IL-33 antagonistic activity. Furthermore, IL-33trap is a stable protein with a monomeric profile both at physiological temperatures and during liquid storage at 4 °C. We also report that IL-33trap specifically targets biologically active IL-33 splice variants and is not affected by inactive variants. Finally, we document the generation and antagonistic activity of a single-chain IL-4/13trap, which inhibits both IL-4 and IL-13 signalling.
Collectively, our results illustrate that single-chain soluble receptor fusion proteins against IL-4, IL-13, and IL-33 are novel biologics that might not only be of interest for research purposes and further interrogation of the role of their target cytokines in physiology and disease, but may also complement monoclonal antibodies for the treatment of allergic and other inflammatory diseases.
Disclosure of Interest: None Declared

LT003

NOVEL REGULATORS OF IFNG-DEPENDENT MHCII EXPRESSION

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Abstract Content: The IFNγ-dependent induction of MHCII expression is critical for CD4+ T cell function in peripheral tissues. Once activated CD4+ T cells induce cytokine production, macrophage activation, B cell responses and immune cell recruitment needed to control infection and disease. Dysregulation of MHCII is associated with autoimmunity, graft versus host disease, and increased susceptibility to specific cancers and chronic infections. Despite its central role in host immunity, the complex and dynamic regulation of IFNγ-induced MHCII is poorly understood. We hypothesize that targeting regulatory networks of MHCII expression will allow the development of new therapies to treat or prevent MHCII-expression related disease states. Using a genome-wide CRISPR screen in macrophages we identified over 200 genes that control IFNγ-mediated MHCII surface expression. We validated these candidates using flow cytometry and qPCR uncovering a range of important MHCII regulators that were previously unknown. We found two parallel pathways that require either the multifunctional kinase GSK3 or the mediator complex subunit Med16. Both pathways were required for IFNγ-mediated Ciita induction, MHCII expression, and CD4+ T cell activation. Using RNAseq analysis we defined the regulons of these distinct transcriptional networks and are using these data to define the mechanisms of MHCII control in macrophages. Our results defining IFNγ-mediated MHCII regulation could provide new therapeutic targets to modify MHCII expression and alleviate disease and infection.

Disclosure of Interest: None Declared

LT004

CHARACTERIZATION OF IL7R ALPHA IN XENOPUS TROPICALIS: IMPLICATIONS FOR EVOLUTION OF CYTOKINE SIGNALING SYSTEMS

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Abstract Content: Signaling through the IL-7 receptor is essential for development of B and T cells, as evidenced by the SCID-like phenotype seen in the absence of IL-7/IL-7 receptor signaling. The IL-7R is a heterodimer comprised of the IL-7R α chain and a γ chain that is shared by other IL-2 family cytokines that utilize the JAK-STAT pathway. We recently cloned the IL-7R α cDNA from *Xenopus tropicalis*. IL-7R α is found in lymphoid (thymus, spleen) and non-lymphoid organs of *X. tropicalis* including intestine, eye, and lungs. A putative Ets binding site is found the promoter region of the *X. tropicalis* IL-7R α gene, demonstrated by others to be important in regulating transcription of the IL-7R α gene in B and T cells. Comparison of the predicted amino acid sequence of the *Xenopus* IL-7R α indicates closest homology with the zebrafish IL-7R α, and significant homology with other characterized IL-7R α including those from humans, mice, chickens, alligators, primitive caecilians, green see turtles, and anole lizards. Analysis of the predicted amino acid sequence indicates an intact WSXWS motif (common to all IL-7R family members). The Box 1 motif, normally required for JAK interaction, has an asparagine replacing aspartic acid (similar variations are found in the zebrafish and anole lizard IL-7R α sequences). There are at least five potential N glycosylation sites. There is a 16 amino acid insert unique to the *Xenopus* sequence that is not found in the reported IL-7R α sequence of any other animal that we have examined. The protein structure model of IL-7R has the typical L-shape of the human and mouse receptors and conserved trio of disulfide bonds. Despite sharing only 20% identity with mouse IL-7R α, the RMSD between the model and the mouse structure (4NN5:B) is only 1.775 Å. The 16 amino acid *Xenopus* insert breaks up the C'2 β-strand in the fibronectin type-3 D2 domain and could potentially affect ligand binding. Synteny analysis failed to identify a gene with homology to IL-7 at the expected position, or anywhere else, in the *Xenopus* genome. Interestingly, we have tentatively identified a transposon in place of the IL-7 gene at the expected position. Signaling through a modified IL-7R using ligands other than IL-7 in *Xenopus* are being examined.

Disclosure of Interest: None Declared

LT005

CONTINUED BCL6 EXPRESSION PREVENTS THE TRANSDIFFERENTIATION OF ESTABLISHED TFH CELLS INTO TH1 CELLS DURING ACUTE VIRAL INFECTION

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Abstract Content: T follicular helper (Tfh) cells are crucial for the establishment of germinal centers (GCs) and potent antibody responses that are elicited during infection and vaccination. In contrast, dysregulated Tfh cells can cause autoimmunity. Despite their importance for humoral immunity, the T cell-intrinsic factors that are required for the maintenance of already established Tfh cells and GCs remain largely unknown. Here, we used temporally guided gene ablation in CD4+ T cells to dissect the contributions of

the Tfh-associated chemokine receptor CXCR5 and the transcription factor Bcl6. Ablation of *Cxcr5* in CD4+ T cells had only minor effects on the function of established Tfh cells. Phenotypical and transcriptional analyses revealed that *Cxcr5*-ablated cells still exhibited most features of CXCR5-positive Tfh cells, including *Il4* and *Il21* expression. In contrast, continued *Bcl6* expression was essential to maintain the GC Tfh cell phenotype and also the GC reaction. Importantly, CD4+ T cell-specific *Bcl6* ablation during acute viral infection resulted in transdifferentiation of established Tfh cells into Th1 cells, e.g. characterized by upregulation of Tbet and concomitant downregulation of *Il6ra*and *Il6st* expression, thus highlighting the plasticity of Tfh cells. These findings have implications for strategies that boost or restrain Tfh cells and GCs in health and disease.
Disclosure of Interest: None Declared

LT006

IL-27 ENHANCES CYTOKINE SECRETION BY TIGIT+ HIVGAG-SPECIFIC T CELLS

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Abstract Content: Although HIV infection can be effectively treated by lifelong administration of combination antiretroviral therapy (cART), novel strategies are required for cART-free HIV control. Recent studies had shown that IL-27, a member of IL-12 family of cytokines, can induce pro- and anti-inflammatory effects and inhibits HIV replication in monocytes, monocyte-derived dendritic cells, and T cells. In this study, we hypothesized that T- cell immune activation driven by HIV will promote IL-27 dependent pro-inflammatory properties enhancing HIV-specific T-cell immunity. We found that overnight *in vitro* stimulation with IL-27 upregulated expression of CD69 and T-bet in CD4 and CD8 T cells from both healthy and HIV infected donors. Accordingly, IL-27 increased IFN-ɣ and TNF-α secretion by $TIGIT^+$ HIVGag specific CD4 and CD8 T cells. Moreover, the RNA-Seq analysis showed that IL-27 induced global transcriptional changes associated with signal transducer and activator of transcription 1 (STAT1)/interferon inducible genes that were enriched in memory CD4 and CD8 T cells from HIV infected patients compared with healthy controls. This study provides new evidence about the effects of IL-27 in enhancing HIVGag-specific T cell responses.
Disclosure of Interest: None Declared

LT007

SENESCENCE-ASSOCIATED □-GALACTOSIDASE ACTIVITY AND OTHER MARKERS OF SENESCENCE ARE PRESENT IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS DURING HEALTHY AGING

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Abstract Content: Aging is associated with a decline in the immune system, termed “immunosenescence”. Cellular senescence is defined by persistent cellular growth arrest and overall loss of function. If immune cells undergo cellular senescence *in vivo* and whether cellular senescence has a role in immunosenesence remains controversial due to the lack of specific markers to reliably identify these cells in blood. Freshly-isolated human PBMC from younger and older donors were fluorescently labeled for senescence-associated b-galactosidase (SA-bGal) activity. CD8+ T cells were sorted based on SA-bGal and analyzed by qRT-PCR, RNA sequencing, and microscopy. We found a higher percentage of cells with high SA-bGal in the older vs. younger cohort. This increase was apparent in NK cells, CD4+ and CD8+ T cells. CD8+ T cells with high SA-bGal displayed increased transcripts of p21, $p16^{INK4a}$, and inflammatory cytokines. They also had greater DNA damage response foci and $p16^{INK4a}$ protein. SA-bGal increased as T cells differentiated into effector memory cells, which was consistent regardless of age. Intriguingly, a portion of T effector cells did not exhibit a senescent phenotype and maintained low SA-bGal; conversely, a portion of naïve T cells expressed high SA-bGal. RNA sequencing of CD8+ T cells revealed alterations in gene expression were associated with SA-bGal and cells with low, medium, and high SA-bGal had distinct profiles. Cells with the same level of SA-bGal shared similar gene expression, independent of age. Functional analysis of the sorted CD8+ T cells showed that cells with high SA-bGal proliferated significantly less than SA-bGal low cells. CD8+ T cells expressing SA-bGal appear to be distinct from exhausted T cells as they do not uniformly express markers of T cell exhaustion such as CD57, PD-1 and KLRG-1. The presence of $p16^{INK4a}$, DNA damage, inflammatory cytokines, and deceased proliferation demonstrate that SA-bGal is a strong indicator of cellular senescence. SA-bGal can be leveraged to further identify senescent immune cells and elucidate cellular senescence’s role in immunosenesence. Understanding this connection in healthy aging will further our understanding of premature aging seen in some chronic diseases.
Disclosure of Interest: None Declared

LT008

DENDRITIC CELLS PRIMED BY MYCOBACTERIUM LEPRAE ARE WEAK STIMULATORS OF T CELLS ACTIVATION

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Abstract Content: Leprosy is a public health problem in endemic areas with 208.641 news cases around the world in 2018. *Mycobacterium leprae*, the causative agent of leprosy, is an obligate intracellular pathogen. The bacillus infects skin macrophages and Schwann cells of peripheral nerves where they proliferate causing neural injury. Several clinical forms of leprosy can arise according to individual immune response since *M. leprae* presents low genetic variability. Tuberculoid patients show a predominance of Th1 cellular immune response against *M. leprae* so the disease is localized. On the other hand, lepromatous patients demonstrate a predominant humoral response that is not efficient resulting in disseminated disease. Dendritic cells (DCs) are crucial for the activation of T cells during the development of adaptive immunity. Although live *M. leprae* seems to be a weak promotor of DCs activation, the role of these cells in the polarization of immune response in leprosy patients is not understood. This study aimed to evaluate the profile of cytokines produced by autologous lymphocytes (LTs) co-cultured with DCs previously infected by live *M. leprae*. Peripheral mononuclear blood cells were obtained from healthy controls (HC) and leprosy patients through a density gradient. Monocytes-CD14+ were positively selected by microbeads and differentiated in DCs using GM-CSF and IL-14 for 6 days. DCs were stimulated by (i) live *M. leprae* at a MOI of 10:1 (bacilli:DC, MLDCs) or (ii) a standard maturation cocktail (MC) composed by IL-1β, IL-6, TNF, PGE_2 (MCDCs) or (iii) remained unstimulated (USDCs) for 48 hours. Then, DCs were co-cultivated with LTs for five days. Intracellular and released cytokines were evaluated by flow cytometry. The production of IFN-y, TNF, IL-9, and IL-22 was higher in MCDCs+LTs cultures compared to LT cultures with MLDCs and USDCs while IL-4 levels were lower in MLDCs+LTs cultures both in leprosy patients and healthy controls. Lepromatous patients showed low IL-2 levels when LTs were primed by MLDCs. In contrast, the frequency of IL-4+ LTs was higher in these patients that presented a lower frequency of IFN-y+ and IL-17A+ LTs in co-cultures with MLDCs. We conclude that DCs primed by *M. leprae* are not able to stimulate the full activation of LTs. Besides, lepromatous patients presented a lower frequency of Th1 and Th17 LTs. This suggests that these patients could present some impairment to develop the profiles of immune response effective against *M. leprae,* favoring its permanence in the host for long periods. Supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Grant: #2015/23256-6).

Disclosure of Interest: None Declared

LT009

PDL1 PROTECTS LCMV-INFECTED TYPE I INTERFERON RECEPTOR DEFICIENT MICE FROM LETHAL IMMUNE MEDIATED DISEASE

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Abstract Content: The type I interferons (IFN-Is) are well recognised for their protective role during viral infection. They induce an antiviral state, promote maturation of leukocytes and thus aid in mediating viral clearance. However, IFN-Is can also provoke an immunosuppressive environment that promotes chronic infection by facilitating functional T cell exhaustion. However, the mechanisms that contribute to this dual role of IFN-Is in balancing inflammatory vs. inhibitory signals are only partially understood.

Using the LCMV-Armstrong (LCMV-ARM) model of acute virus infection, we have recently shown that IFN-Is are required for accumulation and activation of virus-specific CD8+ T cells. Moreover, LCMV-specific CD8+ T cells in LCMV-ARM-infected mice lacking the IFN-I receptor IFNAR (IFNAR KO) were functionally exhausted and unable to clear the virus, while wild type mice mounted a robust CD8+ T cell response and eliminated the virus. Given its importance in regulating T cell responses during LCMV infection, we hypothesised that the PD1/PDL1 pathway contributed to CD8+ T cell dysfunction in the IFNAR KO mice and that loss of PDL1 in IFNAR-deficient mice would prevent T cell exhaustion thereby permitting viral clearance.

To address this, mice lacking IFNAR were crossed with PDL1-deficient mice to generate double-deficient IFNAR x PDL1 DKO mice. In contrast to infection of WT and PDL1 KO mice, which remain asymptomatic, and IFNAR KO mice, which developed minor signs of disease, infection of IFNAR x PDL1 DKO mice with LCMV-ARM resulted in a lethal wasting disease. Whilst disease was accompanied by virus spread and increased expression of cytokines this was not dissimilar to IFNAR KO mice. Importantly, only DKO mice displayed leukocyte infiltration into lungs, which led to necrotising pneumonia. Depletion of CD8+ cells in infected IFNAR x PDL1 DKO mice prevented disease development indicating that a dysregulated CD8+ T cell response mediated lethality. However, phenotypical and functional analysis of CD8+ T cells showed that PDL1-deficiency did not restore numbers or increase effector function of virus specific CD8+ T cells, suggesting that other mechanisms were responsible for mediating lethality.

Together, these findings underpin our limited understanding of the relationship between IFN-Is and the PD1/PDL1 pathway and the impact on T cell responses. This has broader implications for our understanding and improving of immune check point therapy, where the underlying molecular mechanisms presently remain largely unknown.

Disclosure of Interest: None Declared

LT011

MIR-15/16 DEFICIENCY IN T CELLS LEADS TO ACCUMULATION OF CD25-LOW TREGS IN VIVO

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Abstract Content: Introduction: The transcription factor FOXP3 defines the gene expression program of regulatory T cells (Tregs). High expression of the IL-2 receptor plays a key role in Treg suppressor function as it allows Tregs to

consume IL-2 to limit T effector cell activation. Regulation of gene expression by microRNAs (miRNAs) is critical for Treg differentiation and function. Here, we report that mice with miR-15/16 deficiency in T cells accumulate Tregs in multiple tissues with abnormal expression of FOXP3, IL2Rα/CD25 and IL7Rα/CD127.

Methods: Flow cytometry was used for analysis of Tregs ($CD3^+CD8^-CD4^+FOXP3^+$) and T effector cells in thymus, spleen and inguinal lymph nodes isolated from unchallenged miR-15/16$^{fl/fl}$ CD4^{cre} or miR-15/16$^{fl/fl}$ FOXP3^{cre} mice (lacking both *miR-15a/16-1/Mirc30* and *miR-15b/16-2/Mirc10* clusters) and CD4^{wt} or FOXP3^{wt} miR-15/16$^{fl/fl}$ controls.

Results: Mice with miR-15/16 deficiency in all T cells (CD4^{cre}) demonstrated an increased number of FOXP3$^+$ Tregs, but not FOXP3$^-$ conventional T cells, in thymus, spleen and lymph nodes compared to control mice. Interestingly, the expression level of FOXP3 (measured by MFI) was significantly lower in miR-15/16 deficient Tregs, indicating accumulation of functionally defective cells. Indeed, miR-15/16 deficient Tregs expressed low CD25 levels. Additionally, the IL-7 receptor CD127, a direct target of miR-15/16 and positive regulator of Treg survival, was significantly increased in miR-15/16 deficient Tregs, potentially contributing to expanded Treg pool. miR-15/16 deficient Tregs were capable of restricting T effector cell proliferation *in vitro* and *in vivo* by outnumbering T effector cells. The suppressive capacity of miR-15/16 deficient Treg was significantly reduced on a per cell basis compared to wild type Tregs. Spontaneous accumulation of FOXP3^{lo}CD25^{lo}CD127^{hi} Tregs similarly developed in miR-15/16$^{fl/fl}$ FOXP3^{cre} mice which had larger spleen volume and increased numbers of CD44^{hi}CD62L^{lo} T effector cells, suggesting insufficient Treg suppression.

Conclusion: miR-15/16 deficient Tregs compensate for lower suppressive function by enhanced ability to expand.

Disclosure of Interest: None Declared

LT013

IL-12 DERIVED FROM TYPE 1 DENDRITIC CELLS TONICALLY PROMOTES THE DIFFERENTIATION OF INNATE T-BET(HIGH) MEMORY-PHENOTYPE CD4(+) T LYMPHOCYTES IN STEADY STATE

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Abstract Content: CD4$^+$ T lymphocytes consist of naïve, antigen-specific memory, and memory-phenotype (MP) cell compartments in steady state. We recently showed that MP cells exert innate-like effector function in host defense. However, it remained to be defined whether MP CD4$^+$ T cells are functionally heterogenous, and if so, what factors determine the selective differentiation of MP subpopulations under homeostatic conditions. Here we characterize MP lymphocytes as consisting of T-bet^{high}, T-bet^{low}, and T-bet$^-$ subsets, with innate Th1-like host-protecting activity exclusively associated with T-bet^{high} cells. We further show that steady-state differentiation of T-bet^{high} MP cells is promoted by IL-12-producing type 1 dendritic cells (DC1). Finally, we present evidence arguing that this tonic IL-12 production by DC1 requires TLR-MyD88 signaling triggered independently of foreign agonists and is further enhanced by CD40-CD40L interactions with CD4$^+$ T lymphocytes. Together our results argue that optimal differentiation of T-bet^{high} MP lymphocytes in steady state requires IL-12 tonically produced by DC1 in an MyD88 and CD40 signaling-dependent fashion and thus reveal a previously unappreciated homeostatic function of IL-12.

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Disclosure of Interest: None Declared

LT014

RAPID DETERMINATION OF CYTOKINE RELEASE FROM T CELLS WITH NO-TRANSFER, NO-WASH LUMIT™ IMMUNOASSAYS

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Abstract Content: We have utilized NanoLuc® Binary Technology (NanoBiT®) to develop a homogeneous and rapid assay method (≤ 70 min completion time) to measure cytokines released from cells in culture without the need for sample transfer and requiring only a standard, plate-reading luminometer for signal acquisition. In this Lumit™ immunoassay approach, separate antibodies to a specific cytokine are labeled with the small, 11-amino acid subunit of NanoBiT luciferase (SmBiT) or its 17.6 kDa complementary subunit (LgBiT). When SmBiT- and LgBiT-labeled antibodies converge on the target cytokine, the resultant proximity of SmBiT and LgBiT reconstitutes a bright luciferase that produces light proportional to analyte levels. In this manner, Lumit immunoassays have been developed for several cytokines, including human IL-1β, IL-2, IL-4, IL-6, IFN-γ, and TNF-α. These assays typically exhibit a lower limit of detection (LLOD) less than 10 pg/ml and linearity over more than three logs of analyte concentration, with maximal signal-to-background ratios (S/B) greater than 1000.

Following treatment of human peripheral blood mononuclear cells (PBMC) in 96-well plates with vehicle, LPS, R848, or a combination of PMA and ionomycin, the non-lytic cytokine detection reagents were added directly to the culture wells containing cells and medium. Dose- and time-dependent release of cytokines were observed over a wide range of

response levels without the need for sample dilution. Alternatively, replicate aliquots of medium from each cell treatment well were transferred to a separate assay plate for simultaneous determination of multiple cytokines released from individual cell wells. In a cell model comprised of purified CD8+ T cells and target Raji B cells treated with increasing bispecific T-cell engager Blincyto®, release of IL-2 and IFN-γ was observed with an EC_{50} of ~0.2 ng/ml and maximal S/B for IL-2 and IFN-γ of 82- and 168-fold, respectively. In addition, differentiation of purified CD4+ T cells into the Th2 phenotype was confirmed by marked increases in IL-4 vs. IFN-γ release. Following reagent addition, assay signal exhibited glow kinetics with a half-life of approximately two hours. In addition, 384-well, assay performance for determination of cytokine release from treated human PBMC resulted in Z' factors greater than 0.7, indicating the assay format is amenable to screening applications.

The implementation of this novel detection chemistry will enable rapid "add-and-read" determinations of cytokine release from cells for both low- and high-throughput applications, including quantitative assessments of T cell activation and differentiation.

Disclosure of Interest: D. Lazar Employee of: Promega Corporation, K. Hsiao Employee of: Promega Corporation, J. Gilden Employee of: Promega Corporation, C. Sondgeroth Employee of: Promega Corporation, K. Kupcho Employee of: Promega Corporation, D. Thompson Employee of: Promega Corporation, M. O'Brien Employee of: Promega Corporation, J. Cali Employee of: Promega Corporation

LT015

A NOVEL THERAPEUTIC TARGET FOR ARTHRITIS PAIN

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Abstract Content: A GM-CSF→CCL17 pathway was originally identified in vitro using monocyte/macrophage populations. We firstly explored the cellular source of the chemokine CCL17 and its GM-CSF dependence in various inflammation models (zymosan- and antigen-induced peritonitis, and LPS-induced lung inflammation), using *Ccl17*$^{E/+}$ reporter mice and flow cytometry. We present evidence that in these models CCL17 expression is predominately expressed in macrophage-lineage populations, namely MHCII^{+} macrophages and monocyte-derived dendritic cells and is suppressed upon GM-CSF blockade, which is consistent with our in vitro findings. We next investigated the involvement of CCL17 in controlling the pain and disease in various arthritis models, namely models of inflammatory arthritis (zymosan-, antigen- and cytokine-driven monoarticular arthritis) and osteoarthritis (collagenase-induced osteoarthritis). For these particular studies, we utilized CCL17 gene-deficient (*Ccl17*$^{E/E}$) mice and therapeutic protocols using monoclonal antibody administration at different time points. We assessed the development of pain-like behaviour and joint pathology using an incapacitance meter (a measurement of weight distribution between two hind limbs) and histological analysis, respectively. In comparison to wild-type (WT) mice, *Ccl17*$^{E/E}$ mice were protected from pain-like behaviour and joint cartilage damage across all the arthritis models studied, while the degree of joint inflammation was comparable to that seen in WT mice. For the inflammatory arthritis models, neutralizing CCL17 therapeutically (following the onset of pain) rapidly reversed established pain-like behaviour and halted disease progression, as measured by structural changes; for the osteoarthritis model, we used treatment regimens neutralizing CCL17 at different stages of disease (i.e. early vs. late) and consistently found that CCL17 neutralization effectively ameliorates pain-like behaviour. In contrast to the well-recognized chemotactic role of CCL17 (e.g. preferential Th2 cell chemotaxis), these data reveal a novel, non-chemotactic and algesic role of CCL17 in arthritis-associated pain and suggest that it could be a potential therapeutic target in arthritis patients.

Disclosure of Interest: None Declared

LT017

LIPID METABOLISM FUELS IL-17-PRODUCING GAMMADELTA T CELLS AND DRIVES THEIR EXPANSION IN OBESITY AND THE TUMOR MICROENVIRONMENT

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Abstract Content: Metabolic reprogramming is required for T cell activation and effector cytokine production. Recent studies have revealed the metabolic programs that govern αβ T cell subsets, NK cells and myeloid lineages, but little of known about gd T cell metabolism. γδ T cells hold significant promise for immunotherapy in cancer, however, they can be either anti-tumor or pro-tumor, depending on their production of IFNγ (γδ^{IFN}) or IL-17 (γδ^{17}). Therefore, understanding the metabolic pathways that govern these distinct functions is essential. We found that γδ T cell subsets had distinct metabolic requirements. γδ^{17} cells had enhanced oxidative metabolism, increased mitochondrial mass and potential. This was accompanied by enrichment of lipid-related metabolic pathways and intracellular lipid droplets. γδ^{17}, but not γδ^{IFN} cells, had high lipid uptake *in vitro* and administration of lipids *in vivo* by feeding a high fat diet expanded the number of γδ^{17} cells and enhanced their ability to produce IL-17. In the tumor microenvironment, known to be lipid-rich, γδ^{17} cells were enriched, and were further expanded in tumours of obese mice. This study highlights the different metabolic requirements of γδ T cell subsets and suggests an important link between lipids and IL-17 production by γδ T cells. Lipid-driven IL-17 may influence the outcome of γδ T cell-based immunotherapy in

obesity and could be linked with IL-17 related diseases like psoriasis and multiple sclerosis that are increased in obesity.
Disclosure of Interest: None Declared

LT019

STEP-DOSE ESCALATION OF IL-7 TREATMENT LEADS TO SYSTEMIC EXPANSION AND SURVIVAL OF CD4 AND CD8 T LYMPHOCYTES IN RHESUS MACAQUES

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Abstract Content: Background: Interleukin 7 (IL-7) is a homeostatic cytokine playing a central role in the growth and survival of T lymphocytes. In several clinical studies, including HIV infection and cancer, IL-7 was able to efficiently reconstitute the T cell pool. With the rationale of using IL-7 as a vaccine enhancer or in combination with other cytokines in immunotherapy protocols, We have produced human and macaque IL-7 from human human cells. This cytokines have the advantage of being properly glycosylated and, therefore, will not be immunogenic in humans. In this study, we address pharmacokinetics and systemic effects of recombinant macaque IL-7 (rmIL-7) produced from human HEK293 cells.

Study Design: Six rhesus macaques were treated with rmIL-7 delivered subcutaneously using a protocol that included 3 injections of increasing cytokine doses (50, 100 and 200 mg/kg) every three days. Two days after the last injection, animals were sacrificed and the systemic rmIL-7 effects were analyzed on lymphocytes and myeloid cells from blood, lymph nodes (LN) and effector tissues by multi-parametric flow cytometry.
Results and Conclusions: Administration of rmIL-7 was well-tolerated and resulted in increased plasma rmIL-7 levels 4 hrs after each injection. Plasma rmIL-7 remained higher 48 hrs after each injection compared to the pre-timepoint. The increased systemic levels of rmIL-7 were evident by the reduced detection of CD127 (IL-7R) in PBMC and LN mononuclear cells at 48 hrs. rmIL-7 induced rapid movement of central memory T cells from the blood at 4 hr. Repeated rmIL-7 administration induced a gradual and systemic expansion of both CD4+ and CD8+ T cells, particularly in the memory compartment. rmIL-7 led to increased proliferation (Ki67+) and expression of the survival factor (Bcl-2) on T cells from blood, LN and effector sites, such as liver and gastrointestinal tract (duodenum, jejunum, ileum, colon and rectum). Compared to CD4 T cells, higher proliferation among memory CD8 T cells was found. Systemic administration of IL-7 signficantly increased plasma levels of CC chemokines such as CCL2, CCL4, CCL19 and CCL20 and CXC chemokines, CXCL10 and CXCL11. Cytokines, such as IL-6, IL-15 and TPO were also significantly increased in plasma of IL-7 treated macaques. Interestingly, within the lymph nodes, rmIL-7 treatment increased the frequency of plasmacytoid dendritic cells (pDC) that may impact induction of adaptive immune responses in vaccine and immunotherapy protocols. This new IL-7 delivery regimen, alone or in combination with other cytokines, may help to reconstitute the T cell pool in lymphopenic conditions.
Disclosure of Interest: None Declared

LT020

SERTOLI CELLS SUPRESS ACTIVATION AND INFLAMMATORY STATE OF T-CELLS IN VITRO

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Abstract Content: Introduction: Sertoli cells (SCs) are well known as nurse cells for maturating germ cells, their immunological importance has become more and more pronounced last couple of years. They modulate immune system and thus create immunologically privileged site within testis. Mesenchymal stem cells (MSCs) are even better known for their immune-regulatory phenotype and are also studied as a potential therapeutic tool in number of clinical studies. Due to the similarity of actions of these two cell types it was proposed that SCs are kind of MSCs. We aim to compare their ability to regulate the immune system and suppress the inflammatory response in vitro.
Methods: Spleen cells were isolated from BALB/c mice, unstimulated or stimulated with Concanavalin A and cultivated in the presence of SCs or MSCs. After 48 (detection of cytokines) or 72 (detection of transcription factors) hours of incubation cells were analyzed using flow cytometry. Cytokine production and expression of transcription factors was determined intracellularly within spleen cells. Down-regulation of apoptosis and suppression of splenocyte proliferation in the presence of SCs or MSCs was measured using Annexin V and Ki67, respectively.
Results: Percentage of IL-2 and TNFα positive splenocytes decreased after treatment with SCs, whereas co-culture with MSCs reduced the number of IL-17 positive cells. Interestingly, a lower percentage of Th17 cells was observed in the presence of SCs when compared to presence of MSCs in the culture. Both cell types suppressed the proliferation of $CD4^+$ T cells in a similar manner. Apoptosis of splenocytes decreased in the presence of MSCs, in the presence of SCs, the percentage of apoptotic cells within $CD4^+$ population remained similar to controls.
Conclusion: SCs have suppressive effect on T helper cells after activation. In some aspects, this effect was even greater than that of MSCs. Therefore, they may be interesting and important tool in therapies, for example in cases of testicular inflammation accompanied by infiltration of immune cells, which often impairs male fertility.
Disclosure of Interest: None Declared

LT021

T CELL RESPONSES TO PROTEOGLYCAN AGGRECAN PEPTIDES IN KNEE OA PATIENTS

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Abstract Content: Knee osteoarthritis is accompanied by inflammation and infiltration of immune cells eg. T cells and macrophages within the joint-surrounding tissues, such as, the infrapatellar fat pad and synovial linings. Many studies suggest the role of an antigen-driven response in osteoarthritic patients, eg. the presence of an oligoclonal T cell pattern and Ig-producing B cells in the synovium. Previous studies have shown that OA patients were responsive to proteoglycan aggrecan peptides especially the p16-31 and p263-280 peptide fragment. Here, we show that healthy individuals were capable of eliciting T cell responses to the p16-31 and p263-280 peptide fragments, suggesting primed T cells specific to these peptides. Interestingly, these T cells produced the pro-inflammatory cytokine, IL-6. T cells within the infrapatellar fat pad also elicited responses towards both peptide fragments. We also investigated the cytokines that were released from T cell responses in response to these peptide stimulations. In the synovial fluid of knee OA patients, many cytotoxic mediators, including IL-6, were detected at high levels.
Disclosure of Interest: None Declared

LT023

SELECTIVE JANUS KINASE INHIBITION PRESERVES INTERFERON-Λ-MEDIATED BARRIER PROTECTION

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Abstract Content: The ongoing SARS-CoV-2 pandemic clearly illustrates that immune suppressive regimens must be as targeted as possible to reduce the infection risk for patients under treatment to the achievable minimum. Current treatments of patients suffering from inflammatory and other diseases with Janus kinase 1/2 (JAK1/2) inhibitors can lead to inadvertent immune suppression and to increased risks for infections. Here we addressed whether selective JAK inhibition could facilitate a well-tailored immune suppression preserving interferon-λ-mediated barrier protection and its potential therapeutic use as broadly active antiviral. Tyrosine kinase 2 (TYK2), a JAK family member, is required for type I interferon (IFN-α/β) signaling, but its role in type III IFN (IFN-λ) signaling is still unclear. We report here that the selective TYK2 inhibitor BMS-986165 blocks potentially detrimental type I IFN signaling without altering protective IFN-λ-mediated gene expression. In contrast, the clinically used JAK1/2 inhibitor baricitinib was found to be equally potent in blocking responses to both types of IFN. Mechanistically, we show that epithelial cells do not require TYK2 for IFN-λ-mediated signaling or antiviral protection. Lack of TYK2 *in vivo* diminished IFN-α-induced protection against a lethal influenza virus challenge in mice, but did not impair IFN-λ-mediated antiviral protection. Our findings suggest that selective TYK2 inhibitors likely represent a superior treatment option for type I interferonopathies than broadly acting JAK1/2 inhibitors, as selective TYK2 inhibitors may counteract inflammatory responses while preserving the beneficial antiviral effects of IFN-λ and its potential therapeutic use against COVID-19.
Disclosure of Interest: None Declared

LT027

CRYSTAL STRUCTURE OF IL-27 IN COMPLEX WITH SRF388, A FIRST-IN-CLASS IL-27 BLOCKING ANTIBODY UNDER EVALUATION IN A PHASE I CLINICAL TRIAL IN PATIENTS WITH ADVANCED SOLID TUMORS

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Abstract Content: Introduction: IL-27 is a heterodimeric immunoregulatory cytokine composed of EBI3 and p28 subunits. It is produced by activated macrophages and dendritic cells and binds with high affinity to IL-27RA expressed on innate and adaptive immune cells. This interaction also relies on gp130 binding to mediate signaling through phosphorylated STAT1. This results in the downstream transcription of several immunoregulatory receptors (eg, PD-L1) and inhibition of inflammatory cytokines (eg, IFNγ, IL-17, TNFα) that are important in dampening immune responses in certain tumor microenvironments. Here, cytokine/receptor affinity studies and the X-ray crystal structure of the human IL-27 heterodimer are reported. The structure was solved in complex with the Fab of an IL-27 blocking antibody, SRF388, currently being evaluated in a Phase I clinical trial in patients with advanced solid tumors (NCT04374877).
Methods: IL-27 receptor binding and blocking studies were conducted by surface plasmon resonance. Additional binding studies using IL-27 cytokine and receptor subunits were conducted by biolayer interferometry. Biological activity studies using SRF388, recombinant IL-27, or the EBI3 or p28 subunits were tested using the human U937 cell line or human PBMCs. The SRF388 Fab fragment was complexed with the IL-27 heterodimer in a 1:1 stoichiometric ratio, purified, and crystallized.
Results: The binding interactions between the cytokine and receptor subunits showed that IL-27 bound with strong

affinity to IL-27RA (21 pM). IL-27 binding to gp130 was weaker (4 nM) but was still required for signaling. SRF388 blocked the association of IL-27 with IL-27RA and inhibited all downstream signaling events. The EBI3/p28 heterodimer was required for high-affinity binding to IL-27RA and downstream signaling, as each subunit on its own failed to induce STAT1 phosphorylation. While p28 did not bind to either receptor subunit, binding between EBI3 and IL-27RA, but not gp130, was detectable (3.8 nM). The IL-27 subunits had nanomolar affinity for each other (5 nM) and associated into a biologically active heterodimer extracellularly. The structure of the IL-27/Fab complex was solved at 2.02 Å resolution. SRF388 Fab bound exclusively to p28 making contacts with the αA and αC helices of the p28 four-helix bundle and a portion of the poly-Glu sequence. EBI3 bound p28 at the hinge with contacts to the AB loop and the αB and αD helices of p28. Analysis of the p28 binding interface with EBI3 revealed multiple hydrophobic and salt-bridge interactions between the 2 halves.

Conclusion: These data highlight the structural interface responsible for the interactions between EBI3 and p28 that lead to a biologically active heterodimer that has high affinity for IL-27RA. The combination of blocking and structural data suggests that SRF388 inhibits binding of IL-27 to IL-27RA through steric hindrance at Site 2 of p28. Further studies are underway to resolve the structure of the IL-27RA in complex with IL-27.

Disclosure of Interest: J. Strand Shareholder of: Surface Oncology, Employee of: Surface Oncology, D. Logan Shareholder of: SARomics Biostructures AB, Employee of: SARomics Biostructures AB, M. Welin Employee of: SARomics Biostructures AB, J. Hua: None Declared, R. Gilligan: None Declared, M. Rausch Shareholder of: Surface Oncology, Employee of: Surface Oncology, G. Tan: None Declared, P. Holland Shareholder of: Surface Oncology, Employee of: Surface Oncology, J. Hill Shareholder of: Surface Oncology, Employee of: Surface Oncology, D. Moodley: None Declared

LT028

THE IL-2-STAT5 AXIS PROGRAMS HELPER T CELL METABOLISM UPSTREAM OF AKT, MTOR AND MYC

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Abstract Content: Similar to cancer cells, activated lymphocytes adapt their metabolism to meet energetic and biosynthetic demands imposed by rapid growth and proliferation. It has long been known that common gamma (cg) chain cytokines are pivotal in this process but, while numerous downstream mechanisms have been proposed, their relationship is unclear and involvement of the Jak/STAT pathway remains loosely defined. By integrating genome-, transcriptome- and metabolome-wide analyses, we demonstrate that the IL-2-STAT5 axis directly controls key aspects of carbon metabolism, amino acid synthesis and nucleotide synthesis in CD4+ 'helper' T cells. Mechanistically, STAT5 localizes to enhancers and promoters of a 50+ gene module encoding essential, often rate- limiting enzymes transporters and associated proteins, where it instructs transcriptional activity through p300 recruitment and epigenetic landscaping. Beyond autonomous transcriptional programing, systems level analysis also revealed a STAT5-driven signaling network with broad influence on T cell metabolism and involving three prominent metabolic agents, the PI3K-AKT and mToR pathways, and the transcription factor MYC. Taken together, our findings provide a molecular roadmap for transcriptional programing of T cell metabolism downstream of cg cytokines and emphasize the Jak/STAT pathway in powering T cell growth and proliferation.

Disclosure of Interest: None Declared

LT029

WHO IS THE WORST JAK/STAT PROTEIN OF THEM ALL? MODEL SYSTEMS TO EVALUATE THE CONSEQUENCES OF JAK/STAT DRIVER MUTATIONS

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Abstract Content: We know that distinct driver mutations of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway can lead to malignant cell transformation. However, their oncogenicity and lineage-specific transforming capacities have not yet been directly compared. Persistent activation of JAK/STAT signaling proteins promotes aberrant DNA binding, gene expression and chromatin structure. This is especially dominant in the hematopoietic system and in acute leukemias or lymphomas. We demonstrated in mouse models that gain of function JAK or STAT mutations can cause leukemias. Enhanced function of JAKs can lead to uncontrolled phosphorylation of diverse downstream proteins including STATs, whereas activating STAT mutations can increase DNA binding and gene expression. Especially STAT5B gain of function mutations can also promote oligomer formation via the N-terminus. Compared with dimers, STAT5 oligomers are able to bind to different DNA motifs and might be involved in chromatin looping. STAT3 and STAT5 were also shown to regulate the chromatin landscape by recruiting chromatin remodeling enzymes to the DNA. A detailed understanding of these interactions and their consequences will be important for therapeutic intervention strategies.

To gain deeper insights into how different JAK/STAT mutations influence gene expression, chromatin accessibility, oligomerization and chromatin formation in an isogenic background, we established two model systems. We introduced a broad panel of human JAK3, STAT3, STAT5A and STAT5B variants into Ba/F3 cells, a lymphoid/myeloid progenitor, or mouse embryonic fibroblasts lacking Stat5a/b. To investigate the role of the STAT5 N-terminus in oligomerization and oncogene expression, we also introduced oligomerization-deficient mutants. Overexpression of the different JAK/STAT mutants causes vast differences in cell proliferation, cytokine

independent growth and gene expression. We hypothesize that JAK/STAT driver mutations affect chromatin structure differently, through differential STAT oligomerization, DNA binding and interaction with chromatin remodeling enzymes, resulting in distinct oncogene activation and silencing of tumor suppressor genes.
Disclosure of Interest: None Declared

LT030
E3 UBIQUITIN LIGASE VON HIPPEL-LINDAU (VHL) PROTEIN PROMOTES TH17 DIFFERENTIATION
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Abstract Content: VHL is an E3 ubiquitin ligase that targets proteins, including HIF-1a, for proteasomal degradation. VHL and HIF regulate the balance between glycolysis and oxidative phosphorylation, which is critical in highly dynamic T cells. HIF-1a positively regulates Th17 differentiation, a complex process in which quiescent naïve CD4 T cells undergo transcriptional changes to effector cells, which are commonly dysregulated in chronic inflammatory diseases. The role of VHL in Th17 cells is not known. In this study, we hypothesized VHL negatively regulates Th17 differentiation and deletion of VHL in CD4 T cells would elevate HIF-1a and increase Th17 differentiation. Unexpectedly, we found that VHL promotes Th17 differentiation. Mice deficient in VHL in their T cells were resistant to an autoimmune disease, experimental autoimmune encephalomyelitis (EAE), often mediated by Th17 cells. *In vitro* Th17 differentiation was impaired in VHL deficient T cells. In the absence of VHL, Th17 cells had decreased activation of STAT3 and SMAD2, suggesting that VHL indirectly or directly regulates these critical signaling molecules. Gene expression analysis revealed that in Th17 cells VHL regulates many cellular pathways, including genes encoding proteins involved indirectly or directly in the glycolysis pathway. Compared to wild-type, VHL deficient Th17 cells had elevated glycolysis and glycolytic capacity. Our finding has implications on the design of therapeutics targeting the distinct metabolic needs of T cells to combat chronic inflammatory diseases.
Disclosure of Interest: None Declared

LT031
MAPPING AND DISSECTING THE POST-TRANSCRIPTIONAL LANDSCAPE OF CD69
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Abstract Content: Untranslated regions (UTRs) of mRNA transcripts are bound by RNA binding proteins (RBPs) and microRNAs during constitutive RNA metabolism and gene specific regulatory interactions. To study these RNA-protein interactions, we developed a protocol for Global Cross-Linking Protein Purification (GCLiPP) to map RBP occupancy transcriptome-wide. Our method identified RBP binding sites in mRNAs of human Jurkat T cells and mouse primary T cells that corresponded to known cis-regulatory elements. From our data, we identified CD69 as an immune transcript with multiple RBP binding sites in the 3'UTR, suggesting that the gene may be regulated on the post-transcriptional level.

CD69 is an inducible protein that is rapidly up-regulated on the cell surface of T cells during stimulation and swiftly down-regulated after activation. Interestingly, while effector memory cells remain CD69- in circulation, resident memory T cells constitutively express CD69 despite absence of antigen. How CD69 is regulated and the mechanism for the observed differential expression is unknown. Using our GCLiPP dataset, we dissected CD69 3'UTR using CRISPR-Cas9 in human and mouse T cells to identify post-transcriptional cis-elements which may be regulating the transcript and protein expression of CD69. We identified an RBP occupied region in the human and mouse 3'UTR that may regulate transcript stability.
Disclosure of Interest: None Declared

LT031b
CD4+ T CELL RECOGNITION OF HAEMAGGLUTININ EPITOPES ACROSS DIFFERENT INFLUENZA STRAINS
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Abstract Content: The 2019/20 SARS-Cov2 pandemic is a modern-day reminder of the impact that novel viral strains can have on a population without pre-existing immunity. The influenza A virus (IAV) has caused five pandemics in the last 150 years and is particularly prone to mutations in the surface glycoprotein haemagglutinin (HA) which is the primary target of the $CD4^+$ T cell and antibody responses.

$CD4^+$ T cells recognise peptide presented by human leukocyte antigen class II (HLA-II) molecules with their T cell receptor (TCR). Although the pHLA-II-TCR interaction is extremely specific, it may cross-recognise similar peptides leading to a cross-reactive response, providing broad protection against different IAV strains. The ability of $CD4^+$ T cells to cross-react with variants of a HA peptide from different IAV strains, especially those not in circulation, is not well understood. Furthermore, individuals expressing certain HLA-II molecules are known to be more susceptible to severe viral infections. In the context of IAV, HLA-DRB1*07:01 has been associated with poor response to the influenza vaccine whereas HLA-DRB1*11:01 has been associated with protection from viruses such as HIV and HCV. Investigation into the molecular basis underlying this association is warranted.

Using intracellular cytokine staining and HLA-II tetramer staining, we found that HLA-DRB1*11:01^+ individuals have more cross-reactive responses towards HA peptide derived from different IAV strains than HLA-DRB1*07:01^+ individuals, and that the cross-reacting $CD4^+$ T cells generally have lower polyfunctionality than specific $CD4^+$ T

cells. Moreover, we provide the first insight into the molecular and functional basis of IAV epitope presentation by different HLA-II molecules.

Disclosure of Interest: None Declared

Lightning Talk Session 1: Cytokine regulation I

LT032

EPIGENETIC AND TRANSCRIPTIONAL REGULATION OF CCL17 PRODUCTION BY DEXAMETHASONE

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Abstract Content: Clinical trials in rheumatoid arthritis (RA) targeting the cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF) are showing promise although its mode of action remains largely unknown. We have previously shown that that GM-CSF drives CCL17 production via a new interferon regulatory factor 4 (IRF4)-dependent pathway in human monocytes and mouse macrophages.
Glucocorticoids, such as dexamethasone (Dex), are potent anti-inflammatory and immunosuppressive agents broadly used in anti-inflammatory therapy, albeit with adverse side effects associated with long-term usage. The negative consequences of GC therapy provide an impetus for research into gaining insights into the molecular mechanisms of Dex action on immune cells.
We report here that GM-CSF-induced CCL17 expression is inhibited by Dex in human monocytes and mouse macrophages. Moreover, we provide evidence for the first time that Dex suppresses GM-CSF-induced IRF4 expression via regulating the expression and activity of JMJD3, which demethylases trimethylated-H3K27. Further, we demonstrate that Dex suppresses the expression of JMJD3. Significantly, we measured elevated levels of CCL17 in synovial fluid from patients with RA compared to healthy controls. Using synovial fluid mononuclear cells from RA patients, we provide molecular evidence for the anti-inflammatory actions of Dex through epigenetic regulation of IRF4 expression and downstream inhibition of CCL17 production. The delineated pathway potentially provides new therapeutic options for the treatment of inflammatory diseases and their associated pain.
Disclosure of Interest: None Declared

LT033

ARGINASE-2 REGULATES IL-10 MEDIATED METABOLIC REPROGRAMMING IN INFLAMMATORY MACROPHAGES.

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Abstract Content: The activity of metabolic pathways, such as glycolysis, the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) is regulated by cells via various signals, such as nutrient levels and antigen presentation. When it comes to the immune system, specific alterations in these metabolic pathways couple directly with their respective functions. For instance, it has been shown that LPS-stimulated inflammatory macrophages have downregulated oxidative phosphorylation (OxPhos) and upregulated aerobic glycolysis, whereas such cells have been shown to restore their oxidative capacity in the presence of the anti-inflammatory cytokine interleukin-10 (IL-10). We demonstrate here that Arginase-2 (Arg2), the mitochondrial isoform of arginase, is an IL-10 regulated gene in LPS-stimulated macrophages. Our aim was to understand the impact of Arg2 on IL-10's role as a metabolic rheostat in macrophages. We firstly showed that Arg2 is critical for IL-10 induced restoration of oxidative respiration in LPS-stimulated macrophages. This effect was lost in the absence of Arg2 as evidenced by the use of siRNA and Arg2 knock-out cells. Additionally, overexpression of Arg2 was sufficient to significantly enhance OxPhos compared to overexpression of an empty vector or the cytoplasmic isoform, Arg1. We further established that the catalytic activity of Arg2 was crucial for its regulation of oxidative respiration by using a chemical inhibitor of arginase, nor-NOHA, as well as by utilizing a catalytic-dead mutant. Mechanistically, we showed that both Arg2's presence and its enzymatic function is crucial for the activity of Complex II (succinate dehydrogenase (SDH)), a bi-functional enzyme that links the mitochondrial ETC and the TCA cycle. Additionally, an experiment coupling blue-native PAGE to liquid chromatography tandem mass spectrometry (LC-MS/MS) to characterize prospective protein-protein interactions, suggested that Arg2 binds to SDH complexes A and B, providing potential insight into how it regulates SDH activity. Finally, we delineated that Arg2's impact on oxidative metabolism is dependent on its own regulation by ERK1/2 and mTORC1 phosphorylation via site-directed mutagenesis studies. These findings shed light on the importance of a specific arm of metabolic regulation by Arg2, that governs the IL-10 induced oxidative state in inflammatory macrophages.
Disclosure of Interest: None Declared

LT034

HIGH EXPRESSION OF TNF TYPE 1 RECEPTORS INCREASED PRO-APOPTOTIC DOSE-DEPENDENT EFFECTS OF CYTOKINE IN PRESENCE OF TNF TYPE 2 RECEPTORS SIMULTANEOUS EXPRESSION, BUT NOT ALONE

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Abstract Content: Background:

Certain effect of cytokines on cell is mediated by number of parameters including change in receptor expression level or cytokine content. However, the possibility of modulating specific biological effects through the change in expression level remains poorly understood. Since two types of highly specific receptors for TNF (TNFR1 and TNFR2) have the ability to activate fundamentally different effects in cells, this system is a convenient object for studying possible regulation mechanisms. **This study aimed to** investigate the influence of TNF dose-dependent effect on the balance between pro-apoptotic and proliferation reactions depending on TNFR1/2 expression density parameters.

Methods:

Tumor cell lines (HEp-2, K-562, MCF-7, ZR-75/1, MOLT-4, A-549, Raji) cell lines after characterisation for TNFR1/2 co-expression using flow cytometry were studied for cell cycle. The dose-dependent effects of rhTNF on proportion of cells in apoptosis as well as in G1/G0, S, G2/M phases were studied. Associations among studied parameters were estimated using correlation and regression analyses.

Results:

For ZR-75/1 cells (cell line with hith expression of both types) it was found that a dose-dependent increase in the expression of both types of TNFα receptors on cells leads to a decrease in the proliferative activity of cells. For MOLT-4 cell (with lower expression) increase in proliferative response of cells was positively associated with the percentage of both TNFR1 + and TNFR2 + cells. However, for two other linesimilar expression profile was shown to be associated with opposite effect on cells. Both K-562 and MCF-7 expressed mainly 1 type of receptor (the total average percentage of TNFR1 + cells for the MCF-7 line is 66.8%±3.1%, for the K-562 line - 71.5% ±7.2%) and showed a statistically significant change in the relative number of cells in different phases of the cell cycle when different concentrations of TNFα are added to the culture. In both lines, the dose-dependent effect of the cytokine on the increase in TNFR2 + cells with an increase in the dose of TNFα was observed. However, MCF-7 cells responded to the mediator with an increase in proliferative activity, and K-562 cells, on the contrary, with a decrease. However, between lines with similar effects (K-562 and ZR-75/1), a similarity was observed in a large percentage of double-positive cells. No statistically significant dose-dependent effect of TNFα on the functional response of cells was found for the HEp-2, Raji, and A-549 cell lines.

Conclusions:

High expression of type 1 receptor for TNF is not always associated with predominant activation of pro-apoptotic pathways. However, with high simultaneous expression of both types of receptors, the proportion of double-positive cells is crucial for activation either pro-apoptotic or proliferation pathways.

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Disclosure of Interest: None Declared

LT035

SYSTEMIC IL-6, IFN-GAMMA AND TNF AS CANDIDATE BIOMARKERS FOR INFLAMMAGING AND THERAPEUTIC RESPONSE IN HTLV-1 ASSOCIATED NEUROINFLAMMATION

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Abstract Content: **Background**: Human T-cell leukemia virus type 1 (HTLV-1) is the causative agent of Adult T-cell Leukemia, but also causes HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). Although a majority remains asymptomatic, 2-5% of people living with HTLV-1 (PLWH) can develop HAM/TSP, an incapacitating neuroinflammatory disorder with similarity to primary progressive multiple sclerosis. No disease-modifying therapy is available for HAM/TSP but corticosteroids and other immunomodulators provide some clinical benefit. Thus, biomarkers to predict and monitor disease progression and therapeutic outcome are direly needed in HAM/TSP. We aimed to investigate systemic cytokine levels as possible biomarkers of immunopathogenesis in PLWH and of therapeutic response to corticosteroid pulse therapy in HAM/TSP. **Patients and Methods**: PLWH (n=92, of which 49 asymptomatic and 43 HAM/TSP) were recruited at the HTLV outpatient clinic of the "Emílio Ribas" Institute of Infectious Diseases (Sao Paulo, Brazil). For 33 HAM/TSP patients, follow-up samples were obtained after a mean of 4.6 (± 0.5) years of pulse therapy with methylprednisolone, with a total follow-up of 181 person-years. Osame neurological motor scale was measured in all PLWH at diagnosis and at clinical follow-up. Plasma cytokine levels of IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-gamma and TNF were quantified by Cytometric Bead Array (BD Biosciences). Statistical analysis was performed using non-parametric tests (Wilcoxon and Spearman correlation) using Prism 8.0. **Results**: With respect to demographics, gender did not influence systemic cytokine levels, whereas IL-6 (r=0.36; p= 0.00018) was positively correlated with age in PLWH. Regarding clinical status, we observed a significant increase in systemic IFN-gamma (p=0.007) and IL-17A (p=0.0001) in HAM/TSP patients, as compared to asymptomatics. After pulse therapy, patients were classified as responders (n=6) and non-responders (n=26) based on changes in Osame motor scale. All patients significantly decreased IL-17A levels after treatment (p=0.013) but only responders significantly decreased IFN-gamma levels after treatment (p=0.008). In addition, pre-treatment TNF levels allowed significant prediction (p=0.037) of therapeutic outcome. Although none of the cytokines were correlated with CD4, CD8 and NK cell levels quantified by flow cytometry at baseline, lower CD8 levels at diagnosis significantly predicted a stronger IL-17A decrease after treatment. **Conclusions**: 1. An age-related increase in circulating IL-6 levels indicates possible inflammaging in PLWH. 2. Both IFN-gamma and IL-17A are increased in untreated HAM/TSP. 3. Pretreatment TNF predicts therapeutic response to corticosteroid pulse therapy, whereas post-treatment IFN-gamma reliably reflects therapeutic outcome in responders. 4. Inflammatory

cytokines IL-6, TNF and IFN-gamma are promising candidate biomarkers for immunomonitoring of both PLWH and HAM/TSP patients, to be confirmed in independent cohort studies.
Disclosure of Interest: None Declared

LT036
UNIQUE EVOLUTION OF TYPE 3 INTERFERON GENES IN PLACENTAL MAMMALS REVEALS NOVEL REGULATORY MECHANISMS
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Abstract Content: The type 3 interferons (IFNs) – otherwise known as IFN lambdas (λ) - are an important family of antimicrobial cytokines that defend against infection, particularly at barrier tissues such as the gut, lung and placenta. Furthermore, recombinant IFNλs are being trialled against hepatitis D and SARS-CoV-2 infections. However, the development of IFNλ-targeted therapies with improved safety and efficacy is hampered by an incomplete understanding of fundamental IFNλ biology. To facilitate the exploration of IFNλs we apply a novel approach for the unbiased screening of vertebrate genomes (*n*=360) for IFNλ-like sequences, incorporating their genetic relationships and chromosomal location. Finally, we query this dataset to identify specific amino acid positions of evolutionary significance. We identify IFNλs in all major classes of vertebrates, showing that there are two major IFNλ lineages in tetrapods: IFNλ4-like (herein IFNλA) and IFNλ3-like (IFNλB). The IFNλ locus underwent a unique expansion in the ancestor of placental mammals generating the IFNλA and B lineages. These lineages display distinct evolutionary dynamics, with only the IFNλBs undergoing rapid diversification. While nearly all mammalian genomes were found to contain identifiable IFNλ-like sequences, IFNλ gene content differed by mammalian clade with most containing four IFNλs consisting of a single IFNλA and three IFNλBs. Although their early evolution appears to have been shaped by pressure to expand and diverge, it has since evolved by a combination of convergent episodic loss of genes across the entire IFNλ system and pervasive gene conversion at the major IFNλ locus. At a molecular level, we identify amino acid positions in structurally-relevant sites undergoing recurrent diversification indicative of functional importance. This work provides the first systematic understanding of IFNλ evolution across placental mammals, describing the diversity in these critical cytokines to facilitate the development of safe and effective IFNλ-targeted interventions.
Disclosure of Interest: None Declared

LT037
EVALUATION OF THE SERUM CYTOKINES PROFILE AND NITRIC OXIDE IN MURINE EXPERIMENTAL LEPROSY
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Abstract Content: Introduction: Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an obligate intracellular pathogen, thus, the resistance of the host to this pathogen depends on cellular immunity. In experimental leprosy, BALB/c mouse has limited growth of acid-fast bacilli (AFB) without dissemination to organs while nude mouse present bacillary multiplication and dissemination. **Objective:** Considering the scarcity of studies evaluating the immune response in these two strains of mice, this study aimed to evaluate the serum cytokines profile and nitric oxide (NO), at different stages of the infection. **Methods:** BALB/c and nude mice were inoculated in the footpads with *M. leprae* and blood was collected at 3, 5 and 8 months post-inoculation. The cytokines profile (IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ and TNF) and NO were evaluated. The total number of AFB in the footpads was determined**. Results:** Infection was progressive in the nude mice with bacillary multiplication in the footpads (2.5×10^7, 6.9×10^7 and 1.9×10^8 at 3, 5 and 8 months). In BALB/c mice, bacillary multiplication was more intense at 5 months (8.4×10^5) and there was no significant difference in the number of bacilli at 3 and 8 months (2.6×10^5 and 2.5×10^5). BALB/c mice showed increased levels of IL-2, IL-17, IFN-γ and TNF at 8 months when compared to the control group and decreased levels of IL-6 and NO, at the same times. Nude mouse showed increased levels of TNF at 8 months compared to the control group. No differences in NO levels were observed between these two leprosy experimental models. **Conclusion:** The results suggest the development of an active immune response in BALB/c mice able to control *M. leprae* multiplication in comparison to a progressive and disseminate infection in nude mice.
Disclosure of Interest: None Declared

LT038
SERUM LEVELS OF ADIPOKINES AND PRO-INFLAMMATORY CYTOKINES IN MALNOURISHED AND NOURISHED BALB/C MICE INFECTED BY LACAZIA LOBOI
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Abstract Content: Introduction: Adipose tissue presents neuroendocrine and immune functions, producing adipokines (adiponectin, leptin and resistin), as well as pro-inflammatory mediators such as TNF and IL-6. There is no study regarding the role of adipokines and its interaction with immune response in Jorge Lobo's disease (JLD), a chronic granulomatous cutaneous-subcutaneous fungal infection caused by *Lacazia loboi*. Aim: To evaluate the serum levels of adipokines and cytokines in malnourished and nourished

BALB/c mice infected by *Lacazia loboi*. Methods: Mice were divided into four groups: G1: infected with restricted diet, G2: not infected with restricted diet, G3: infected with regular diet,
G4: not infected with regular diet. Mice from G1 and G2 were submitted to malnutrition for 20 days before infection and kept with restricted diet during the experiment. After 4 months, mice were euthanized for the collection of serum and footpads. Serum levels of adipokines and cytokines was evaluated. The number of fungi and viability were
evaluated in the inoculated footpads. Results: Viability and number of fungi were higher in nourished than in malnourished mice. No differences in adiponectin levels were found. Leptin production was decreased in the malnourished groups (G1 and G2) while resistin levels was lower in infected groups (G1 and G3). Besides, IL-6 and TNF levels were lower in malnourished groups (G1 and G2). Conclusion: The process of malnutrition promoted a decrease in the production of leptin and pro-inflammatory cytokines in spite of infection, suggesting some interaction among these mediators, while the infection by *L. loboi* caused a decrease in resistin release. In an unexpected way the malnutrition status seems to favor the control of *L. loboi* infection by the host. Altogether, the results reveal a complex scenario in JLD involving nutrition and immunity. Supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (nº 2017/15664-2).
Disclosure of Interest: None Declared

LT039

NEIGHBORHOOD DEPRIVATION ASSOCIATES WITH DECREASED MONOCYTE TLR2 EXPRESSION VIA TNF-ALPHA POTENTIALLY CONTRIBUTING TO SARS-COV-2 DISPARITIES IN AFRICAN AMERICANS

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Abstract Content: Neighborhood deprivation (ND) as a measure of neighborhood-level socioeconomic status (SES) has been implicated as an important social determinant of SARS-CoV-2. SARS-CoV-2 disproportionally affects African Americans (AA) as well as individuals residing in more deprived neighborhoods. An upregulation of monocyte toll-like receptor (TLR2) surface marker expression (SME) has been shown in individuals infected with SARS-CoV-1, suggesting its importance for an adequate immune response to coronaviruses. In animal models of social defeat TLR2 and TLR4 SME were modulated, indicating their relevance in the body's response to social factors. But little is known about the relationship between ND and TLR2/4 SME modulation. Therefore, we utilized *ex vivo* and *in vivo* approaches to test the hypothesis that increasing ND is associated with monocyte TLR2 SME. NDI is defined by a U.S. Census-derived ND index (NDI; higher NDI=lower NSES). Furthermore, we aimed to identify a potential mediating role for pro-inflammatory cytokines as measured by an ELISA-based technique. In a first step, monocytes derived from a healthy donor were treated with serum from AA participants of a community-based cohort study in Washington DC without SARS-CoV-2 (n=84 AA (95% female), mean age 59.7 yr, BMI 33.5). Subsequently, TLR2 SME was determined by flow cytometry. Multivariable regression was used to examine the association between NDI and TLR2 SME. Sera-treated monocytes revealed that higher NDI was associated with a downregulation of TLR2 SME (ß=-0.34, p=0.03) after adjusting for atherosclerotic cardiovascular disease (ASCVD) 10-year risk score, BMI, and individual-level income. In a second step, monocyte TLR2 SME was determined *in vivo* from freshly drawn blood. Again, higher NDI was associated with lower monocyte TLR2 SME (ß=-0.34, p=0.02) after adjustments verifying our previous *ex vivo* results. In a third step, we determined that monocyte TLR2 SME displayed a significant negative association with serum-level tumor necrosis factor-alpha (TNF-α) (ß=-0.46, p=0.01) after adjustments. Structural equation modeling showed that TNF-α significantly mediated the relationship between NDI and *in vivo* monocyte TLR2 SME by 67.5% when using ASCVD 10-year risk score and BMI as covariates. In summary we determined that increasing NDI, potentially via increasing TNF-α levels, was associated with decreased monocyte TLR2 expression, a phenomenon which has been associated with decreased survival in SARS-CoV-1. Further studies are needed to identify the connection of monocyte TLR2 levels and SARS-CoV-2 severity or mortality as well as potential underlying mechanisms. However, understanding immunomodulating features of NDI may provide therapeutic insights in the fight against SARS-CoV-2, especially for individuals living in deprived neighborhoods.
Disclosure of Interest: None Declared

LT041

PLASMACYTOID DENDRITIC CELLS ENHANCE TLR-MEDIATED B-LYMPHOCYTE ACTIVATION AND DIFFERENTIATION IN AN STAT1 AND TYPE I IFN-DEPENDENT MANNER

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Abstract Content: B cells function to secret antibodies upon activation and differentiation, which play an important role in adaptive immunity. Plasmocytoid dendritic cells (pDCs), on the other hand, are a subset of DCs known for robust type I IFN (IFN-I) production upon TLR stimulation or viral infection and are crucial for antivirus response of innate immunity. B cells and pDCs can cooperate to boost the humoral immunity. However, the detailed mechanisms remain elusive. Here, we set up an in vitro coculture system of splenic pDCs and B cells and stimulated with, R848, a TLR7 agonist. pDCs enhanced TLR7-mediated B cell activation with increased expression of MHCII, CD86 and CD69. Moreover, increased proliferation and plasma cell differentiation but decreased survival and germinal center B cell (GC B) formation were also found through both soluble

factor- and cell-to-cell contact-dependent manner. In addition, follicular B cells (FO B) are more sensitive to pDC/R848 stimulation than marginal zone B cells (MZ B). Among soluble factors secreted in the coculture system, IFN-I was found to be critical for the enhanced response. B cells lacking IFNAR1 displayed impaired responses. Interestingly, IFN-I receptor signaling in pDCs contributed to the enhanced responses as well. A similar phenotype was also observed for cell-intrinsic requirement of STAT1 signaling for both B cells and pDCs. STAT1 was activated by serine 727 phosphorylation (pS727) in B cells directly by R848 stimulation. S727 to A mutation in STAT1 of B cells attenuated the synergism between pDC and B cells. Adoptive transfer of B cells into *Rag1*$^{-/-}$ mice that had been depleted of pDCs also reduced IgM production in response to R848, suggesting an essential role of pDC in T-independent antibody response in vivo. Collectively, we have identified that murine splenic pDCs directly enhance B cell activation, proliferation and plasma cell differentiation in vitro and in vivo, and define a crucial role of IFN-I and STAT1 signaling in both pDCs and B cells to positively modulate the humoral immunity.

Disclosure of Interest: None Declared

LT042

REVEALING THE HIDDEN HELP FROM IL-21: FOLLICULAR HELPER T CELLS RE-SENSITISE GC B CELLS TO IL-21 FOR PLASMA CELL DIFFERENTIATION

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Abstract Content: The differentiation of plasma cells from germinal centre (GC) B cells requires the selection signals from follicular helper T (Tfh) cells. However, how the competent GC B cells commit the plasma cell differentiation remains unclear. In this study, we demonstrate that the magnitude of GC B cells to plasma cell differentiation is in proportion to the signalling potency of Interleukin-21 (IL-21), a key effector cytokine produced by Tfh cells. Importantly, we revealed that the sensitivity of GC B cells to IL-21 is not determined by IL-21 receptor (IL-21R), but by the expression level of a novel coreceptor, which enhances IL-21 signalling strength by increasing the cellular bioavailability to IL-21. In GC B cells, the expression of this putative coreceptor is stringently regulated and progressively increased upon receiving the help signals from Tfh cells. GC B cells with the deficiency of this coreceptor showed prolonged retention in GCs and impaired GC B cells to-plasma cell differentiation. We propose a model whereby Tfh cell-mediated upregulation of IL-21 coreceptor enhances the sensitivity of GC B cells to IL-21 and thus promotes plasma cell differentiation. This model is complementary to antigen affinity-based selection of plasma cells and provide new insights in the formation of broad neutralising antibodies in infection and vaccination, and also for the generation of autoantibodies in autoimmune diseases.

Disclosure of Interest: None Declared

LT043

THE ROLE OF DNMT1 AND G9A METHYLTRANSFERASE IN VIRUS-ACTIVATED PDC IFN-ALPHA PRODUCTION IN HEALTHY DONORS AND COVID-19 PATIENTS

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Abstract Content: In chronic viral infections such as HIV, pDC initially produce a robust amount of IFN-alpha but then lose their ability to produce IFNs, with the cells themselves being rapidly turned-over. Our lab is investigating the role of DNA and histone methylation in virus-activated pDC cytokine response from healthy donors and individuals with COVID-19. DNMT1 proteins are known for their dual functions including 1) methylation at cytosine phosphate guanine (CpG) sites and, 2) recruiting histone deacetylases (HDACs), promoting histone methylation both of which are known to silence cytokine gene expression. To investigate the effects of virus activation on pDC, we isolated fresh PBMC from healthy donors and COVID-19 patients and stimulated with RNA viruses IAV or HIV-1, and DNA virus, HSV-1, which signal in pDC through TLR7 and -9, respectively, for 8 and 24 hrs. Flow cytometry was used to quantify the expression of DNA methyltransferase-1 (DNMT1) in pDC. We also treated pDC with the G9a methyltransferase inhibitor, BIX01294, and measured IFN-alpha and DNMT1 6-hr post stimulation with HSV and IAV. RNA from purified pDC was analyzed for expression of DNMT1 and G9a methyltransferase via qRT-PCR. More recently, pDC from hospitalized COVID-19 patients were stimulated with virus, and expression of DNMT1 and IFN-alpha was measured by flow cytometry. IAV and HSV-stimulated pDC, DNMT1 was significantly upregulated at 24-hrs compared to 8-hr stimulated and unstimulated controls. IFN-alpha production and DNMT1 expression in IAV-activated pDC, but not in HSV, was significantly inhibited by the G9a methyltransferase inhibitor, BIX01294. G9a methyltransferase mRNA was elevated in IAV and HIV-1-stimulated pDC, but minimally expressed in HSV-stimulated cells. Furthermore, hospitalized COVID-19 patients had decreased numbers of pDC as well as decreased IFN-alpha production, while DNMT1 proteins were elevated compared to healthy controls. Our studies indicate that DNMT1 is increased in virus-activated pDC at 24-hrs post-stimulation, a time when IFN-a production is turning off, possibly suggesting a role for DNMT1 in controlling excessive immune activation. Further, the reduction of DNMT1 in IAV, but not HSV-activated pDC upon treatment with BIX01294 implies a virus or TLR pathway (TLR7 vs -9)-specific role of DNMT1. In addition, decreased IFN-alpha production and elevated DNMT1 expression by COVID-19 patient pDC suggests that DNMT1 may play a vital role in silencing genes associated with IFN-alpha production, resulting in pDC dysfunction.

Disclosure of Interest: None Declared

LT044

IL-27 REGULATES THE MAGNITUDE OF ECTOPIC LYMPHOID STRUCTURES IN EXPERIMENTAL SIALADENITIS VIA CONTROL OF TH17 CELLS

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Abstract Content: Introduction: IL-27 is a key regulator of adaptive immunity and has both pro- and anti-inflammatory roles. One of its main functions is to exert immunomodulatory effects on CD4 T cells. For example, IL-27 inhibits the expansion of Th17 cells which secrete the pro-inflammatory cytokine IL-17. CD4 T cells, and in particular Th17 cells have been implicated as a driver of many autoimmune diseases and were previously shown to contribute to ectopic lymphoid structure (ELS) development. These organised aggregates of immune cells can harbour a functional germinal centre and are often present in autoimmune diseases where they correlate with worse disease progression. Here, we aim to elucidate the role of IL-27 in ELS formation in a murine model of inducible sialadenitis, using transcriptomic data.
Methods: We administered a replication deficient adenovirus (AdV) to the salivary glands (SG) of C57BL6 (WT) and IL27Rα-deficient (KO) mice via retrograde cannulation. IL-17A signalling was blocked using a neutralising antibody. Salivary gland samples were analysed using immunofluorescence, flow cytometry and RNAseq.
Results: In experimental sialadenitis in WT mice, the immune cell infiltration in the SG, was organised in aggregates by 12 days post cannulation (dpc) and fully formed ELS by 19 dpc. Temporally tracking the top upregulated genes showed that at early time points (1 and 5 dpc) genes involved in inflammation (*Cxcl11, Acod1, S100a8/a9 and Saa2/3*) were the most upregulated, whereas at 12 and 19 dpc T and B cell signature genes (*Icos, Cd19, Cxcr5 and Pax5*) were highly upregulated. IL-27 producing cells and IL-27 family gene expression were upregulated in the SG of WT mice. KO mice had larger and more numerous SG ELS and presented an expansion of Th17 cells compared to WT. Transcriptomic data corroborated this showing a strong and sustained ELS gene signature in the KO mice, between 12 and 19 dpc. IL-17 blocking in KO mice resulted in the reduction of ELS size and activity to levels comparable to WT, without affecting the ELS number. IL-17 blockade in KO mice also resulted in a gene signature more similar to WT with a reduction in expression of the ELS-related genes including *Aicda*, *Ccl19*, *Ltb* and *Cxcr5*. Furthermore, we detected an upregulation of Tfh cell and T regulatory cell-related genes at 19dpc in the KO compared to WT, that was only partially reduced upon IL-17A blockade.
Conclusion: Our data shows that IL-27 plays a critical role in regulating the magnitude and function of ectopic lymphoid structures, through control of Th17 cell expansion. This may provide a viable therapeutic option for treating patients with autoimmune diseases.
Disclosure of Interest: None Declared

LT045

TISSUE SOURCE OF ISOLATION INFLUENCES MESENCHYMAL STROMAL CELLS CHEMOKINE SECRETION AND THEIR IMMUNOMODULATORY PROPERTIES

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Abstract Content: Since the discovery of mesenchymal stromal cells (MSCs) ability to repair craniofacial defects and immunomodulatory properties, they became focus of research due to their cellular therapeutic potential. The Advanced Therapeutics Department within the Scottish National Blood Transfusion Service (SNBTS) has developed standardised methodologies for the isolation of MSCs from pancreatic islets (Is MSCs), adipose tissue (Ad MSCs) and bone marrow (BM MSCs). However, studying and comparing their *in vivo* function and the immunomodulatory potential is essential prior to their use within a clinical setting. The immunomodulatory abilities of human MSCs have been studied using mouse models that lack a control for mismatched major histocompatibility complex molecule expression. Thus, this study aimed to objectively compare the immune cell recruitment and potential immunomodulatory functions *in vivo* of murine BM, Is and Ad MSCs in a stringent, standardised manner, without species or gender mismatch that could lead to both cell–mediated and humoral immune responses.

Under resting conditions, MSCs from the three tissue sources were able to secrete chemokines at similar levels, were CCL2 was secreted the most; however, this secretion did not produce a recruitment of leukocytes above control levels. Inflammatory stimulation of MSCs with a cytokine cocktail (TNF-α, IL-1β, IFN-γ) led to the upregulation of the secretion of chemokines where Is MSCs secreted the highest levels of CCL2, CCL5, CXCL1 and CXCL10 while CXCL12 was secreted at higher levels by BM MSCs. CCL2, CCL5 and CXCL1 are strong chemoattractans, but despite the higher secretion by Is MSCs under inflammatory conditions, Ad MSCs were able to recruit significantly more leukocytes *in vivo* than BM and Is MSCs. More importantly, Ad MSCs were the only MSCs able to produce the recruitment of T cells. Recipient cytotoxic cells are considered detrimental in clinical settings but they are essential to initiate MSC–mediated immunosuppression; thus, we could hypothesize that Ad MSCs have a greater immunosuppression potential than BM and Is MSCs.

Chemokines not only have leukocyte recruitment properties as examples, CXCL1, CXCL2 and CXCL12 have angiogenic properties while CXCL10 has angiostatic potential. Chemokines are not the only molecules secreted by MSCs with the potential to regulate angiogenesis. Ad MSCs secreted the most IL–6, which can promote VEGF secretion,

but Is MSCs secreted the most VEGF under resting and stimulatory conditions, which combined with the increased secretion of CXCL1, CXCL2 and CXCL12, we hypothesize that Is MSCs could have greater re–vascularisation potential.

Altogether, this study highlights that MSCs from different sources differ in their ability to recruit and immunomodulate surrounding immune cells *in vivo*. These differences have the potential to influence their clinical performance.
Disclosure of Interest: None Declared

LT046

AMBRA1 REGULATES CYTOKINE SIGNALLING BY TARGETING THE SUPPRESSORS OF CYTOKINE SIGNALLING FAMILY FOR PROTEASOMAL DEGRADATION

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Abstract Content: Introduction: The canonical suppressors of cytokine signalling (SOCS) family proteins include CIS and SOCS1-7, with CIS and SOCS1-3 acting as classical negative feedback inhibitors of cytokine signalling pathways. The SOCS proteins possess an N-terminal domain of varying length, a central SH2 domain, and a C-terminal SOCS box motif. In response to cytokine activation of downstream signalling pathways, SOCS proteins bind to phosphorylated target proteins via their SH2 domain and facilitate proteasomal degradation of the target by recruiting an E3 Cullin 5-RING ligase (CRL5) complex through their SOCS box motif. The CRL5 complex is utilised by more than 80 human SOCS box-containing proteins and consists of the adaptors Elongins B and C, the RING protein Rbx2 and Cullin 5. Recently, the E3 Cullin4-RING ligase (CRL4) AMBRA1 was shown to regulate $CRL5^{SOCS3}$ activity by targeting Elongin C for proteasomal degradation [1]. However, the exact mechanism/s by which SOCS3 and other SOCS family members are regulated, remains to be elucidated.

Methods: An affinity purification-mass spectrometry (AP-MS) approach was utilised to identify proteins which might regulate SOCS levels. Co-immunoprecipitation and immunoblotting were used to validate AP-MS findings and investigate cytokine signalling responses. CRISPR/Cas9 gene editing was used to fully or partially delete AMBRA1 in HEK293T and A549 cell lines. Q-PCR was used to assess SOCS transcriptional profiles.

Results: AMBRA1 was identified by AP-MS in CIS protein complexes, together with known $CRL5^{CIS}$ complex members, Elongin B, Elongin C, Cullin-5, and Rbx2. Co-IP studies confirmed that, in addition to CIS, SOCS1-7 were able to interact with AMBRA1 and this interaction occurred via their respective SOCS box motifs. Reduction of AMBRA1 levels in HEK293T and A549 cell lines resulted in enhanced SOCS inhibition of interferon gamma (IFNγ), growth hormone (GH), and leukaemia inhibitory factor (LIF) signalling. Ongoing work will assess cytokine induction of SOCS mRNA levels in these cell lines and determine whether the SOCS proteins themselves are direct substrates for $CRL4^{AMBRA1}$ mediated ubiquitination and proteasomal degradation.

Conclusion: In this study AMBRA1 was validated as a prominent interactor of the CRL5 SOCS box complex and shown to modulate SOCS1 and SOCS3 activity, two important negative regulators of inflammatory cytokine signalling. This further suggests that AMBRA1 will have a key role in regulating multiple members of the greater SOCS box family, most likely by ubiquitinating and directing them to proteasomal degradation.

Disclosure of interest: None declared

References

1. Chen, S.H., et al., *CRL4(AMBRA1) targets Elongin C for ubiquitination and degradation to modulate CRL5 signaling.* EMBO J, 2018. **37**(18).

Disclosure of Interest: None Declared

LT047

IL-6 TYPE CYTOKINE SIGNALLING FROM AN INFORMATION THEORETIC POINT OF VIEW – MECHANISMS TO REDUCE UNCERTAINTY OF SIGNALLING

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Abstract Content: Cytokines of the IL-6-type cytokine family, which are characterised by the shared use of the signal transducing β-receptor gp130, are involved in a variety of processes in both health and disease. The orchestration of these processes critically depends on reliable intracellular signalling. It is remarkable that the expression and activation of signalling proteins differ markedly even between isogenic cells of the same cell type within a living organism. Obviously, this cell-to-cell variability does not prevent reliable IL-6-type cytokine signalling.
The aim of this study is to elucidate molecular mechanisms, that allow reliable IL-6-type cytokine signal transmission despite cell-to-cell variability. Despite the common activation of the signal transducing β-receptor gp130 and subsequent JAK/STAT signalling, individual IL-6-type cytokines execute distinct functions. Whether these distinct functions are accompanied by different molecular mechanisms governing

reliable signalling is unclear. To address these questions we apply multiplexed single cell analyses and information theoretic measures to investigate robustness and Channel Capacity of IL-6-type cytokine-induced JAK/STAT signalling.
Interestingly, different mechanisms that enable robustness of IL-6-induced JAK/STAT signalling against varying STAT3 expression complement each other at different time scales and cytokine doses. Early IL-6-induced STAT3 activation is robust as long as cytokine-concentrations are low to intermediate. Robustness at high cytokine concentrations is ensured by high STAT3 protein copy-number or by STAT3 serine phosphorylation. At late time-points the kinase-inhibitor SOCS3 increases robustness. Time-complementary to the late-acting SOCS3, the protein tyrosine phosphatase SHP2 increases robustness of IL-6-induced STAT3 signalling at early time-points.
The amount of information transmitted by JAK/STAT signalling is quantified by Channel Capacity. Channel Capacity of IL-6-induced JAK/STAT signalling is limited by cell-to-cell variability in STAT3 expression. Additionally, Channel Capacity is affected by the same mechanisms that govern robustness. An increase in STAT3 copy number increases both Channel Capacity and robustness, whereas an increase in STAT3 tyrosine phosphorylation reduces robustness but increases Channel Capacity.
In summary, the application of information theory is an elegant and intuitive way to elucidate the mechanisms that prevent disease-associated dysregulated signalling by enabling reliable JAK/STAT signalling despite cell-to-cell heterogeneity.
Disclosure of Interest: None Declared

LT048

CHRONIC PAIN INDUCED UPREGULATION OF NLRP 2 INFLAMMASOME IN THE RAT SPINAL DORSAL HORN

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Abstract Content: Background
Long-term noxious stimulation related to chronic inflammation lead to central sensitization of neural circuit within the spinal dorsal horn. It is widely accepted that inflammasomes play a cardinal role in this process. Inflammasomes are regarded as key signaling platforms that detect pathogenic microorganisms and activate the proinflammatory cytokines such as IL-1β. Precursor of the IL-1β is cleaved by the protease caspase-1 converting it to a mature biologically active form. In our previous results we demonstrated a significant enhancement in the IL-1β expression in astrocytes, therefore our next aim was to identify the potential inflammasomal type(s), which may orchestrate the formation of IL-1β.
Materials and methods
Male Wistar rats (n=12) were used for animal experiments. Inflammatory pain was evoked by unilateral intraplantar injection of complete Freund adjuvant (CFA). The mechanical nociceptive threshold of control and CFA-treated animals was measured daily for withdrawal responses before and after CFA administration with modified von Frey method. The distribution of NLRP receptors was investigated with immunoperoxidase method, and single immunofluorescent labelings. After CFA injection expressional changes of NLRP1, NLRP2 and NLRP3 proteins were investigated by double immunofluorescent labelings and Western blotting. The colocalisational values with GFAP marker were obtained by confocal microscopy and IMARIS software.
Results
After CFA injection the mechanical withdrawal threshold of the treated animals was significantly reduced. Studying the distribution of NLRP receptors, immunoperoxidase reactions confirmed the presence of NLRP proteins in the pain-processing Rexed lamina I-II of the spinal gray matter. We found significant increase of the NLRP2 but not NLRP1 or NLRP3 protein levels within the spinal dorsal horn three days following CFA injection. According to the findings of double fluorescent labelings only NLRP2 protein was abundantly expressed by astrocytes in chronic pain conditions.
Conclusion
Here we provide evidence that the spinal astrocytes are the major source of IL-1β in CFA-induced inflammatory pain and the production of mature IL-1β is induced by the NLRP2 inflammasome at the peak of mechanical pain sensitivity. The currently available literature on NLRP2 protein in CNS is scanty, and these works mainly focus on its effects in reproductive system and embryonic development.

Keywords: interleukin-1β, NLRP2, inflammatory pain, spinal cord, astrocyte,
Disclosure of Interest: None Declared

LT049

REVERSE-SIGNALING OF SOLUBLE TNF RECEPTOR I BINDING TRANSMEMBRANE TNFA INDUCES A WAKE-LIKE STATE IN VITRO

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Abstract Content: Introduction
Tumor necrosis factor alpha (TNF) is involved in brain development and regulating physiological sleep and sleep responses to pathological insult. Cell activity affects neuronal TNF expression; increased action potentials (APs) enhance expression. Neuron/glia co-cultures treated with TNF exhibit a sleep-like state. Binding of soluble TNF receptors (sTNFR) to transmembrane (tm) TNF induces reverse cell signaling, contrary to conventional signaling induced by sTNF binding tmTNFRs. We previously inhibited sleep *in vivo* by administering sTNFR. Thus, we hypothesized tmTNF-sTNFR binding would generate reverse signaling *in vitro* and induce a wake-like state.
Methods
Wildtype (WT) mice and mice lacking either TNF (TNFKO) or both TNFRs (TNFRKO) provided somatosensory cortical neurons/glia for co-cultures on six-well multi-electrode arrays (MEAs). Cells were allowed three days of undisturbed, incubated development. Developmental

analyses used daily one-hour MEA recordings taken on days four through 14. A one-hour baseline was recorded on day 14 immediately prior to sTNFR1 treatment (0.0 ng/µL-120 ng/µL). Additional one-hour recordings were made immediately after treatment and 24 hours later. Measures for *in vitro* development rates and treatment-induced changes were identical to those defining *in vivo* sleep: synchronization of electrical activity (SYN), APs per second (APs/s), slow wave delta power (SWP; 0.25-3.75 Hz), and burstiness index (BI).

Results

Developmental rates were highest in TNFKO cells, lowest in WT cells. sTNFR1 treatment response was decreased in TNFKO cells compared to TNFRKO and WT cells. Dose-dependent response varied across time and between TNFKO and TNFRKO cells. TNF-expressing TNFRKO cells showed decreased SYN and SWP indicating a wake-like state that was abolished 24 hours post-treatment.

Conclusion

We provide the first evidence of reverse TNF signaling in sleep/wake states and offer a new perception of sleep state regulation for potential clinical applications.

Disclosure of Interest: None Declared

LT050

MICROBIAL- AND HOST-DERIVED STIMULI ACTIVATE REACTIVE OXYGEN SPECIES AND GENERATE ROBUST CYTOKINE RESPONSES IN HUMAN INTESTINAL ORGANOIDS

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Abstract Content: Background: The epithelium of the gastrointestinal tract represents a key interface between the luminal environment of the gut and the host immune system. Intestinal organoids provide a unique system to explore this epithelial-cross-talk. Organoids can be derived from human intestinal stem cells and differentiated into all the intestinal cell types: enterocytes, goblet cells, paneth cells, enteroendocrine cells, *etc*. Moreover, they are non-transformed, non-cancer-derived and propagatable. Based on their similarity to normal intestine, many researchers have begun to use this tool to examine epithelial signaling. However, current human organoid systems have been refractory to stimulation of cytokine production due to the presence of antioxidants in culture media. We have made several technical refinements to create human organoids capable of producing measurable amounts of cytokines. Using this new system, we hypothesize that human intestinal organoids will respond to pro-inflammatory stimuli derived from host and microbes in unique patterns. **Methods & Results:** Human intestinal organoids derived from the jejunum of healthy volunteers were grown as two-dimensional monolayers. RNAseq revealed expression of TLRs, cytokine receptors and NOD receptors. To assess cytokine responses, monolayers were treated apically with microbial stimuli (lipopolysaccharide (LPS), lipoteichoic acid (LTA), and flagellin), host stimuli (IL-1α, and TNF), or microbial- and host-produced histamine in either complete organoid media or an optimized minimal media. Secreted cytokines were examined by Luminex Magpix in the supernatant after 16 hours of incubation. Minimal cytokine production was observed in organoids incubated in the commonly used complete human intestinal organoid medium. In contrast, our optimized organoid media resulted in the production ROS, as assessed by H_2DCFDA fluorescence, and robust cytokines in response to select microbial- and host-derived stimuli. Of the host-derived stimuli, 1 µg/ml TNF stimulated the largest number of epithelial cytokines (13 cytokines; >2 fold change to media control); TNF stimulated IL-8, IL-1α, IL-1α, IL-1Rα, IL-7, IL-15, EGF, GRO, GM-CSF, IP-10, MCP-1, MCP-3, MDC, and TGF-α. IL-1α (1 µg/ml) only stimulated 9 cytokines: IL-8, TNF, IL-1Rα, EGF, G-CSF, IL-15, IL-7, GRO, and MDC. While LPS has been shown in a number of cancer-derived cell lines to stimulate IL-8 and other cytokines, in human organoids LPS (1 µg/ml) only stimulated TNF. Moreover, LTA (1 µg/ml) only stimulated GRO. In contrast, flagellin (100 ng/ml) stimulated several cytokines: IL-8, TNF, GRO, MCP-1, and MCP-3. The functionality of organoid secreted cytokines was confirmed using Boyden chambers with human neutrophils. Interestingly, although histamine receptor 1 was identified by RNAseq, no concentration of histamine (0.1-100 µg/ml) stimulated any cytokine production. **Conclusions**: This work is among the first to identify the optimal media conditions to generate human organoids that are amenable to microbial and host-derived stimuli. These findings pave the way for studying host and microbial interactions in a physiologically relevant model.

Disclosure of Interest: None Declared

LT051

PBMC AND LYMPH NODE TRANSCRIPTIONAL RESPONSES TO ZIKA VIRUS INFECTION IN PIGTAIL MACAQUES

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Abstract Content:

Background: Zika virus (ZIKV) is a pathogenic flavivirus primarily transmitted by *Aedes* mosquitoes that causes an acute febrile illness. Monocytes are a major cellular target during acute ZIKV infection, with innate immunity shaping ZIKV tropism and persistence. Here, we used a nonhuman primate model to investigate innate immune responses in whole blood and lymphoid tissue during acute ZIKV infection. We performed whole transcriptome sequencing on longitudinal PBMC samples and lymph node, a known anatomical site of ZIKV persistence.

Materials & Methods: Four adult male PTM were inoculated with ZIKV subcutaneously at multiple sites in the forearms. Whole blood and lymph node biopsies were collected throughout the 30-day experimental time course. We performed total RNA-seq on PBMC samples at baseline (5 days before inoculation), 1-4, 7, 14 and 23 days post-inoculation (dpi), and lymph node tissue biopsied at baseline, 7, 14, and 21 dpi. Gene Set Enrichment Analysis (GSEA) of the whole transcriptome was performed for each time-point to identify functional categories responding to ZIKV infection. A ZIKV qRT-PCR assay was used to detect viral RNA copy numbers in samples.

Results: Viremia peaked 2-3 dpi and was completely resolved in the blood in all animals by day 7 p.i. In contrast, ZIKV RNA persisted in the peripheral lymph node (PLN) to 21 dpi. Differential expression analyses of PBMC samples revealed gene enrichment related to *interferon signaling* and *RIG-I-like receptor signaling* pathways, with increased differential gene expression between day 2-4 p.i. There was a prominent innate immune and cytokine signaling gene signature at 4 dpi. Differentially expressed genes involved in the PBMC antiviral innate immune response included *DDX58* (encoding RIG-I) and *TRIM25*. Several interferon-stimulated genes were strongly upregulated, including *IFIT1/3/5*, *OAS1-3* and *STAT1/2*. By 7 dpi an inflammatory gene signature had evolved, with gene enrichment related to *IL-1 production*, *granulocyte activation*, *neutrophil-mediated immunity*, and *chemokine production*. In contrast to PBMCs, there was little change in gene expression in PLN samples, despite the persistence of ZIKV RNA in the lymphoid tissue.

Conclusions: Our data sets and analyses define immunologic profiles linked with acute ZIKV infection. While PBMCs mount a strong antiviral innate immune response early in ZIKV infection, we hypothesize that the muted response in the PLN contributes, in part, to the persistence of viral RNA in this anatomic site. This may have consequences on the transmission of ZIKV after clinical symptoms resolve.

Disclosure of Interest: None Declared

LT052

CHRONIC PSYCHOSOCIAL STRESS AGGRAVATES SOCIAL FEAR CONDITIONING: THE ROLE OF THE IMMUNE SYSTEM

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Abstract Content: Chronic psychosocial stress is a risk factor for somatic and affective disorders, including posttraumatic stress disorder (PTSD). Human and animal studies suggest that an over-reactive immune system accompanies stress-associated disorders and is involved in their pathogenesis. The mouse model of chronic subordinate colony housing (CSC) mimics this type of health-compromising stress and induces behavioral, physiological, and immunological alterations described in PTSD patients. However, developing PTSD requires a traumatic event that is actively avoided. The social fear conditioning paradigm (SFC) is a mouse model that induces social fear and social avoidance by pairing social investigation with electric foot shocks. Here, we tested the hypothesis that (i) prior CSC exposure potentiates the effects of SFC, and (ii) this is accompanied by central and peripheral changes in immune activation. Therefore, male mice were exposed to 19 days of CSC before they were tested in the SFC paradigm. During social fear acquisition (day1), fewer shocks were needed to reliably evoke social fear in CSC mice compared to single housed controls. Moreover, CSC mice displayed a delayed extinction of social fear (day2), which was still visible during recall (day3). Regarding the immune system, *in vitro* spleen and lymph node responsiveness, plasma cytokine levels as well as central immune markers including microglia and cytokine expression were differentially regulated following CSC, SFC, or their combination. Supported by DFG Ne465/33-1 and BMBF (OptiMD).

Disclosure of Interest: None Declared

LT053

ZIKA VIRUS DYSREGULATES TROPHOBLAST INVASION VIA INHIBITION OF LIF-MEDIATED STAT3 SIGNALING

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Abstract Content: Zika virus (ZIKV) is a flavivirus that causes fetal infection and disease including fetal demise. During pregnancy ZIKV infects the placenta leading to alteration of the maternal-fetal interface and placental damage but the virus/host interactions that underlie placental infection and disease are not defined. Human placenta has three layers, with distinct cell types in each; trophoblasts, mesenchymal cells, macrophages (Hofbauer cells) and fibroblasts. Trophoblasts are epithelial cells that form the outer layer of the blastocyst and are major cell types of the placenta that are responsible for invading maternal decidua to facilitate implantation. Trophoblast differentiation and invasion are critical for implantation and proper exchange of oxygen and nutrients during the development. Trophoblast invasion is mediated by specific trophoblast type and is controlled through JAK/STAT signaling by developmental cytokines. During development Leukemia inhibitory factor (LIF) binds to LIF receptor and gp130 on trophoblasts to induce JAK/STAT signaling to mediate the phosphorylation and activation of STAT3. Active STAT3 then directs the expression of genes that mediate trophoblast invasion and implantation. Our previous studies show that ZIKV imparts a broad blockade to JAK/STAT signaling that includes a block to STAT3 activation. During infection of first trimester trophoblast cell lines we hypothesize that ZIKV blocks STAT3 activation to control LIF-regulated JAK/STAT signaling. We used an in vitro invasion/migration model, immunoblotting and immunofluorescence (IF) staining to test this hypothesis. We

show that ZIKV infection disrupts LIF-induced trophoblast-matrix interactions concomitant with STAT3 suppression. Our findings suggest that ZIKV infection of trophoblasts and suppression of LIF dependent STAT3 signaling can impact critical steps of implantation to disrupt placental development.
Disclosure of Interest: None Declared

LT054

ADARZA'S ZIVA MULTIPLEX PROTEIN DETECTION PLATFORM OFFERS A NEW ULTRA-SENSITIVE ASSAY FORMAT FOR FEMTOGRAM LEVELS OF SENSITIVITY

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Abstract Content: We have used Arrayed Imaging Reflectometry (AIR™), a label-free protein microarray technology that relies on the target binding-induced perturbation of an antireflective coating on the surface of a silicon chip, to create a multiplex antibody array for the detection of both low and highly abundant targets. ZIVA is a new multiplex protein detection platform that leverages AIR™ technology and can multiplex up to 100 unique protein targets.We have previously detailed our Human Cytokine Screening Array, an assay that footprints a large number of high and low abundant proteins with sensitivity ranging from picogram to nanogram/ml (analyte dependent and optimized).In this presentation, we demonstrate a newly developed mass enhancement option that increases sensitivity down to low picogram and femtogram/ml levels. Using this mass enhancer, we have developed an 18-plex Human Ultra-Sensitivity Cytokine Array and have validated the array to include linearity, spike-recovery, and reproducibility. With array capabilities offering affordable assays from one to hundreds of plex combined with a hands-free, automation-friendly workflow enabled by the ZIVA instrument, the technology has been designed to achieve "tunable" levels of sensitivity empowered by AIR™ technology and novel mass enhancers.
Disclosure of Interest: None Declared

LT055

IDENTIFICATION OF IL-18BP-PRODUCING CELLS USING NEWLY DEVELOPED TDTOMATO REPORTER MICE

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Abstract Content:
Introduction: Interleukin (IL)-18 is a pro-inflammatory cytokine, the activity of which is regulated by its natural inhibitor IL-18 binding protein (IL-18BP). If the balance between IL-18 and IL-18BP is dysregulated, abnormal levels of free bioactive IL-18 (fIL-18) are present in the circulation, as is the case in sera of patients with macrophage activation syndrome (MAS). We showed that endogenous Il-18bp exerts a protective role in a murine model of MAS induced by repeated injections of the TLR9 agonist CpG. Il-18bp production is strongly increased in liver, lung, and spleen in this model. *In vitro* studies have shown that IL-18BP mRNA is expressed by a variety of cell types (myeloid, endothelial and epithelial). However, IL-18BP-producing cells have been poorly described *in vivo*. This study aimed to identify Il-18bp-producing cells at steady state and following CpG injections in blood, spleen, liver and lung.
Methods: As Il-18bp is a secreted protein, the identification of Il-18bp-producing cells by immunostaining is bound to be unreliable. Thus, we generated an Il18bpTomato knock-in (KI) mouse line carrying a nuclear tdTomato reporter inserted upstream of the first coding exon of the *Il18bp* gene to visualize Il-18bp-producing cells by flow cytometry and immunofluorescence (IF). The tdTomato reporter and Il-18bp coding sequences are separated by an A2 self-cleavable peptide, allowing independent production of the reporter and the Il-18bp protein.
Results: In comparison to WT littermate controls, Il-18bp expression was impaired in Il18bpTomato KI/KI mice suggesting that insertion of the targeting construct partially interferes with basal and CpG-induced Il-18bp expression. Nevertheless, tdTomato mRNA levels reliably mirrored relative Il-18bp expression in different organs of naïve and CpG-treated mice and the reporter protein production allowed for identification of Il-18bp producing cells. In naïve mice, we identified three Tomato $(Tom)^+$ cell populations based on the intensity of fluorescence of the reporter: Tom^{low}, Tom^{int} and Tom^{high} by flow cytometry. The Tom^{low} cells likely correspond to $F4/80^+$ $CD11b^+$ monocytes, macrophages, and/or granulocytes. A large population of $Ly6G^+$ Tom^{int} neutrophils was identified in spleen and in the circulation. Interestingly, all neutrophils analyzed were Tom^{int}. In liver and lung, Tom^{int} cells corresponded respectively to $CD146^+$ Liver Sinusoidal Endothelial cells (LSEC) and $CD31^+$ endothelial cells. In addition, 44% of $F4/80^+$ $CD11b^-$ red pulp macrophages (RPM) and 34% of Kupffer cells (KC) were Tom^+ in spleen and liver, respectively. Finally, we observed red fluorescence in liver $F4/80^+$ macrophages and in stabilin-2^+ LSEC by IF.
Conclusion: We identified neutrophils, endothelial cells and resident macrophages as major Il-18bp-producing cells in the circulation, spleen, liver and lung of naïve mice. This project is currently pursued for a precise description of the different cell types involved in Il-18bp production in CpG-induced MAS.
Disclosure of Interest: None Declared

LT056

MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IS RELEASED BY NECROTIC MONOCYTES/MACROPHAGES

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Abstract Content: Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory molecule with both cytokine and non-cytokine activity. MIF is constitutively released from multiple cell types via an unconventional secretory pathway that is not well defined. Here, we show

that both mouse immortalised bone marrow-derived macrophages (iBMM) and human THP-1 monocytic cells constitutivelt release relatively high levels of MIF in culture and rapidly replenish extracellular MIF following a change of culture medium. Previous studies have also shown that MIF can be released in response to different inflammatory stimuli, including the TLR4 agonist lipopolysaccharide (LPS). Here, we looked at MIF release from human and mouse monocytes/macrophages in response to different stimuli. In iBMM and THP-1 cells, MIF release was not significantly altered in response to LPS, the TLR2 agonist PAM3CYSK3, the TLR7/8 agonist resiquimod (R848) or heat-killed *Escherichia coli.* However, cytotoxic stimuli strongly promoted release of MIF. In particular, MIF release was highly upregulated in cells undergoing necrosis, necroptosis and NLRP3 inflammasome dependent pyroptosis. Our data suggest that cell death represents a major route for MIF release from myeloid cells. The functional significance of these findings and their potential importance in the context of autoimmune and inflammatory diseases, particularly those in which cell death and defective clearance of apoptotic cells are drivers of pathology, warrants further investigation.

Disclosure of Interest: None Declared

LT057

GENDER DIFFERENCES IN INFLAMMATION-RELATED BLOOD PRESSURE SETTING

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Abstract Content: The relationships between inflammation and hypertension have long been observed. Although a major trigger to inflammation-related hypertension is obesity and/or unhealthy lifestyle, many mechanistic details remain unclear. This study aimed to examine possible relationships between blood pressure (BP) and levels of inflammatory cytokines among healthy eastern Taiwanese (a Southeast Asian [SEA] population).

Methods: Adults subjects were recruited at the Health Checkup Center of Taitung MacKay Memorial Hospital (TT-MMH), with approval of the MMH Institutional Review Board. Besides the general physical assessment, recruited subjects were asked to fill out a questionnaire that assessed their lifestyle. Their plasma samples were tested for IL-1a, IL-6, and IL-18.

Results: The data from age-matched, healthy subjects (116 male & 110 female) were selected for analyses. The averaged body-mass indices (BMI) for both gender groups were similar (male: 25.7+/-3.9; female: 25.2+/-5.7). The averaged BPs for male subjects were 126.4+/-15.9 mmHg (systolic) and 84.0+/-12.7 mmHg (diastolic), while the averaged BPs for female were 115.8+/-13.9 mmHg (systolic) and 73.5+/-9.9 mmHg (diastolic). Both gender groups reported similarly scores of sleep quality. The male group in average exercised twice more often than the female group per week. By correlation analyses, we observed a roughly direct relation between BMI and systolic BP (SBP) as expected. The relations between IL-1a and SBP, between IL-6 and SBP, and between IL-18 and SBP, were all loosely directly correlated in these healthy SEA subjects. Since BP is affected by gender differences, we repeated the analysis by separating male and female groups. The direct correlations between SBP and each of the inflammatory cytokines were more pronounced in female than in male subjects. But the healthy male subjects showed significantly higher levels of plasma IL-1a, IL-6, and IL-18 than their female counterparts ($p^* < 0.0001$ for each cytokine comparison).

Conclusion: From analyzing the SEA data, the blood pressure setting of healthy individuals could be directly correlated with the plasma levels of inflammatory cytokines IL-1a, IL-6, and IL-18. Yet the levels of these cytokines likely were also affected by sexual hormones, as demonstrated here that the levels were generally much higher for similarly healthy male subjects than female subjects. Our findings suggest that the gender factor should be taken into consideration for inflammation-related hypertension.

Disclosure of Interest: None Declared

LT057b

CYTOKINE RESPONSES AND TISSUE PATHOLOGY IN NLRP12-DEFICIENT MICE IN A MODEL OF DIET-INDUCED OBESITY (DIO)

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Abstract Content: Metabolic syndrome is named for a group of risk factors that predisposes patients to cardiovascular disease and type 2 diabetes (T2DM). These risk factors include obesity, elevated serum cholesterol, triglycerides, blood pressure and elevated fasting glucose. Significantly increased visceral adipose tissue depots (VAT) can cause central obesity, hallmarked by a chronic, low grade inflammation. Obese, diabetic patients have increased levels of circulating pro-inflammatory cytokines, such as TNF, IL-6, MCP-1, and the IL-1 family members, IL-1β and IL-18. These pro-inflammatory cytokines originate from multiple sites, including both the liver and from infiltrating phagocytes of the white VAT. Though our understanding of the pathways that trigger insulin resistance has grown, and includes activation of several innate immune signaling pathways, including Toll-like receptors (TLR) and the Nod-like receptor (NLR), NLRP3, the complex interaction between innate immune signaling and metabolism is only starting to be appreciated. We tested the role of NLRP12 in a model of diet-induced obesity (DIO) and metabolic disease. Compared to wildtype mice, NLRP12-deficient mice gained more weight, were more insulin resistant and glucose intolerant. NLRP12-deficient mice had higher levels of serum cholesterol, leptin, and insulin as well as the appearance of fatty liver not observed in WT mice. When we performed both co-housing experiments and littermate control experiments between NLRP12 and WT mice, we found the phenotype to be transferable.

Pro-inflammatory cytokine levels, specifically IL-18, were generally higher in NLRP12-deficient mice serum. Analysis

of tissues revealed that NLRP12-deficient mice had significantly higher levels of pro-inflammatory cytokines in the VAT and pancreas. Histological analysis of tissues revealed that NLRP12-deficient mice had significantly increased area of pancreatic islets as compared to wildtype mice. These studies further identify NLRP12 as a novel suppressor of metabolic disease.
Disclosure of Interest: None Declared

LT057c
CHARACTERIZATION AND PREDICTION OF ISRE BINDING PATTERNS ACROSS CELL TYPES UNDER TYPE I INTERFERON STIMULATION
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Abstract Content: Many studies have noted cell-type specificity in the ISGs differentially expressed in response to type I IFN stimulation. Non-canonical IFN signaling has been clearly associated with variation in the ISGs across cell types, for example in cases where STAT3 is activated and shifts signaling away from ISGF3 associated regulation. The existence of variation associated with binding to ISREs by ISGF3 and its impact on ISGs is less clear. We examined ISRE binding patterns under IFN stimulation across six cell types using existing ChIPseq datasets available on the GEO and ENCODE databases. We find that ISRE binding is cell specific, particularly for ISREs distal to transcription start sites, potentially associated with enhancer elements, while ISRE binding in promoter regions is more conserved. Given variation of ISRE binding across cell types, we investigated associations between the cell type, homeostatic state and ISRE binding patterns. Taking a machine learning approach and using existing ATACseq and ChIPseq datasets available on GEO and ENCODE, we show that the epigenetic state of an ISRE locus at homeostasis and the DNA sequence of the ISRE locus are predictive of the ISRE's binding under IFN stimulation in a cell type, specific manner, particularly for ISRE distal to transcription start sites.

Reference:

Leviyang S. (2020) Characterization and Prediction of ISRE Binding Patterns Across Cell Types Under Type I Interferon Stimulation. BioRxiv. https://t.co/5ILY5rKUT3
Disclosure of Interest: None Declared

Lightning Talk Session 1: Innate Immunity I

LT059
SP140 EPIGENETIC READER REPRESSES TOPOISOMERASE ACTIVITY TO MAINTAIN MACROPHAGE IDENTITY
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Abstract Content: Dysregulated epigenetic enzymes and aberrant chromatin landscapes and architecture are sentinel events in cancer. Yet virtually nothing is known about how altered epigenetic regulators directly contribute to immunological disease. Speckled Protein 140 (SP140) is a chromatin "reader" protein, with high homology to autoimmune regulator AIRE, that is restricted to the immune system. Loss-of-function mutations within *SP140* associate with Crohn's disease (CD) (*1, 2*) multiple sclerosis (MS) and chronic lymphocytic leukemia (CLL) (*3*). We previously identified SP140 as a key orchestrator of macrophage identity and function through repression of lineage-inappropriate genes such as *HOX* but the mechanisms by which this novel epigenetic reader functions are unknown. Here we reveal SP140 as a repressor of topoisomerase activity and DNA accessibility *via* its PHD and Bromodomain. We employed a global proteomic strategy to find SP140 in complex with DNA unwinding and chromatin remodeling machinery, including topoisomerases (TOP1, 2A), FACT and SMARCA complexes. We find that SP140 acts to repress topoisomerases to maintain gene silencing. Loss of SP140 in CD patients bearing SP140 mutations, led to disruption of the SP140-TOP complex, increased topoisomerase activity, increased DNA double-stranded breaks and irregular gene expression programs in leukocytes that could be successfully rescued with topoisomerase inhibitors. Our results illustrate how epigenetic and transcriptional alterations induced by SP140 mutations disrupt immune cell identity and function. This comprehensive understanding of how SP140 exerts transcriptional repression for macrophage identity, encourages development of precision medicine strategies for manipulation of the aberrant immune transcriptome due to loss of SP140 in immunological disease.

Disclosure of Interest: None Declared

LT061
NOVEL MECHANISMS OF NUCLEIC ACID SENSING: IMPLICATIONS FOR ANTIVIRAL RESPONSES
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Abstract Content: Intracellular detection of viral nucleic acids leads to the production of type I interferons (IFNs) and subsequent establishment of an antiviral state in infected and neighboring cells. Viruses have evolved multiple mechanisms to counteract IFN responses in infected cells, however, viral nucleic acids released from dying cells can stimulate IFN production in surrounding or distal uninfected cells. We have previously shown that class A scavenger receptors (SR-As) internalize extracellular double-stranded RNA (dsRNA) to mediate an IFN response. Cytosolic sensors are essential in mediating an antiviral response against the endocytosed dsRNA, but the mechanism of endoplasmic release and cytoplasmic entry of dsRNA remains an enigma. In this study, we hypothesized that a dsRNA-channel protein facilitates entry of dsRNA into the cytoplasm after endocytosis. To test our hypothesis, we studied the role of SIDT2, a mammalian ortholog of *Caenorhabditis elegans* SID-1 dsRNA transporter in

facilitating dsRNA entry into cytoplasm and subsequent activation of antiviral responses. We generated and assessed antiviral responses to dsRNA in SIDT2 deleted human cells. Wildtype and SIDT2 deleted cells did not respond differently to dsRNA. Preliminary observations suggest that SIDT2 might not be necessary in facilitating extracellular dsRNA recognition in mammalian cells. We are currently using high-throughput assays to identify cellular molecules that may be involved in the antiviral response to extracellular dsRNA. Data from our studies will allow us to identify mechanisms of antiviral immune response that are mediated by extracellular nucleic acids. We shall harness this knowledge to identify mechanisms to boost innate immune responses against viruses and develop nucleic acid-based therapies that can bypass endosomal entrapment and lysosomal degradation.

Disclosure of Interest: None Declared

LT062

SLAM MEDIATES MYCOBACTERIUM TUBERCULOSIS RECOGNITION AND ENDOLYSOSOMAL MATURATION IN HUMAN MONOCYTE-DERIVED MACROPHAGES

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Abstract Content: Tuberculosis is the leading cause of death from a single infectious agent in the world. *Mycobacterium tuberculosis* has smartly manipulated the immune system to survive within host macrophages over ages. The Signaling Lymphocytic Activation Molecule (SLAM) is a self-ligand receptor that internalizes Gram-bacteria and regulates macrophages' phagosomal functions. In Tuberculosis, SLAM promotes Th1 protective immune responses. In this work, we studied SLAM role on macrophages' functions against *M. tuberculosis* infection.

Human monocyte-derived macrophages were obtained from healthy donors by CD14 positive selection. After 2h of adherence, cells were cultured in complete media overnight before stimulation with sonicated-*M. tuberculosis* (*Mtb*). THP-1 cells were differentiated with PMA for 24h before *Mtb* stimulation.

We observed that both *Mtb* and rhIFN-g stimulation induce SLAM expression in monocyte-derived macrophages and THP-1 cells, as determined by flow cytometry (FC). No changes where observed with rhIL-4 and rhIL-10 treatment. Moreover, rhIFN-g increased TNF-α secretion in *Mtb*-stimulated THP-1 cells. However, SLAM did not regulate TNF-α nor IL-1β production as measured by ELISA.

Rhodamine-stained *Mtb* (*Mtb-R*) or live *M. tuberculosis*-RFP were used to evaluate SLAM role on bacterial uptake by FC. Costimulation trough SLAM with an agonistic antibody further induced *Mtb-R* and *M. tuberculosis*-RFP endocytosis by macrophages and THP-1 cells ($p<0.05$).

To elucidate SLAM role as a microbial sensor, we first demonstrate a direct interaction between SLAM and *Mtb* by FC and fluorescence microscopy. Furthermore, we found that SLAM colocalized with *Mtb* (Manders' index (MI) of 0.74) and with early (EEA1) and late (LAMP2) endosomes/lysosomes markers by confocal microscopy (MI of 0.6 and 0.7 respectively). These results indicate that SLAM could recognize *Mtb* and participate in the endolysosomal maturation process.

Notably, higher SLAM levels were detected in CD14 positive cells from pleural effusions compared to peripheral blood CD14 positive cells from Tuberculosis patients, indicating a possible active function of SLAM at the site of infection.

Taken together, our results provide evidence of SLAM as a potential microbiological sensor that improves *M. tuberculosis* uptake in human monocyte-derived macrophages.

Disclosure of Interest: None Declared

LT063

CHARACTERIZATION OF INNATE AND ADAPTIVE IMMUNE IMPACTS OF CHEMICALLY DISSIMILAR STING ADJUVANTS

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Abstract Content: Vaccination represents the most effective and adaptable strategy for combating viral infections. Live-attenuated vaccines generate a potent and long-lived immune response; however, they can be associated with contraindications, especially in immune-insufficient individuals. Protein subunit vaccines offer a safer solution, yet these do not activate all necessary facets of an immune response and in some cases confer shorter protection than their replication-competent counterparts. These limitations can be mitigated through the co-administration of adjuvants, non-antigenic molecules capable of activating innate immune cells through the induction of PRR-mediated, cytokine-terminal innate signaling pathways. Unfortunately, few adjuvants are licensed in the U.S. and identification of new ones represents an unmet clinical need. These may additionally facilitate the development of novel vaccines through precise T helper polarization of the immune response. The PRR STING recognizes cyclic dinucleotides (CDN) synthesized by the cytosolic enzyme cGAS in response to microbial or cellular DNA. The activation of STING stimulates innate antiviral activity characterized by a localized type I IFN and proinflammatory cytokine response and, as such, exhibits characteristics of an effective adjuvant target. However, the activation of STING also drives transcription-independent

processes such as apoptosis and autophagy. As a result, the general phenotype elicited in response to STING activation can vary substantially between STING-inducing stimuli such as CDN species and small molecule agonists. While pharmacologic STING activation enhances immunogenicity of vaccine antigens, the impact of differences in STING-associated intracellular responses on processes important for establishment of adaptive immunity has not been explored. To address this, we utilize a mouse model of vaccination against Chikungunya virus. This is done in combination with chemically diverse STING agonists including CDNs and small molecules. While all CDNs and small molecules directly activate STING, the downstream immune reactivity to the antigen varied significantly. Furthermore, our exploration of STING-mediated innate correlates of these differences led to unexpected observations regarding agonist-specific cytokine induction, APC maturation, follicular helper T cell enhancement, and antigen trafficking and presentation. This has important implications in at least two areas: 1) It is crucial to filling the numerous gaps that exist with regard to understanding mechanisms that drive STING-associated immune enhancement; 2) It will reveal properties desirable for STING-based adjuvants to help direct future optimization.
Disclosure of Interest: None Declared

LT064
GUANYLATE-BINDING PROTEINS AND CASPASE-4 COOPERATE FOR NON-CANONICAL INFLAMMASOME ACTIVATION
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Abstract Content: The human non-canonical inflammasome enables caspase-4 activation and Gasdermin-D-dependent pyroptosis in response to cytosolic bacterial lipopolysaccharide (LPS). LPS has been reported to bind and oligomerizes caspase-4 and the pathway is thought to proceed without dedicated LPS sensors or an activation platform. Here we report that interferon-inducible guanylate-binding proteins (GBPs) are required for non-canonical inflammasome activation following cytosolic *Salmonella* infection or upon cytosolic delivery of LPS. In epithelial cells, GBP1 associates with the surface of cytosolic *Salmonella* seconds after bacterial escape from their vacuole and initiates the recruitment of GBP2-4 to assemble a GBP coat on the bacteria. The GBP coat recruits caspase-4 to the bacterial surface and allow caspase dimerization and activation, without lysing the bacteria. Mechanistically, GBP1 binds LPS directly with high affinity through electrostatic interactions. LPS recognition increases GBP1 activity and promote oligomerisation on the bacteria. Our findings indicate that in human epithelial cells GBP1 acts as a cytosolic LPS sensor and assembles a platform for caspase-4 recruitment and activation at LPS-containing membranes as the first step of non-canonical inflammasome signalling.
Disclosure of Interest: None Declared

LT065
THE ANTIVIRAL EFFECTOR RTP4 IS ENGAGED IN A HOST-VIRUS ARMS RACE IN BATS AND OTHER MAMMALS
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Abstract Content: Viruses and their hosts continuously impose evolutionary pressure on one another. Host antiviral genes frequently underlie these evolutionary conflicts, with examples in primates, but fewer in other mammals. Mammalian cells deploy a range of antiviral effectors as part of the interferon (IFN) response, a critical component of antiviral immunity. While the IFN pathway is conserved across vertebrates, many antiviral IFN-stimulated genes (ISGs) exhibit species-specific properties. In screens to identify antiviral effectors in bats, which harbor many zoonotic viruses, we identified black flying fox Receptor Transporter Protein 4 (RTP4) as a potent interferon-induced inhibitor of human pathogens in the *Flaviviridae* family, including Zika, West Nile, and hepatitis C viruses. Mechanistically, RTP4 associates with the flavivirus replicase and disrupts interactions required for replication. Comparative approaches demonstrate that RTP4 is undergoing positive selection, that a flavivirus can mutate to escape RTP4-imposed restriction, and that diverse mammalian RTP4 orthologs exhibit striking patterns of specificity against distinct *Flaviviridae* members. Our findings reveal an antiviral mechanism that has likely adapted over 100 million years of mammalian evolution to accommodate unique host-virus genetic conflicts.
Disclosure of Interest: None Declared

LT066
METFORMIN EFFECT ON HUMAN MACROPHAGE INFLAMMATORY RESPONSE AND PHAGOCYTOSIS OF MYCOBACTERIUM TUBERCULOSIS
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Abstract Content: Metformin (MTF) has a well-documented ability to control hyperglycemia, which has been shown to have effects on macrophage and lymphocyte functions that are key to controlling tuberculosis (TB) infection.
Here, we aimed to better understand the effects of MTF on the phagocytosis of *Mycobacterium tuberculosis* (Mtb) by human macrophages. PMA-differentiated THP-1 cells with two reporters for nuclear factor-κB (NF-κB), and interferon-regulatory factors (*IRFs*) were treated with 2mM of MTF for 4 hours, and then inoculated with Mtb from various lineages. Since MTF can also directly inhibit key metabolic processes of Mtb, we controlled this variable by using of gamma-

irradiated mycobacteria. Phagocytosis was assessed by immunofluorescent assay.
Phagocytosis of Mtb increased in MTF-treated macrophages. NF-κB activation after Mtb stimulation was lower in MTF-treated macrophages. The effect on IRF activation was minimal.
Our results indicate that MTF improves phagocytosis of Mtb by macrophages, while it at the same time modulating their inflammatory response. Downregulation of type I IFN pathways, associated with active TB infection, could allow for improved activation of macrophages in the presence of TB infection. These results support the effects of MTF in keys steps TB infection control, and support its use as an additional treatment for TB.
Disclosure of Interest: None Declared

LT067

DISSECTION OF NLRP3 INFLAMMASOME ACTIVATION BY INFLUENZA A VIRUS SC35M (H7N7) USING A NOVEL VIRUS-ENCODED REPORTER TOOL

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Abstract Content: Inflammasome sensors are an important part of the innate immune system mediating ASC-dependent cytokine secretion and pyroptotic cell death. Influenza A virus is known to activate the NLRP3 inflammasome, however the exact mechanism, the importance of the ASC speck assembly and the existence of additional inflammasome sensors are still debated.

Inflammasome activation is often evaluated by the secretion of IL-1b and IL-18 from infected cells while the quantification of ASC speck assembly often is neglected. We have developed a novel, fluorescent inflammasome reporter, which allows the visualization and quantification by flow cytometry of ASC nucleation, upstream of cytokine secretion.

We detected high inflammasome activity in primary macrophages and macrophage-like THP-1 reporter cells upon infection with the SC35M (H7N7) strain, which was not observed upon infection with lab-adapted WSN (H1N1) strain. The SC35M-induced inflammasome was dependent on a late stage of the virus replication cycle but independent of virus release or activation of the type I IFN pathway. To dissect the inflammasome response further, we introduced our inflammasome reporter into the genome of the SC35M strain, allowing expression of the reporter tool at early time points of infection. By screening a range of THP-1 KO cells we confirmed the existence of IAV-induced NLRP3 inflammasomes in myeloid cells. However, reporter virus infection of primary human airway epithelial cells activated an NLRP3-independent inflammasome response, indicating that multiple inflammasome sensors may detect influenza viruses.
Disclosure of Interest: None Declared

LT068

NEURONAL HYPEREXCITABILITY IS A DLK-DEPENDENT TRIGGER OF HSV-1 REACTIVATION THAT CAN BE INDUCED BY IL-1B

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Abstract Content: Herpes simplex virus-1 (HSV-1) establishes a latent infection in neurons wherein viral transcription is restricted and the viral promoters are associated with heterochromatin. Periodically, HSV-1 can reactivate in response to certain stimuli to induce lytic gene expression and permit transmission. The physiological triggers of reactivation and the intrinsic neuronal signaling pathways that mediate reactivation are not well-understood. Previously, we found that dual leucine zipper kinase (DLK)-mediated activation of c-Jun N-terminal kinase (JNK) was essential for reactivation in response to inhibition of PI3K signaling, which mimics loss of nerve growth factor (NGF) support. Moreover, reactivation was associated with a DLK/JNK-dependent histone phospho/methyl switch on lytic gene promoters. Given that the same histone phospho/methyl switch occurs in cortical neurons following hyperexcitability (triggered by forskolin), we examined whether HSV reactivation was linked to neuronal hyperexcitability and the contribution of DLK/JNK activity and histone phosphorylation. Using our primary neuronal model of HSV-1 reactivation, we demonstrate that HSV reactivation is triggered by stimuli that induce neuronal hyperexcitability. Neuronal hyperexcitability-induced reactivation was dependent on voltage-gated ion and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, demonstrating that neuronal excitability is required for HSV-1 reactivation. Additionally, activation of DLK/JNK was essential for HSV-1 reactivation triggered by neuronal hyperexcitability. The initial burst of HSV-1 lytic gene expression in response to neuronal hyperexcitability occurred independently of histone demethylase activity and was accompanied with a histone phospho/methyl switch. Neuronal hyperexcitability can also be induced by IL-1β, an inflammatory cytokine released during chronic psychological stress and fever; both are triggers of clinical HSV-1 reactivation. Notably, in mature sympathetic neurons, IL-1β triggered HSV-1 reactivation that was dependent on DLK activation and neuronal excitability. Thus, HSV co-opts an innate immune pathway resulting from IL-1 stimulation of neurons to induce reactivation.
Disclosure of Interest: None Declared

LT069

CLASS IIA HISTONE DEACETYLASES DRIVE THE TOLL-LIKE RECEPTOR-INDUCIBLE GLYCOLYTIC SWITCH AND MACROPHAGE INFLAMMATORY RESPONSES VIA PYRUVATE KINASE M2

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Abstract Content: The metabolic state of innate immune cells is intrinsically linked to their functions. Toll-like Receptor (TLR) signaling enhances aerobic glycolysis during macrophage activation, resulting in skewing of cytokine production towards proinflammatory responses. Here, we use metabolomic, pharmacological, genetic and proteomic approaches to demonstrate that class IIa histone deacetylases (HDACs) play key roles in fuelling metabolism-mediated inflammation. Inhibition of these enzymes in macrophages attenuates lipopolysaccharide (LPS)-inducible production of lactate and other glycolytic intermediates, reduces proinflammatory IL-1b and CCL2, and amplifies immunoregulatory IL-10. We have previously reported a role for Hdac7 in driving macrophage inflammatory responses. Myeloid-specific overexpression of Hdac7 in transgenic mice amplifies LPS-inducible lactate, and promotes glycolysis-associated inflammatory cytokine release. Conversely, pharmacological or genetic targeting of Hdac7 and other class IIa HDACs attenuates LPS-inducible glycolysis and associated inflammatory responses in macrophages. A proteomic screen identified the glycolytic enzyme, pyruvate kinase M isoform 2 (Pkm2) as an interacting partner of proinflammatory Hdac7 in murine macrophages. We show that an Hdac7-Pkm2 complex is a novel immunometabolism signaling hub, where both Pkm2 and Hdac7 are required for optimal macrophage inflammatory functions. Screening for potential lysine residues that might be a target for Hdac7, we report that Pkm2 deacetylation at lysine 433 is crucial for its proinflammatory functions. The interaction of Hdac7 with Pkm2 was increased when using an enzyme-dead variant of Hdac7, consistent with an enzyme-substrate interaction. Pharmacological targeting of this complex also suppresses inflammatory responses *in vivo*. Our findings therefore highlight a novel role of class IIa HDACs (HDAC4/5/7/9) as key molecular links between toll-like receptor (TLR)-inducible aerobic glycolysis and macrophage inflammatory responses.

Disclosure of Interest: None Declared

LT071

IQGAP1 CLEAVAGE AND MICROTUBULE LOSS DURING PYROPTOTIC CELL DEATH.

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Abstract Content: Pyroptosis and apoptosis are two forms of programmed cell death that eliminate unnecessary, dysfunctional, infected, or damaged cells; and whereas apoptosis is considered immunologically silent, pyroptosis is inflammatory to release interleukin-1 beta (IL-1β) to stimulate a downstream inflammatory response. We recently discovered that as in apoptosis, the cytoskeleton is destroyed during pyroptosis. Moreover we demonstrated that loss of intermediate filaments was driven by the calcium-dependent protease, calpain. However, as in apoptosis, the mechanism driving pyroptotic microtubule loss is unknown. Here we show that pyroptotic microtubules dissociate from their connections at the cell periphery, suggesting that microtubules detach from tethers that link them to the periphery and prevent depolymerization. Consistent with this idea, we demonstrate that the microtubule-actin tether protein, IQGAP1, is cleaved during both pyroptotic and apoptotic cell death of both primary and tumor derived cells from mice and humans, and we show that cleavage of IQGAP1 is modulated by calpain. Together our data support a model whereby apoptotic and pyroptotic calcium fluxes activate calpain, to regulate cleavage of IQGAP1 which dissociate microtubules from stabilizing connections at the periphery and drive microtubule depolymerization. We hypothesize that this process may enhance the release of IL-1β and other damage associated molecular pattern molecules for triggering the inflammatory response.

Disclosure of Interest: None Declared

LT072

WHEN VIRUSES AND THE INNATE IMMUNE SYSTEM MEET: COMPARISON OF DIFFERENT PESTIVIRAL E-RNS IN EVASION OF INNATE IMMUNITY

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Abstract Content: The genus Pestivirus, family Flaviviridae, includes four approved species named bovine viral diarrhea virus (BVDV)-1 and -2, classical swine fever virus (CSFV), and Border disease virus (BDV). In addition to these species, currently named as pestivirus A-D, some of the related viruses previously grouped as “unassigned pestiviruses” are now classified as pestivirus E-K. A common feature to all the members is the presence of two unique proteins that pestiviruses evolved to regulate the host’s innate immune response: the nonstructural protein N^{pro} (only missing in the virus isolated from P. phocoena), and the envelope glycoprotein E^{rns}.

Particularly, the E^{rns} is also released in the extracellular space in a soluble form, where it is taken up by endocytosis into neighboring cells to exert its function. E^{rns} is an RNase able to cleave viral ss- and dsRNAs, thus preventing the stimulation of the immune response by this potent pathogen-associated molecular pattern (PAMP). This activity is at the basis of the ability of BVDV to establish persistent infections and immunotolerance, with relevant implications for the epidemiology of the infection. Therefore, this study aims to characterize the basic features of the E^{rns} proteins of different classified and unassigned pestiviruses that have not been described yet. To that end, we purified a wide variety of E^{rns} proteins and characterized them in vitro and in cell cultures. The ability to dimerize, the enzymatic activity on different RNA substrates and the intracellular localization of E^{rns} and its ability to inhibit dsRNA-induced interferon (IFN) synthesis, were the main features we analyzed in this project.

Overall, we found large differences between the various E^{rns} proteins that cannot be predicted solely based on their

primary amino acid sequences. This provides valuable information to delineate the structure-function relationship of pestiviral endoribonucleases, and on their potential to regulate the host's IFN response.
Disclosure of Interest: None Declared

LT073
TBK1 AND IKK-EPSILON ACT REDUNDANTLY TO MEDIATE STING-INDUCED NF-KAPPA-B RESPONSES IN MYELOID CELLS
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Abstract Content: STING is a critical component of host innate immune defense, but can contribute to chronic autoimmune or autoinflammatory disease. Once activated, the cGAS-STING pathway induces both type I IFN expression and NF-kB-mediated cytokine production. Currently these two signaling arms are thought to be mediated by a single upstream kinase, TBK1.
Here, using genetic and pharmacological approaches we show that TBK1 alone is dispensable for STING-induced NF-kB responses in human and mouse immune cells, as well as *in vivo*. We further demonstrate that TBK1 acts redundantly with IKKe to drive NF-kB upon STING activation. Interestingly, we show that activation of IRF3 is highly dependent on TBK1 kinase activity, while NF-kB is significantly less sensitive to TBK1/IKKe kinase inhibition.
Our work redefines signaling events downstream of cGAS-STING. Our findings further suggest cGAS-STING will need to be targeted directly to effectively ameliorate the inflammation underpinning disorders associated with STING hyperactivity.
Disclosure of Interest: None Declared

LT074
INNATE IMMUNE BARRIERS TO INTRA- AND INTER-SPECIES TRANSMISSION OF VIRUSES
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Abstract Content: Viruses emerging from zoonotic reservoirs represent a major public health concern. While identifying potential zoonoses is paramount to pandemic preparedness, there are few experimental models that are able to characterize virus and host factors necessary for cross-species transmission. Here, we leverage a model whereby pet store mice—which harbor a myriad of mouse pathogens—are co-housed with specific pathogen-free laboratory mice. This 'dirty' mouse model offers a platform for studying the acute transmission of viruses between hosts via natural mechanisms—through direct contact, air, and saliva and other fluids. We co-housed pet store mice with wild type laboratory mice and mice that are deficient in interferon-I or interferon-III receptors and harvested various organs for RNA sequencing analysis. After identifying viruses present in both the reservoir and new host, we used an amplicon-based strategy to quantify variance in viral populations and assessed the constrains the interferon response places on transmission and dissemination bottlenecks. We have also co-housed our laboratory mice with the bedding of pet store rats to analyze species-specific immune and non-immune barriers to transmission. We have identified viruses present in the pet store rats that do and do not transmit to mice. Overall, this mouse model allows for the analysis of barriers to transmission of natural rodent viruses.
Disclosure of Interest: None Declared

LT075
ANALYSIS OF THE UNIQUE BIOLOGICAL FUNCTIONS ELICITED BY TYPE III INTERFERONS IN THE EPITHELIUM
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Abstract Content: The epithelium is a highly proliferative tissue under constant exposure to pathogenic and commensal microbes where type I and III interferons (IFNs) activate similar downstream signaling cascades that result in kinetic differences in the expression of antiviral IFN-stimulated genes (ISG). Our study addresses the fundamental question of why these two IFN families are required to prevent viral dissemination at barrier sites. While previous observations had suggested type I IFNs (IFNα/β), type III IFNs (IFNλ) do not elicit strong inflammatory responses *in vivo*, this study provides some of the molecular mechanisms underlying this disparity. IFNα/β uniquely induce expression of the transcription factor IRF1, with little to no IRF1 induction observed after IFNλ treatment. Through genome-wide expression analysis, we demonstrate that IRF1 expression is dispensable for the antiviral activity of IFNα/β, but necessary for the induction of inflammatory chemokines and immune cell infiltration. The muted induction of IRF1 by IFNλ is due to insufficient STAT1 activation and homodimerization given the limited IFNλ receptor 1 subunit (IFNLR1) expression. We show that in the epithelium, the expression of IFNLR1 is not affected by inflammatory stimuli or viral challenge, although exogenous overexpression of IFNLR1 can enhance both antiviral and chemokine gene expression through IRF1 induction. This suggests that sustaining low IFNLR1 expression in the epithelium is crucial for preventing deleterious inflammation. On the other hand, our gene expression profiling identified genes that were expressed uniquely in IFNλ stimulated cells. Gene set enrichment analyses suggested that many

of these genes are involved in pathways consistent with the resolution of inflammation and the maintenance of barrier function. Many of these pathways, likely regulated independently from canonical JAK/STAT signaling. Overall, we show that IFNα/β and IFNλ work in concert to sustain antiviral immunity and limit tissue damage at sites of pathogen exposure. The transient inflammatory responses to IFNα/β help recruit immune effectors to promote protective immunity while IFNλ act as potent antiviral effectors and remodelers of the epithelial barrier.
Disclosure of Interest: None Declared

LT076
NON-HAEMOLYTIC ENTEROTOXIN INDUCES PORE-FORMATION AND ACTIVATION OF THE INFLAMMASOME AND PYROPTOSIS
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Abstract Content: Non-haemolytic enterotoxin induces pore-formation and activation of the inflammasome and pyroptosis

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Inflammasomes are central to innate immunity mediating host defence against pathogens and maintaining homeostasis with commensal microbes. The sensing of pathogens, danger, or homeostasis-altering signals by inflammasome sensor proteins is the molecular basis driving assembly of the inflammasome complex. Here, we identify non-haemolytic enterotoxin (NHE) as an activator of the NLRP3 inflammasome. We show that NHE, found in the neglected human foodborne pathogen *Bacillus cereus*, is a non-redundant toxin to haemolysin BL (HBL) despite exhibiting a similar mechanism of action. NHE-induced activation of NLRP3 has a delayed kinetic to that induced by HBL. Subunit C of NHE, via its putative transmembrane region, initiates binding to the plasma membrane, leading to the recruitment of subunit B and subunit A, forming a tripartite lytic pore permissive to efflux of potassium. NHE mediates killing of cells from multiple lineages and hosts, highlighting its versatile functional repertoire in different host species. Moreover, our data suggest that both NHE and HBL operate synergistically to induce inflammation in the host. Overall, our results highlight that multiple virulence factors from the same pathogen exhibiting conserved function and mechanism of action can be exploited for sensing by a single inflammasome, ultimately leading to increased capacity of the host to detect and defend against naturally-occurring genetic variants.
Associated Reference:

Fox, D*., Mathur, A*., Xue, Y., Liu, Y., Tan, W., Feng, S., Pandey, A., Ngo, C., Hayward, J., Atmosukarto, I., Price, J., Johnson, M., Jessberger, N., Robertson, A., Burgio, G., Tscharke, D., Fox, E., Leyton, D., Kaakoush, N., Märtlbauer, E., Leppla, S. and Man, S.M., 2020. *Bacillus cereus* non-haemolytic enterotoxin activates the NLRP3 inflammasome. ***Nature Communications***, 11(1).
Disclosure of Interest: None Declared

LT078
RATIONAL DESIGN OF ANTISENSE OLIGONUCLEOTIDES MODULATING THE ACTIVITY OF TLR7/8 AGONISTS
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Abstract Content: Oligonucleotide-based therapeutics have become a reality, and are set to transform management of many diseases. Nevertheless, the modulatory activities of these molecules on immune responses remain incompletely defined. Here, we show that gene targeting 2′-O-methyl (2′OMe) gapmer antisense oligonucleotides (ASOs) can have opposing activities on Toll-Like Receptors 7 and 8 (TLR7/8), leading to divergent suppression of TLR7 and activation of TLR8, in a sequence-dependent manner. Through a screen of 192 2′OMe ASOs and sequence mutants, we characterized the structural and sequence determinants of these activities. Importantly, we identified core motifs preventing the immunosuppressive activities of 2′OMe ASOs on TLR7. Based on these observations, we designed oligonucleotides strongly potentiating TLR8 sensing of Resiquimod, which preserve TLR7 function, and promote strong activation of phagocytes and immune cells. We also provide proof-of-principle data that gene-targeting ASOs can be selected to synergize with TLR8 agonists currently under investigation as immunotherapies, and show that rational ASO selection can be used to prevent unintended immune suppression of TLR7. Accordingly, we propose that rational selection of TLR8-potentiating ASOs could present new opportunities in the therapeutic development of bifunctional ASOs with gene-targeting and immunostimulatory activities. Taken together, our work characterizes the immumodulatory effects of ASOs to advance their therapeutic development. doi: 10.1093/nar/gkaa523
Disclosure of Interest: None Declared

LT079
MONOCYTE-DERIVED DENDRITIC CELLS FROM LEPROSY PATIENTS PRODUCED LOW LEVELS IL-12P70 AFTER STIMULUS WITH VIABLE MYCOBACTERIUM LEPRAE
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Abstract Content: Leprosy is a chronic infectious disease caused by *Mycobacterium leprae,* an obligate intracellular pathogen, with a predilection for Schwann cells in peripheral nerves. In the absence of treatment, nerve injuries bring significant physical disabilities that affect the quality of life. Tuberculoid leprosy patients present a strong Th1 immune response that controls bacilli multiplication resulting in localized disease while lepromatous patients show a predominant and ineffective humoral immune response that favors disease dissemination. Dendritic cells (DCs) play a crucial role in the activation of specific immune responses by antigen presentation, expression of costimulatory molecules and production of cytokines during immunological synapses. Accordingly, the cytokines released by DCs during infection by *M. leprae* can prevent or favor its survival and spread. The aim of this study was to analyze the cytokine profile produced by DCs from healthy subjects and leprosy patients after infection with viable *M. leprae*. Peripheral blood mononuclear cells were obtained by gradient density and submitted to positive selection employing anti-CD14 microbeads and magnetic columns to monocytes isolation. Monocytes were cultivated with IL-4 and GM-CSF for differentiation of DCs. After 6 days, immature DCs were infected by live *M. leprae* obtained from athymic mice at a MOI of 10:1 (bacilli:DC) and cultivated for 48 hours. A standard maturation cocktail (MC) composed by IL-1β, IL-6, TNF, PGE2 was used as a positive control for DC maturation. In order to evaluate the capability of IL-12p70 production, *M. leprae*-primed DCs were co-cultured with J588 transfected cells expressing CD40L. The cytokines profile was determined in the supernatant by cytometric bead array. Our results showed that *M. leprae* stimulus did not induce an increase in the production of IL-1β, IL-6, TNF and IL-23, while MC elicit high production of these cytokines, proving the effective potential of activation of DCs from both leprosy patients and healthy controls. DCs from lepromatous patients produced a higher amount of IL-1β in response to *M. leprae* in comparison to tuberculoid ones and larger amounts of IL-23 and IL-10 if compared to healthy controls. Of special interest, DCs from leprosy patients produced lower levels of IL-12p70 in response to *M. leprae* than healthy controls. In conclusion, our results show to *M. leprae* as a weak inducer of DCs activation and leprosy patients as unable to produce the cytokine essential to induce the development of Th1 immune response, the most effective against intracellular pathogens. Altogether these findings highlight a complex pathogen-host interaction, pointing a role for human and bacillary factors in the outcome of leprosy. Supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Grant: #2015/23256-6).

Disclosure of Interest: None Declared

LT080

A COMPARATIVE STUDY OF NOVEL MICRORNAS IDENTIFIED IN INTERLEUKIN-27-INDUCED HIV-RESISTANT T CELLS

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Abstract Content: Interleukin-27 (IL-27) is known as an anti-HIV cytokine. We have recently demonstrated that IL-27-pretreatment promotes phytohaemagglutinin (PHA)-stimulated CD4(+) T cells into HIV-1-resistent cells. In the current study, we investigated profiles of known and unknown microRNA in the IL-27-treated CD4(+) T cells and characterized anti-viral effect of microRNAs.

PHA-stimulated CD4(+) T cells were treated with or without IL-27 for three days. MicroRNA profiles were analyzed using MicroRNA sequencing. To assess anti-HIV effect, T cells or macrophages were transfected with synthesized microRNA mimics and then infected with HIVNL4.3 (T cell-tropic HIV) or HIVAd8 (Macrophage-tropic HIV). Anti-HIV effect was monitored by a p24 antigen ELISA kit. IFN-a, -b, or -l production was quantified using each subtype-specific ELISA kit.

A comparative analysis of microRNA profiles indicated that expression of known miRNAs was not significantly changed in IL-27-treated cells compared to untreated T cells, however, a total of 15 novel microRNAs (miRTC1~miRTC15) were identified .The over-expression of each novel microRNA revealed that 10 nM miRTC14 remarkably suppressed HIV replication by 99.9$\pm$0.04% (n=5) in macrophages but not in T cells. The inhibition was associated with induction of >1000 pg/mL of multiple subtypes of IFN-a, ~600pg/ml of IFNl1, and ~50 pg/ml of IFN-b.

As a conclusion, we discovered a total of 15 novel microRNAs in T cells and characterized that miRTC14, one of the novel microRNAs, was a potent IFN-inducing anti-HIV miRNA, implicating that regulation of the expression of miRTC14 may be a potent therapeutic tool for not only HIV but also other IFN-sensitive virus infection.

Disclosure of Interest: None Declared

LT081b

IL-25 EXACERBATES CHRONIC AIRWAY INFLAMMATION BY PROMOTING PATHOGENIC TH2 CELLS

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Abstract Content: Chronic allergic disorders are difficult to treat due to the complex nature of heterogenous cells in the inflammatory tissues after repetitive re-exposures to allergens. Pathogenic TH2 cells (peTH2) are a key driver of allergen recall-induced exacerbations of chronic atopic diseases such as allergic asthma. peTH2 cells are a subset of antigen-specific memory T helper cells that are phenotypically distinct from conventional TH2 cells. Furthermore, peTH2 cells show enhanced Type 2 effector functions compared to conventional TH2 cells. However, the mechanisms underlying the pathogenicity of peTH2 cells are poorly understood. Using mouse model of chronic airway inflammation, we show that IL-25 amplified the generation of

allergen-induced peTH2 cells and exacerbated pulmonary inflammation. IL-25-induced peTH2 cells were antigen-specific and expressed higher levels of IL-17RB (IL-25 receptor) and ST2 (IL-33 receptor) than non-peTH2 cells. In normal human PBMCs, the frequency of peTH2 positively correlated with the ability of IL-25 to enhance allergen-induced IL-13 production. Furthermore, IL-25 amplified stronger allergic immune responses in normal human PBMCs than either IL-33 or TSLP. Together, these findings indicate IL-25 as a critical inducer of peTH2 cells during chronic airway inflammation, and may be a therapeutic target for chronic comorbid allergic disorders.
Disclosure of Interest: None Declared

LT081c

CCL2-MEDIATED REVERSAL OF IMPAIRED SKIN WOUND HEALING IN DIABETIC MICE BY NORMALIZATION OF NEOVASCULARIZATION

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Abstract Content: Patients with diabetes frequently present complications such as impaired skin wound healing. Skin wound sites display a markedly enhanced expression of CCL2, a potent macrophage chemoattractant, together with macrophage infiltration during the early inflammatory phase in skin wound sites of healthy normal individuals, but it remains elusive on the association of CCL2 with delayed skin wound healing in diabetic patients. Compared to control mice, streptozotocin-induced diabetic mice displayed impaired healing after excisional skin injury, with decreased neovascularization, CCL2 expression, and macrophage infiltration, as compared with control mice. Compromised skin wound healing in diabetic mice was reversed by the administration of topical CCL2 immediately after the injury, as evidenced by normalization of wound closure rates, neovascularization, and infiltration of macrophages expressing vascular endothelial growth factor, a potent angiogenic factor. Eventually, CCL2 treatment increased the accumulation of endothelial progenitor cell at the wound sites of diabetic mice and eventually accelerated neovascularization. Thus, the tropical application of CCL2 can be an effective therapeutic option for the treatment of diabetic patients with defective wound repair, promoting neovascularization at skin wound sites.
Disclosure of Interest: None Declared

LT081e

PHENOTYPIC CHARACTERISTICS OF TYPE 2 INNATE LYMPHOID CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH T2-DRIVEN BRONCHIAL ASTHMA COMPARED WITH HEALTHY DONORS

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Abstract Content: Background: Group 2 innate lymphoid cells (ILC2s) are considered to be the most significant mediators during the orchestration of immune responses in asthma. ILC2s are able to produce Th2-cytokines rapidly in early immune responses to allergens and non-specific stimuli. ILC2s are important for initiation of adaptive 2 immune responses and regulation of persistent airway inflammation. It was also shown that ILC2s dynamically express the checkpoint inhibitor molecules PD-1 and PD-L1. However, it is still largely unexplored how the immunophenotypic characteristics of ILC2s change in patients with asthma and if they are clinically relevant. The role of PD-1 and PD-L1 expression on ILC2s seems to be controversial. According to recent studies PD-1 can be an important negative regulator of iILC2s, whereas activating function of PD-L1 in type 2 immune responses was described . Therefore, it is of the utmost importance to explore PD-1 and PD-L1 expression on ILC2s in different diseases, particularly T2-driven, e.g. bronchial asthma, allergic rhinitis etc.
Materials and methods: The study included 9 patients (51.1 ± 4.7 years) with a T2-high asthma endotype, whose blood samples were obtained from the Clinic of Immunopathology of RIFCI, Novosibirsk, and 11 healthy donors (44.3 ± 4.7 years). ILC2s were identified as a $Lin^-FceRI^-CD127^+CRTH2^+$ cell population distinct from human peripheral blood mononuclear cells. We investigated phenotypic characteristics of ILC2s, i.e. expression of PD-1 and PD-L1 on inflammatory ($KLRG1^+$) and natural ($KLRG1^-$) ILC2 cells .
Results: Frequencies of inflammatory ILC2s were significantly increased in patients with asthma (median 91,1% (IQR 84,6-93,5)) compared with healthy donors- 83,3% (71,1-88,7), p=0,04. The percentages of PD-1^+ iILC2s were three times higher in patients- 17,9 % (15,4-18,0) than in healthy donors- 4,3% (2,2-6,6, p=0,008. There also was significant decrease of PD-1^-nILC2s and of PD-$L1^-$nILC2s in patients (4,9% (4,0-7,4); 6,0% (2,9-7,7) resp.) in comparison with healthy donors (11,5% (9,1-19,5); 13,45% (9,4-22,0) resp.), p=0,006 and p=0,003 respectively.
Conclusion: With the present study we observed significant changes of ILC2s phenotypic characteristics in patients with T2-driven bronchial asthma: increase in inflammatory ILC2 cells and frequencies of PD-1^+ iILC2s, significant decrease in frequencies of $PD1^-$ and PD-$L1^-$nILC2s. It is yet to be clarified if such changes may play a role in therapy success and can be used as a diagnostic marker. Further studies are required to assess functional characteristics of ILC2s and to establish the role of PD-1 and PD-L1 expression on ILC2s in patients with bronchial asthma and allergy.
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Disclosure of Interest: None Declared

LT081f

NEUTROPHILS PROMOTE ANTIBODY ISOTYPE SWITCHING IN RESPONSE TO THE PNEUMOCOCCAL CONJUGATE VACCINE IN AN INTERFERON GAMMA-INDEPENDENT MANNER

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Abstract Content: *Streptococcus pneumoniae* (pneumococcus) cause serious infections including pneumonia and invasive pneumococcal disease. Neutrophils or polymorphonuclear cells (PMNs) are innate immune cells that are important for initial control of pneumococcal infection. However, we recently showed that PMNs are also required during immunization with the pneumococcal conjugate vaccine (PCV) for subsequent host protection against infection. Depletion of PMNs in mice at the time of vaccination impaired protective antibody responses, resulting in a decrease in anti-pneumococcal antibody class switching to IgG2c and IgG3 and reduced antibody opsonophagocytic activity. However, the mechanism by which PMNs mediate production of protective antibodies during vaccination is unknown. As interferon gamma (IFN-g) promotes switching to IgG3 and IgG2, we explored its role during vaccination. We first tested whether PMNs produced IFN-g in response to PCV. We found no increase in IFN-g production in vaccinated hosts as compared to naïve controls by mature PMNs in the spleen and vaccine draining lymph nodes of mice 18- or 48-hours following vaccination. We also used antibodies to block IFN-g at the time of vaccination and assessed antibody production as well as host protection following pneumococcal lung infection. IFN-g-blocked vaccinated mice had comparable anti-pneumococcal antibody production, bacterial burden in the lung and blood as well as survival as compared to isotype-treated vaccinated controls. These data suggest that IFN-g does not play an important role in the ability of PMNs to induce protection during vaccination. However, when we investigated the phenotypes of PMNs, we found that upon vaccination, PMNs expressed molecules involved in T cell interaction and antigen presentation. As compared to naïve controls, the phenotype of circulating PMNs remained unchanged, however, PMN in the spleen and vaccine draining lymph nodes upregulated expression of CD11b, MHC-II, CD80 and CD86 following vaccination. These findings suggest that PMNs contribute to PCV responses by potentially acting on T cells. Exploring the mechanisms by which PMNs induce protective anti-pneumococcal antibodies is important for designing more effective vaccines against *Streptococcus pneumoniae.*

Disclosure of Interest: None Declared

LT081g

FUNCTIONAL GENETIC APPROACHES UNCOVER A CRITICAL REQUIREMENT FOR MITOCHONDRIAL RESPIRATION FOR ANTIGEN-PRESENTING CELLS TO RESPOND TO IFN-GAMMA

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Abstract Content: The immunological synapse allows antigen presenting cells (APC) to convey a wide array of distinct signals to the T cell, which ultimately shape the immune response. The strength of stimulatory/inhibitory signals is influenced by the activation state of the APC, which is determined by an interplay between signal transduction and metabolic pathways. While TLR ligation relies on glycolytic metabolism for the expression of inflammatory mediators, less is known about the metabolic dependencies of other signals, such as IFNγ. Using CRISPR-Cas9, we performed a series of genome-wide knockout screens in macrophages to identify the regulators IFNγ-inducible T cell-stimulatory/inhibitory proteins MHCII, CD40, and PD-L1. Our multi-screen approach identified new pathways that uniquely associate with each marker. In addition, meta-analysis implicated complex I of the mitochondrial respiratory chain in the expression of all three markers, and by extension IFNγ signaling. We report that the IFNγ response requires mitochondrial respiration and APCs are unable to activate T cells upon genetic or chemical inhibition of complex I. Further characterization found that complex I loss alters T-cell mediated control of *M. tuberculosis* but did not inhibit TLR-mediated inflammation or type I IFN responses. These findings suggest a dichotomous metabolic dependency between IFNγ and toll-like receptor signaling; implicating mitochondrial function as a fulcrum of innate immunity and central to critical interactions between APCs and T cells.

Disclosure of Interest: None Declared

Lightning Talk Session 2: Autoinflammation and autoimmunity

LT082

INCREASED METFORMIN DOSAGE SUPPRESSES PRO-INFLAMMATORY CYTOKINE LEVELS IN SYSTEMIC CIRCULATION AND MIGHT CONTRIBUTE TO ITS BENEFICIAL EFFECTS

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Abstract Content: Background: Type 2 diabetes Mellitus (T2DM) is a metabolic disorder, characterized by persistent elevation of blood glucose either due to insulin resistance or insulin insufficiency. Inflammatory responses plays significant role in the pathogenesis and progression of T2DM. Cytokines play a pivotal role in modulation of immune reactions and disease pathogenesis. T-helper type 1 (Th1) and type 17 (Th17) cells, are important pro-inflammatory $CD4^+$ T cell subsets secreting TNF-α, and INF-γ (Th1), and interleukin 17 (Th17). These cytokines has been shown to play a crucial role in inflammation, insulin resistance, and development of T2DM. Metformin is the recommended first line of medication for the management of T2DM and is known to improve insulin sensitivity and prevents hyperglycemia by reducing chronic inflammatory responses. Here, we provide the first report on the therapeutic effect of metformin dosage on pro-inflammatory cytokine levels among T2DM patients.

Methodology: Two hundred and ninety eligible male and female participants admitted to or receiving treatment at the diabetic unit of the Komfo Anokye Teaching Hospital (KATH) in the Ashanti region of Ghana were recruited in a hospital-based cross-sectional study design. Serum samples collected from the participants were analyzed for the concentrations of TNF-α, INF-γ, GM-CSF and IL-17 cytokine levels by solid phase sandwich ELISA
Results: We found that participants on 3000 mg/day dose of metformin had significantly lower levels of TNF-α ($p<0.001$) and IFN-γ ($p=0.014$) compared to the other dosages (1000 mg and 2000 mg/day). However, GM-CSF and IL-17 levels were not affected by increased metformin dosages. After adjusting for age, gender, dose and duration of metformin use, we observed that participants who took higher doses of metformin had significantly reduced levels of TNF-α (β= -0.0297, 95% CI = (-0.005 to -0.002) $p<0.001$. Metformin dosage independently predicted TNF-α levels which explained 14.4% of the variations in the dosage levels.
Conclusion: Increased metformin dosage suppresses pro-inflammatory cytokine levels in systemic circulation and hence might contribute to its beneficial effects.
Key words: Type 2 diabetes mellitus, pro-inflammatory cytokine, metformin dosage
Disclosure of Interest: None Declared

LT083

BLOCKADE OF CYTOKINE PRODUCTION AND ATTENUATION OF EXPERIMENTAL ARTHRITIS PROGRESSION BY NOVEL SMALL MOLECULE INHIBITORS OF SEC61-DEPENDENT PROTEIN SECRETION

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Abstract Content: The Sec61 membrane protein complex gates translocation into the endoplasmic reticulum (ER) and subsequent targeting of nearly all proteins destined for secretion or membrane incorporation. Entrance to the ER via Sec61 is negotiated by signal sequences or transmembrane anchors unique to each translating protein. This interaction can be impeded by small molecule translocation inhibitors. Although biologics targeting individual cytokines and their receptors have been highly successful in the treatment of inflammatory and autoimmune diseases, there is increasing interest in the simultaneous suppression of multiple targets for improved efficacy. While Sec61 inhibitors such as mycolactone reduce cytokine secretion, cytotoxicity associated with their broad Sec61 client specificity has been observed. Here we describe subset-selective protein secretion inhibitors capable of targeting multiple pro-inflammatory cytokines, yet with limited cytotoxicity.
HEK293 cells stably overexpressing TNFα fused to a luciferase reporter were utilized to screen over 1000 Sec61 inhibitors from three distinct chemotypes to identify potent suppressors of this critical cytokine (IC_{50} < 3μM). Compounds were counter-screened for cytotoxicity in the myeloma cell line, H929, known to be sensitive to Sec61 inhibition. Activity of potent TNFα translocation inhibitors with a range of H929 cytotoxicity levels was confirmed in primary cell assays. Following LPS or anti-CD3/anti-CD28 stimulation of isolated human PBMCs or mouse splenocytes, inhibition of multiple pro-inflammatory cytokines was observed, including GM-CSF, IFNγ, IL-2, IL-6, IL-23, and TNFα, but not IL-1β, which is known to be secreted in a Sec61-independent manner. Cytokine inhibition potencies in primary cells were comparable to or higher than those observed for transfected luciferase reporter lines of the same cytokines. Of these confirmed inhibitors, representatives from each chemotype were selected for inflammatory mouse model testing based on anti-cytokine potency (IC_{50} < 100-250nM), low toxicity against unstimulated primary immune cells (IC_{50} > 25μM), and in vitro metabolic stability.
Dose-dependent decreases in serum pro-inflammatory cytokines were observed following injection of compounds two hours prior to administration of a mitogen to mice. Furthermore, dosing in a mouse model of rheumatoid arthritis revealed dose-dependent amelioration of disease activity at doses <1/8th of the compound MTDs. Paw lysates obtained at the peak of clinical scoring (observed for vehicle-treated animals) exhibited significant reductions in pro-inflammatory cytokines and chemokines subsequent to Sec61 inhibitor treatment, including IL-6, KC/GRO, and TNFα.
These data support Sec61 as a novel therapeutic target for autoimmune disorders via simultaneous suppression of multiple pro-inflammatory cytokines. We continue to optimize Sec61 inhibitors for development as oral therapies for the treatment of chronic inflammatory conditions.
Disclosure of Interest: J. Anderl Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, R. A. Fan Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, J. Jiang Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, H. Johnson Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, A. Kanicki Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, C. Kirk Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, E. Lewis Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, E. Lowe Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, D. McMinn Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, B. Millare Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, T. Muchamuel Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, M. Rao Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, J. Taunton Shareholder of: Kezar Life Sciences, Employee of: University of California, San Francisco, C. Tun Employee of: Kezar Life Sciences, J. Wang Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, J. Whang Shareholder of: Kezar Life Sciences, Employee of: Kezar Life Sciences, J. Zhang Shareholder of: Kezar Life Sciences, Employee of: Kezar

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LT086

CHARACTERIZATION OF AUTOIMMUNE OVARIAN FAILURE UPON REMOVAL OF THE IFN-GAMMA 3' UTR AU-RICH ELEMENT – A NOVEL MOUSE MODEL.

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Abstract Content: Premature ovarian failure (POF), which is when the ovaries cease to function, affects approximately 1-10% of women less than 40 years of age and is associated with increased risks of osteoporosis, cardiovascular pathologies, and diminished quality of life. Hence, laboratory studies using animal models for the development and testing of effective POF therapies are therefore necessary. Existing drug-induced POF animal models lack some clinical features and exhibit variable POF. In this regard, the poor reproductive capacity observed in mice with homozygous loss of the 3' untranslated region (3'UTR) AU-rich element (ARE) of the interferon gamma (IFNγ) gene and clinical characteristics of systemic lupus erythematosus led us to investigate the possibility of autoimmune POF.

A day before experiments, mice were estrus synchronized using urine-infused spent male bedding. On the day of the experiment, the ovaries, uteri, and spleen (controls) were collected, weighed, and dissociated. Vaginal epithelial cells were also collected. Using flow cytometry and immunohistochemistry (IHC), the distribution of immune cells in the ovaries, uteri, vaginal epithelial cells (VECs), and spleen (controls) were assessed. Histopathology was used to assess the morphological presentation of the organs. Estrus cycle stages were determined, and organ weights assessed. From the results obtained thus far, flow cytometric analysis showed increased $CD8^+$-, $CD4^+$ -T cells, $CD11c^+$- and $F4/80^+$ cells in the ovaries and uteri of $ARE^{-/-}$ compared to the wildtype (WT) and heterozygous ($ARE^{+/-}$) mice. $MHCII^+$ increased in the $ARE^{-/-}$ uteri. VECs showed decreased neutrophils with a simultaneous increase in eosinophils, macrophages, and MHCII populations in $ARE^{-/-}$. IHC revealed a dominance of $CD8^+$ and $CD4^+$ T cells in the ovaries and uteri with infiltration of the zona pellucida and granulosa cells of maturing follicles and corpora lutea while sparing the primordial and primary follicles. Histopathology showed atrophied ovaries and uteri in the $ARE^{-/-}$ mice while ovary and uterine weights were also decreased, indicative of atrophy. The $ARE^{-/-}$ and $ARE^{+/-}$ also showed reduced susceptibility to estrus synchronization.

This study has shown that the female $ARE^{-/-}$ mice exhibit features consistent with autoimmune ovarian failure phenotype with clinical POF features. While further characterization of POF continues, the ARE mouse model holds the potential to advance research in the management of autoimmune POF.

Disclosure of Interest: None Declared

LT087

EBI3 AND IL-1BETA PLAY A MAJOR ROLE IN THROMBOSIS DURING BEHÇET DISEASE BY REGULATING ACTIVATED PROTEIN C RESISTANCE

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Abstract Content: Behcet disease (BD) is a chronic systemic inflammatory disorder that is considered as a vasculitis touching both veins and arteries of all sizes. The underlying mechanism is under investigation with emphasis on the role of inflammation. In this study, we intended to explore the role of IL-1b with two other proinflammatory cytokines, IL-32 and EBI3, in the regulation of coagulation during BD.

Eighteen patients with BD (6 active and 12 inactive stage) and 10 control subjects were enrolled in this study. Freshly collected blood on citrated tubes was used in the study. Hemostasis parameters and factors were assessed in freshly collected plasma. IL-1b, IL-32 and EBI3 were measured by ELISA (Biotechne). Statistical analyses were measured by Mann Witney U test for group comparison while Spearman test was used for correlation analyses.

We observed a significant increase in EBI3 and IL-32 levels during BD in comparison to controls ($p<0.05$). IL-1b and IL-32 were increased during active stage ($p<0.05$) but not EBI3 ($p=0.123$). All Hemostasis parameters didn't show any differences between patients and control subjects ($p>0.05$). However, correlation studies showed significant relationships between cytokines and some hemostasis parameters in patients and not in control subjects. Especially, EBI3 was positively correlated with fibrinogen ($r= 0.583$, $p=0.018$) and Activated protein C resistance ($r=0.626$, $p=0.01$). IL-1b was positively associated with Activated protein C resistance ($r=0.782$, $p=0.013$) but negatively correlated with FIX ($r= - 0.846$, $p=0.008$). IL-32 showed no significant correlation with the tested parameters ($p>0.05$).

Our results showed that EBI3 and IL-32 were increased in BD in comparison to control subjects while IL-1b and IL-32 were more associated to disease activity. Even if we didn't observe any alteration in the hemostasis in patients with BD, we observed that IL-1b increase is associated the intrinsic coagulation pathway suggesting its implication in the alteration of endothelial cells. EBI3 was more associated with fibrinogen genesis. The two cytokines were associated with Activated protein C resistance reflecting a state of hypercoagulability during Behçet disease. These findings highlight the role of inflammatory cytokines, especially IL-1b and EBI3, in the prothrombotic state during BD.

Disclosure of Interest: None Declared

LT088

TYPE I INTERFERON INHIBITS GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ) EXPRESSION AND UPREGULATION BY GLUCOCORTICOIDS

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Abstract Content: Background

Glucocorticoids (GC) are broadly used in the treatment of inflammatory diseases, including systemic lupus erythematosus (SLE). Despite their widespread use, most SLE patients do not reach a state of low disease activity on GC treatment. Currently it is not completely understood what factors play a role in the response to this treatment. It is thought that many anti-inflammatory effects of GC are mediated through upregulation of glucocorticoid-induced leucine zipper (GILZ). GILZ expression is decreased in the blood of SLE patients compared to healthy controls. Interestingly, we have previously shown that this is inversely correlated with the interferon (IFN) signature induced by type I IFN, including IFNα and IFNβ. Given the important role of type I IFN in SLE pathogenesis, we studied whether IFNα could suppress GILZ and thereby reduce the effectiveness of GC.

Methods

Human peripheral blood mononuclear cells (PBMC) were isolated from healthy individuals and treated with 1000 IU IFNα2a, 100 nM dexamethasone (DEX) or both, with or without the Jak1/Tyk2 inhibitor tosylate salt (TS). GILZ expression in these cultures was analysed using RT-PCR. STAT1 binding sites were analyzed from public datasets and using chromatin immunoprecipitation followed by RT-PCR. Public datasets were also used to study the effect of IFNα and the interplay between GC and IFNα on GILZ expression in SLE patients.

Results

IFNα treatment reduces the expression of GILZ in human PBMC in a dose- and time-dependent manner. Interestingly, it also reduces the DEX-induced upregulation of GILZ. This corresponds to data in SLE patients, where GC treatment is less effective at inducing GILZ in patients with a high IFN score than in patients with a low IFN score. Mechanistically, we found that IFNα2a reduces GILZ expression via the Jak1/Tyk2 signalling pathway, as treatment with the specific inhibitor TS reversed the effects of IFNα2a. In public datasets, and then confirmed via ChIP, we subsequently found that the transcription factor STAT1, downstream of Jak1/Tyk2, has multiple DNA binding sites surrounding the GILZ locus. These STAT1 binding sites coincide with binding sites of the glucocorticoid receptor (GR), which may explain the mechanism by which IFNα2a reduces the DEX-induced GILZ upregulation.

Conclusion

In human PBMC, IFNα2a reduces GILZ expression and the DEX-induced upregulation of GILZ via the Jak1/Tyk2 signalling pathway and direct DNA binding of STAT1 to the GILZ locus. These data reveal a potential mechanism by which type I IFN suppress the effectivity of GC, which could be targeted to improve therapeutic efficacy in SLE.

Disclosure of Interest: None Declared

LT089

IL-10 PROMOTES REMYELINATION IN AN EX-VIVO MODEL OF MYELIN DAMAGE

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Abstract Content: Multiple sclerosis (MS) is an autoimmune disease characterized by peripheral immune infiltration of the CNS and subsequent demyelination of axons, leading in turn to neuron damage and symptoms in patients. This damage can be repaired via a process of remyelination of previous damaged neurons. The cytokine IL-10 has been shown to promote a micro-environment conducive to myelin repair in MS, however the underlying mechanism remains poorly understood. Remyelination can be modelled ex vivo by application of the demyelinating drug lysolecithin to cultured organotypic cerebellum and brain slice cultures, then applying factors and assessing the extent of remyelination via Immunofluorescent microscopy. We show brain slice cultures were successfully demyelinated using lysolecithin and subsequently remyelination was observed ex vivo. Demyelinated slices were then treated LPS, IL-10 and a combination of both, and we observe a trend towards greater remyelination in brain slices treated with the combination of both stimuli. Our lab has previously identified Arginase 2 as a downstream immunometabolic target of IL-10. In brain slices obtained from an Arg2 knockout strain, this observed LPS+IL-10 effect is lost. Arg2 is also a target of miR-155, and in brain slices lacking miR-155, remyelination is enhanced compared with controls, suggesting a mechanism via which miR-155 may mediate the observed LPS + IL-10 effect via Arg2.

Disclosure of Interest: None Declared

LT090

THE ASSOCIATION BETWEEN TMAO AND NEIGHBORHOOD DEPRIVATION IS MEDIATED BY PRO-INFLAMMATORY CYTOKINES AMONG AFRICAN-AMERICANS AT RISK FOR CARDIOVASCULAR DISEASE.

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Abstract Content: Trimethylamine N-oxide (TMAO), is identified as a biomarker for atherosclerosis. Likely mechanisms for TMAO and atherosclerosis include the promotion of pro-inflammatory cytokines such as Il-1β and TNF-α from *in vitro* studies. The role of TMAO in cardiovascular (CVD) risk among resource limited communities that are most impacted by neighborhood risk factors, such as limited food access or exposure to pollutants has not been fully investigated. We therefore sought to examine the relationship of TMAO at both an

individual and neighborhood level and to evaluate a mediating role for pro-inflammatory cytokines.
Plasma samples were utilized from African-American study participants who lived in neighborhoods surrounding and including Washington, D.C. TMAO plasma levels were determined by ELISA (Biohippo). Cytokine levels were determined by the ELISA-based technique, U-Plex (MesoScale). Clinical CVD risk factor data included BMI, cholesterol levels and ASCVD risk score calculation. Demographic data collected included addresses, which allowed for the calculation of the census linked variable, neighborhood deprivation index (NDI). Associations between variables were determined by bivariate and multivariate ANOVA using STATA. Mediation analysis was also conducted using STATA.
Results from 60 African-American individuals (female 93%, median age 60.8, median BMI = 33.0) showed a mean TMAO level of 37.54 ng/ml. Bivariate analysis showed a significant association between NDI and TMAO, after adjusting for BMI and ASCVD risk score (β= 0.314, p=0.017). There were significant associations between NDI and pro-inflammatory cytokines TNF-α (β=0.485, p<0.001) and IL-1β (β=0.434, p<0.001). As well as with TMAO and TNF-α (β=0.368, p=0.004) and IL-1 β (β=0.222, p=0.053). Together both TNF-α and IL-1β explained 61.7% of the relationship between NDI and TMAO. Interestingly, other pro-inflammatory cytokines, such as IL-6 did not contribute to the mediation model (p=0.895).
In conclusion, by using cross-sectional data available from African-American adults with CVD risk factors living in variable neighborhood environments, we found an association between plasma TMAO levels and neighborhood deprivation and that the relationship was mediated by cytokines, IL-1β and TNF-α. Findings from this study may serve to identify biological mechanisms related to neighborhood deprivation and CVD risk, but also serve to provide insight on TMAO, a previously unidentified biomarker for adverse health outcomes at the neighborhood level.
Disclosure of Interest: None Declared

LT091
METABOLIC AND STRUCTURAL MUSCULAR CHANGES DRIVEN BY CHRONIC DISEASE-LIKE IFN-G EXPOSURE
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Abstract Content: The effect of cytokines on non-traditional immunological targets under conditions of chronic inflammation have been fruitful at explaining many comorbidities in disease. Cardiovascular myopathies and fatigue have been associated with chronic inflammatory responses and activated cell-mediated immunity. We report aberrant cardiovascular activity in the form of reduced ejection fraction *in vivo* in male mice with persistent low-level expression of IFN-g (ARE mice). ARE mice have a deletion and replacement with random nucleotides in the 3' UTR of the IFN-g gene that serves to remove a regulatory element that is integral at controlling the stability of IFN-g mRNA. ARE mice had increased cellular infiltrates into heart tissue and mitochondria linked metabolic changes that were concomitantly observed following a stress- exacerbated decrease in heart function. Our data also demonstrate an increase in glucose expenditure and lower general mobility of ARE mice as well as structural and ultrastructural changes in the heart. We also observe a decrease in the transcripts and function of aerobic respiratory components in the muscle of male mice. Notable, we observe a decrease in X chromosome linked pyruvate dehydrogenase, a key component in pyruvate's entry to the TCA cycle, and other downstream mitochondrial genes. Furthermore, the *in vivo* data revealed an increase in factors associated with fatigue, such as lactic acid production and changes in expression of genes associated with an increase in anaerobic respiration in cardiac musculature. These results indicate that the chronic expression of IFN-g results in a model for male biased heart failure under stress in an autoimmune environment, similar to the idiopathic cardiomyopathy found in male Systemic Lupus Erythematosus patients. Considering that fatigue is a symptom often associated with autoimmune diseases, we hypothesize that cytokine-driven changes in cardiac musculature may play a critical role in fatigue-related disease associated with autoimmune disorders. This work provides new insights into the diverse actions of IFN-g in fatigue and cardiomyopathy.
Disclosure of Interest: None Declared

LT092
PATHOGENIC, GLYCOLYTIC PD-1+ B CELLS ACCUMULATE IN THE HYPOXIC RA JOINT.
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Abstract Content: Background:
Rheumatoid arthritis (RA) often has a progressive and debilitating course, with significant impact on the patient's quality of life. Despite the long-known association with autoantibodies, knowledge of the role of B cells and their potential direct contribution to RA pathogenesis remains elusive.
Methods:
Synovial tissue biopsies from RA patients were obtained through key-hole arthroscopy followed by single cell flow cytometric analysis of B cell subpopulations and chemokine receptor expression. SPICE analysis was performed for the chemokine receptor expression of synovial B cells and B cell invasion assays. Functional characterization of sorted RA B cells, cultured *in vitro* under hypoxia, simulating the inflamed joint environment. Characterization of B cell metabolism and transcriptional regulation of pSTAT3 by flow cytometry.

Novel, non-invasive Fluorescent Lifetime Imaging Microscopy (FLIM) assay for the characterisation of the metabolic status of sorted RA patient PD-1 B cells coupled with RNAseq analysis was performed.

Results:

Extensive flow-cytometric characterisation and SPICE algorithm analyses of single-cell synovial tissue from RA-patients revealed the accumulation of switched and double negative memory PD-1 expressing B cells at the site of inflammation. Accumulation of memory B cells is mediated by CXCR3, evident by the observed increase in CXCR3 expressing synovial B cells compared to the periphery, differential regulation by key synovial cytokines, and restricted B cell invasion demonstrated in response to CXCR3 blockade. Notably, under 3% O_2 hypoxic conditions that mimic the joint-microenvironment, RA B cells maintain marked expression of MMP-9,-TNF and IL-6, with PD-1$^+$ B-cells demonstrating higher expression of CXCR3, CD80, CD86, IL-1β and GMCSF than their PD-1$^-$ counterparts. Following functional analysis and flow cell sorting of RA PD-1$^+$ vs PD-1$^-$ B-cells, we demonstrate using RNAseq and novel FLIM visualisation of cellular NADH, a significant shift in metabolism of RA PD-1$^+$ B-cells towards glycolysis, associated with an increased transcriptional signature of key cytokines and chemokines that are strongly implicated in RA pathogenesis.

Conclusions:

The accumulation of pro-inflammatory PD-1 B cells in the synovium of RA patients, is CXCR3 mediated and offers an opportunity for early therapeutic intervention. Once in the hypoxic environment of the inflamed RA joint, PD-1 B cells show altered activation, pro-inflammatory cytokine production and metabolism that could prove important for understanding the role of B cells in RA pathogenesis.

Disclosure of Interest: None Declared

LT093

STING GAIN-OF-FUNCTION IN RADIORESISTANT CELLS SUPPORTS A LYMPHOCYTE DEPENDENT AUTO-INFLAMMATORY LUNG DISEASE

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Abstract Content: cGAS-STING is a cytosolic dsDNA sensing pathway whose regulation is vital to immune homeostasis. Pediatric patients with constitutively active STING mutations develop an autoinflammatory syndrome known as STING Associated Vasculopathy with onset in Infancy (SAVI) and suffer from treatment resistant lung fibrosis. Interestingly, SAVI patients develop immune abnormalities including lymphopenia concomitant with hyperactivation, and lung biopsies from SAVI patients show lymphocyte predominant immune aggregates which strongly resemble bronchus associated lymphoid tissues (BALT), suggesting a role for lymphocytes in SAVI lung disease. We have genetically engineered a mouse model of SAVI (STING V154M or VM mice) that recapitulates the development of lymphocyte rich BALTs in the lung as early as 6 weeks of age and develops progressive fibrotic disease by 16 weeks.

To test the requirement of lymphocytes in SAVI disease, we bred VM Rag1$^{-/-}$ mice and found they were protected from lung and systemic inflammation, as indicated by histology, splenomegaly, and body weight. To define which lymphocyte subsets were required for disease, we genetically and pharmacologically ablated T and B cells respectively using VM TCRβ$^{-/-}$ mice or VM mice treated with αCD20 antibodies. Systemic inflammatory disease persisted in both cohorts, suggesting neither αβ T cells nor B cells were required for disease. Additionally, lung disease persisted in VM TCRβ$^{-/-}$ mice, and it has been reported prior that lung disease persists in B cell deficient μMT$^{-/-}$ STING N153S SAVI mice. These results support the notion that T and B cells could independently contribute to SAVI lung disease. Moreover, we found that VM splenic αβ T and B cells depended on each other for maximal hyperactivation, suggesting a potential synergistic interaction as well.

Next, we generated chimeric mice by transferring donor VM bone marrow (BM) into irradiated WT mice to test if immune abnormalities were due to hematopoietic intrinsic STING Gain-of-Function (GOF). Although BM intrinsic STING GOF was sufficient to drive lymphopenia, it was not sufficient for lymphocyte activation. To test if lymphocyte activation could instead be driven by extrinsic STING GOF in radioresistant cells, we transferred WT BM into irradiated VM recipients and found WT splenic T cells developed hyperactivation that phenocopied VM mice. WT B cells in these chimeras also became activated, although to a lesser degree. Importantly, progressive lung disease persisted in these chimeric mice, indicating radio-resistant cell STING GOF was sufficient to perpetuate SAVI inflammatory lung fibrosis.

Together, these findings suggest that STING GOF in radioresistant cells organizes and activates a profibrotic inflammatory lymphocyte response in the lung. We are currently investigating candidate radioresistant cell populations as initiators of this process including endothelium and lung epithelium, as well as mechanisms by which lymphocytes drive SAVI lung pathology. These findings are clinically relevant for SAVI patients, as well as more broadly for patients suffering from interstitial lung disease associated with rheumatic disease and suggest that lymphocyte depleting therapies may mitigate further lung injury and worsening of fibrosis.

Disclosure of Interest: None Declared

LT094

ASTROCYTE, BUT NOT MICROGLIA UNRESPONSIVENESS TO TYPE I INTERFERON IS PROTECTIVE IN A MOUSE MODEL OF CEREBRAL TYPE I INTERFERONOPATHIES

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Abstract Content: Cerebral type I interferonopathies are an expanding group of neuroinflammatory and

neurodegenerative disorders associated with the overproduction of interferon-α (IFN-α) in the central nervous system (CNS). Aicardi-Goutières Syndrome (AGS) is a monogenic disorder that is considered the prototypical cerebral type I interferonopathy. Clinically, patients with AGS suffer cognitive deficits and motor dysfunction, as well as other pathological features such as leukocytosis, intracerebral calcifications and chronic encephalitis. Other cerebral type I interferonopathies include autoimmune disorders such as CNS-lupus and congenital and chronic viral infections. Currently, there is no cure for these disorders.

In the CNS, astrocytes and microglia are key effectors of innate immune defenses against infection and injury and play important roles in the maintenance of a normal, homeostatic environment. However, recent evidence has implicated both astrocytes and microglia in the pathogenesis of neuroinflammation and neurodegeneration. Transgenic mice with CNS-restricted chronic production of IFN-α (termed GIFN mice) recapitulate many of the hallmark features seen in human patients with cerebral type I interferonopathies. To determine the contribution of astrocytes and microglia in the pathogenesis of this disease, we generated novel triple transgenic GIFN mouse lines in which either astrocytes or microglia are unresponsive to IFN-I, using Cre/lox-mediated recombination of the *Ifnar1* gene.

Astrocyte-specific deletion of IFNAR in GIFN mice delayed onset and progression of disease and improved survival compared to GIFN mice. However, no improvement was seen in GIFN mice with microglia-specific deletion of IFNAR. Despite these differences, both triple transgenic mice showed protection from CNS pathology including neurodegeneration, calcifications, aneurysms and microgliosis observed in GIFN mice. These findings indicate that while microglia responses to IFN-α appear not to contribute to disease pathogenesis in cerebral type I interferonopathies, astrocytes may play a greater role in the pathogenesis of disease. This is a key step for the development of cell-specific, targeted therapies.
Disclosure of Interest: None Declared

LT095

IL-38 ABLATION REDUCES INFLAMMATION AND DISEASE SEVERITY IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Abstract Content: **Introduction:** Interleukin-38 (IL-38) is a 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 and downstream T lymphocyte IL-17 production by blocking X-linked IL-1 receptor accessory protein-like 1 (IL1RAPL1) signaling. IL-38 polymorphisms are associated with increased susceptibility for auto-inflammatory diseases such as psoriasis, spondyloarthritis and rheumatoid arthritis, but its role in IL-17-driven inflammation *in vivo* remained largely obscure.
Methods: The experimental autoimmune encephalomyelitis (EAE) mouse model was used as an IL-17-dependent model of autoimmune disease of the central nervous system, which closely resembles human multiple sclerosis. EAE was induced in WT and IL-38 KO mice by immunization with MOG_{35-55} peptide emulsified with Complete Freund's Adjuvant (CFA), followed by intraperitoneal administration of pertussis toxin (PTX) 2 h and 22 h after immunization. Mice were examined daily for body weight and clinical signs of EAE using clinical scores that follow different degrees of paralysis. Immune cell composition and cytokine profiles of the spinal cord and blood were analyzed by flow cytometry. IL-38 and pro-inflammatory mRNA expression levels were evaluated by quantitative PCR and RNAScope. Spinal cord histology was performed by PhenOptics. Statistical comparisons between two groups were performed using Mann Whitney test, unpaired two-tailed Student's t test. One- or two-way analysis of variance (ANOVA) followed by appropriate post-tests.
Results: IL-38 was shown to be expressed in the spinal cord and its absence in IL-38 KO mice, against expectations strongly reduced clinical scores in the EAE model indicating attenuated disease development. This finding was substantiated by a better spinal cord structure in IL-38 KO mice, including increased number of neurons and reduced myelin deposition, as well as less immune cell infiltration. Indeed, we observed less macrophages, monocytes, neutrophils and T helper cells in the spinal cord of IL-38 KO mice compared to WT mice, while the percentage of these immune cell populations tended to be higher in the blood. Moreover, IL-38 KO mice showed lower mRNA expression of inflammatory markers in the spinal cord, as well as lower concentration of TNFα at protein level. Alteration of the macrophage number and macrophage-related gene expression in the spinal cord of IL-38 KO mice was related to the absence of IL-38 in a cell-intrinsic manner. Indeed, classically activated IL-38 KO macrophages expressed lower levels of inflammatory genes such as TNFα or IL-23, compared to WT macrophages.
Conclusion: Unlike other auto-inflammation models, ablation of IL-38 dramatically reduced disease severity in the EAE model. This appeared to be due to IL-38 expression by macrophage themselves, as opposed to IL-38 by other sources acting on macrophage, which usually dampens inflammation. suggest IL-38 as a potential target for CNS autoimmune diseases such as multiple sclerosis or autoimmune encephalopathies.
Disclosure of Interest: None Declared

LT096

B LYMPHOCYTE-DERIVED CCL7 AUGMENTS NEUTROPHIL AND MONOCYTE RECRUITMENT, EXACERBATING ACUTE KIDNEY INJURY

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Abstract Content: Acute kidney injury (AKI) is a serious condition affecting a fifth of hospital in-patients. B lymphocytes have immunological functions beyond antibody production and may produce cytokines and chemokines which modulate inflammation. Here we investigated leucocyte responses in a mouse models of AKI and observed an increase in circulating and kidney B cells, particularly a $B220^{low}$ subset, following AKI. We found that B cells produce the chemokine CCL7, with the potential to facilitate neutrophil and monocyte recruitment to the injured kidney. Siglec-G-deficient mice, which have increased numbers of $B220^{low}$ innate B cells and a lower B cell activation threshold, had increased *Ccl7* transcripts, neutrophil and monocyte numbers in the kidney and more severe AKI. CCL7 blockade in mice reduced myeloid cell infiltration into the kidney and ameliorated AKI. In two independent cohorts of human patients with AKI, we observed significantly higher CCL7 transcripts compared to controls, and in a third cohort, an increase in urinary CCL7 levels in AKI, supporting the clinical importance of this pathway. Together our data suggest that B cells contribute to early sterile inflammation in AKI via the production of leucocyte-recruiting chemokines.
Disclosure of Interest: None Declared

LT097

PROLONGED RESIDENCE OF ALBUMIN-FUSED ANTI-INFLAMMATORY CYTOKINES IN THE SECONDARY LYMPHOID ORGANS AMELIORATES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND RHEUMATOID ARTHRITIS

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Abstract Content: Multiple sclerosis (MS) is a common and severe demyelinating autoimmune disease of the central nervous system. Although interleukin (IL)-4, an immunosuppressive cytokine, suppresses development of pathology in a murine MS model, experimental autoimmune encephalomyelitis (EAE), IL-4 has not been clinically translated to the clinic. We hypothesized that the poor efficacy of recombinant IL-4 administration is due to insufficient residence of IL-4 in the secondary lymphoid organs (SLOs), because antigen-specific T cell priming and Th17 cell development primarily occur in the SLO. We have engineered a fusion protein of serum albumin (SA) and IL-4 (SA-IL-4) to increase the persistence of IL-4 in the SLOs. SA-IL-4 showed greater accumulation and residence time in lymph nodes (LNs) and spleen compared to wild-type (wt) IL-4, which was dependent on neonatal Fc receptor (FcRn) binding. Administration of SA-IL-4 prevented EAE disease development in all mice and demonstrated higher therapeutic efficacy compared to FTY720, a clinically used drug (fingolimod), and wt IL-4. SA-IL-4 prevented immune cell infiltration into the spinal cord, facilitating maintenance of spinal cord structure and resulting neurological function. SA-IL-4 decreased integrin expression in antigen-reactive $CD4^+$ T cells, indicating impaired cell migration capability. SA-IL-4 increased the number of and programmed death ligand-1 expression on granulocyte-like myeloid-derived suppressor cells, a key EAE disease suppressor, in the spinal cord-draining LN (dLN). SA-IL-4 decreased the number of Th17 cells, a pathogenic cell population for EAE disease. In the chronic phase of EAE, SA-IL-4 also showed marked therapeutic effects, accompanied by inhibition of immune cell infiltration into the spinal cord and complete abrogation of immune response to the myelin antigen in the spleen. Engineered SA-IL-4 demonstrate translational promise for MS as both a preventative and therapeutic treatment via accumulation in SLOs.
To extend our study, we also made SA fusion to IL-10, an immunosuppressive cytokine. Rheumatoid arthritis (RA) is a major autoimmune disease that causes synovitis and joint damage. Although clinical trials using IL-10 have been performed as a potential treatment of RA, its therapeutic effects have been limited. After intravenous injection to mice, SA fusion to IL-10 led to enhanced LN accumulation compared with unmodified IL-10. Intravenous SA-IL-10 treatment restored immune cell composition in the paws to a normal status, elevated the frequency of suppressive M2 macrophages, reduced IL-17A amount in the paw- draining LN, and protected joint morphology. Intravenous SA-IL-10 treatment showed similar efficacy as treatment with an anti-TNF-a antibody. SA-IL-10 was equally effective when administered intravenously, locally or subcutaneously, which benefits clinical translation of this molecule. SA fusion to IL-10 is a simple but effective engineering strategy for RA therapy and holds clinical translational potential. Overall, we are the first to show that SA fusion to the immunosuppressive cytokines would increase the residence in the SLO, and strongly suppresses autoimmune diseases symptoms through decreasing pathogenic cell population.
Disclosure of Interest: None Declared

LT098

LUPUS-LIKE DISEASE IN B6.NBA2 MICE SHOWS A BIPHASIC PROGRESSION

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Abstract Content: Lupus is an autoimmune disease with a diverse pattern of symptoms affecting approximately 1.5 million Americans. Over the past decade, new biologics (f.ex. Belimumab, Anifrolumab) have emerged targeting cytokines and/or cells known to be pathogenic in lupus, however, response-rates among varied patient populations remain, low supporting the heterogeneity of the disease. B6.Nba2 mice exhibit a spontaneously occurring constitutive progressive lupus-like disease pattern driven by type I interferons (IFNα). Using this model, ablation of plasmacytoid dendritic cells (pDCs) is effective if initiated before, but not after, 4 months of age, suggesting a biphasic disease pattern with pDCs/IFNα playing a role during the early phase only. To investigate the second phase, we

compared disease manifestations in 4 and 9 month old B6.Nba2 mice. As expected, IFNα-induced gene transcripts diminished after 4 months of age as did levels of IL-6; another product of pDCs, while we saw no changes in IL-21 levels. In contrast, splenic BAFF levels increased over time both at the transcriptional and protein level as did levels of *Irf4* and *Bcl6* transcripts, supporting an upregulation in specific B cell subsets including germinal center B cells and plasma cells in aging mice. These data indicates that pDCs and their products, IFNα and IL-6, play a critical role in early lupus like disease progression, while BAFF and B cells are involved in the later stage of the disease progression. Thus, treatment with different biologics may be needed at different time points of disease. We suggest that investigating patterns of gene expression combined with obtaining an inclusive patient history is necessary to determine the treatment approach needed for each individual lupus patient.
Disclosure of Interest: None Declared

LT099
IMPAIRED IL2/STAT5 SIGNALLING IN NAÏVE CD4 T CELLS PROMOTES TFH DIFFERENTIATION IN GRANULOMATOSIS WITH POLYANGIITIS PATIENTS

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Abstract Content: Granulomatosis with Polyangiitis (GPA) is a systemic autoimmune disease, classified as an anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis. Accompanying elevated levels of ANCA in patient's blood, an imbalance in circulating functional effector CD4 T cell subsets is strongly associated with GPA pathogenesis. Recent studies have observed a suppression of regulatory T cell (Treg) function and an increase in follicular helper T (FH) / Th17 cells in GPA. However, the mechanism(s) responsible for this imbalance in GPA remain unknown. In this study, we hypothesise that transcriptomic changes may occur in naïve CD4 T cells in GPA that lead to an imbalanced effector CD4 T cell population. Bioinformatic analysis of microarray data revealed a dysregulation of IL2Rβ/JAK-STAT signalling pathway, and higher expression of BCL6 (a key TFH transcription factor) and BCL6-related genes in GPA naïve CD4 T cells compared to healthy controls (HC). In naïve CD4 T cells pre-activated with anti-CD3 and anti-CD28 antibodies, IL2 induced STAT5 activation (which suppresses BCL6 expression) was significantly reduced in GPA compared to HC as measured by western blot analysis. In contrast to STAT5 activation, no differences in STAT3 activation (which upregulates BCL6 expression) by IL6 and IL2 were observed. *In vitro* CD4 T cell differentiation assays showed that BCL6 is highly expressed in response to T cell activation in GPA naïve CD4 T cells. Moreover, under conditions designed to skew CD4 T cell differentiation towards a TFH phenotype, GPA naïve CD4 T cells exhibited a more pronounced increase in TFH characteristics; higher $CXCR5^+$ $PD1^+$ frequencies, and higher BCL6, IL21 and IL6 expression. In conclusion, perturbed signalling pathways in GPA naïve CD4 T cells in response to T cell activation result in decreased STAT5 activation versus STAT3 activation, and lead to aberrant BCL6 expression, promoting enhanced TFH differentiation. Strategies targeting BCL6 expression and/or IL6/STAT3 activation may have therapeutic benefit in the treatment of GPA/ANCA associated vasculitis.
Disclosure of Interest: None Declared

LT100
TYPE III INTERFERON LIMITS RECOVERY AND PROMOTES AXONAL INJURY DURING AUTOIMMUNE NEUROINFLAMMATION

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Abstract Content: Multiple sclerosis (MS) is a chronic autoimmune disease characterized by pathologic infiltration of lymphocytes and macrophages into the central nervous system (CNS) that leads to demyelination and axonal injury. Extent of axonal injury is strongly correlated with disease progression and permanent disability in MS patients. Currently available treatments for MS have varying efficacy, do not impact transition to progressive disease, or promote axonal recovery. Here, we define a role for type III interferon (interferon lambda or IFNλ) in the maintenance and progression of CNS autoimmune diseases.
Interferons (IFN) are a class of cytokines that play a role in antiviral immunity, as well as in immune-mediated diseases. In particular, type I IFN has been highly studied and implicated in CNS autoimmune diseases, such as MS; however, less is known about the closely related type III IFN (IFNλ) in this context. IFNλ is most known for its antiviral properties at mucosal barriers, promotion of Th1 adaptive immune responses, and ability to alter blood-brain barrier permeability. Based on these characteristics, we hypothesized that IFNλ's immunomodulatory properties may alter immune-mediated inflammation during CNS autoimmune disease.
We found that IFNλ signaling impacts recovery in mice with experimental autoimmune encephalomyelitis (EAE), a well-established murine model of CNS autoimmunity. Genetic deletion of the IFNλ receptor (*Ifnrl1*) and antibody mediated neutralization of IFNλ both result in clinical recovery during EAE. Recovery was linked to resolution of CNS inflammation, with animals deficient in IFNλ signaling exhibiting fewer infiltrating, cytokine-producing lymphocytes and decreased microglial activation compared to wildtype animals. Deletion of *Ifnlr1* also protected animals from axonal injury of the spinal cord, while surprisingly having no effect on demyelination or remyelination. Cell specific deletion of *Ifnlr1* revealed $CD11c^+$ antigen presenting cells, critical for T cell reactivation, to be targets of IFNλ during EAE. Finally, in human tissue, we found increased levels of IFNλ in lesions of secondary progressive MS patients compared to relapsing remitting MS patients. These data reveal a novel role for IFNλ in maintaining chronic

neuroinflammation and as a potential therapeutic target in MS patients.
Funded by: NIH grant F31 NS108629-01A1 (SM) and NMSS Research Grant (RSK)
Disclosure of Interest: None Declared

LT101
DETERMINING HNRNP-A2/B1'S ROLE IN CONTRIBUTING TO INFLAMMATION IN RHEUMATOID ARTHRITIS
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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 which 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, a ubiquitous RNA processing protein, is emerging as an important player in a wide variety of autoimmune diseases such as Rheumatoid Arthritis (RA). Loss of tolerance to native and citrullinated forms of hnRNP-A2/B1 is a hallmark of RA; abundance of autoantibodies against it in patients' sera as well as it's abundance in patients' synovial tissue are well documented. Despite its use as a biomarker for RA, the degree of its involvement in disease pathogenesis is not yet studied. The aim of our work is to determine if hnRNP-A2/B1 is playing a direct role in contributing to the inflammation associated with RA. We performed siRNA mediated knock down of hnRNP-A2/B1 in fibroblast-like synoviocytes (FLS) obtained from RA patients to investigate whether knockdown of hnRNP-A2/B1 leads to changes in expression of ISGs and inflammatory genes associated with RA. Our gene expression analysis using RNA-seq and Nanostring technology uncovered a role for hnRNP-A2/B1 in driving inflammation, where loss of this protein dampened the inflammatory circuit associated with RA pathogenesis. We are currently working to determine the mechanism by which hnRNP-A2B1 contributes to controlling inflammation within the human RA samples. In addition we will utilize our recently generated hnRNP-A2/B1 conditional knock out mouse to further characterize this protein's involvement in amplifying the inflammatory circuit in the arthritic joint *in vivo*.
Disclosure of Interest: None Declared

LT102
NANOMODULATION OF MICRORNAS IN MACROPHAGES
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Abstract Content: Multiple Sclerosis (MS) is a chronic demyelinating autoimmune disease characterised by central nervous system (CNS) infiltration of peripherally derived immune cells, the largest fraction of which are macrophages. The macrophage role in MS is multifaceted, in a pro-inflammatory or 'M1' state instructing demyelination and axonal loss, while the anti-inflammatory 'M2' state holds a key role in promoting tissue repair and regeneration. There is considerable interest in elucidating and manipulating factors that mediate switching between these phenotypes. Previously, we have identified that IL-10 inhibition of miR-155 is a prominent mechanism utilised by macrophages to maintain an M2 state. Moreover, using a miR-155 floxed x LysMCre model, where miR-155 is specifically deleted from myeloid cells, there was reduced disease onset and less lesion burden in the experimental autoimmune encephalomyelitis (EAE) animal model.
Therefore, we hypothesise miR-155 inhibition may favourably modulate the macrophage population to an 'M2' or pro-repair phenotype, reducing inflammation, alleviating disease progression, mimicking an IL-10 mediated effect. Thus, the objective of this study is to investigate the therapeutic potential of a miR-155 anti-miRNA oligonucleotide (AMO) packaged in nanoparticle-based carriers to enhance uptake into macrophages. From a range of anti-miRNA oligonucleotide (AMO), we have identified the best miR-155 AMO to inhibit mir-155 in Raw 264.7 and in murine bone marrow derived macrophages (BMDMs). We investigated a range of macrophage polarisation parameters, including pro-inflammatory cytokine expression, Nitric Oxide production, expression of M2 markers Arginase-1 and CD206, and identified that Arginase-2 is the most robust miR-155 target that mimics an IL-10 M2 mediated phenotype. We also demonstrate the capacity of miR-155 AMO for nanoparticle encapsulation into PLGA and delivery using novel star shaped polypeptides *in vitro.* Our future studies will examine the capacity for *in vivo* delivery and directing macrophage specific uptake.
Disclosure of Interest: None Declared

LT103
INTEGRIN ALPHA 3 PROMOTES THE EXTRAVASATION OF PROINFLAMMATORY TH17 CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS.
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Abstract Content: The disruption of blood-brain barrier and the infiltration of leukocytes into the central nervous system (CNS) parenchyma are critical in the pathogenesis of multiple sclerosis (MS). Because integrin α 4 mediates lymphocyte tethering and transendothelial migration, integrin α 4 has been therapeutically targeted by natalizumab to inhibit the infiltration of $CD4^+$ T cells in MS. However, natalizumab is less effective in inhibiting the migration of Th17 cells which play major pathogenic roles in MS due to the low expression of integrin α 4 on Th17 cells. Moreover, long-term use of natalizumab down-regulates

Th1-mediated immune surveillance and increases the risk of opportunistic JC-virus infection in the brain leading to progressive multifocal leukoencephalopathy. Therefore, it is necessary to understand Th17 cell trafficking during inflammation in order to identify new therapeutic targets in MS treatment. In this study, we identified that integrin α 3 is expressed by inflammatory Th17 cells that infiltrate into the CNS parenchyma in experimental autoimmune encephalomyelitis (EAE) through an IL-6-Stat3 signaling pathway. Moreover, the deletion of integrin α 3 in $CD4^+$ T cells or IL-17A-fate-mapped cells attenuated the disease severity of EAE by impeding $CD4^+$ T cell infiltration into the CNS parenchyma. Immunofluorescence staining of spinal cord sections revealed an increased retention of integrin α 3-deficient $CD4^+$ T cells in laminin-rich perivascular spaces. The significance of integrin α 3 in Th17 cell migration is further corroborated by the enhanced migration of Th17 cells in the presence of laminin-α5 in transwell migration assay. In conclusion, these data collectively demonstrate that integrin α 3 promotes the extravasation of inflammatory Th17 cells in the context of EAE and suggest integrin α 3 as a therapeutic target in MS.

Disclosure of Interest: None Declared

LT105

PLEIOTROPHIN SERUM CONCENTRATIONS CORRELATE WITH CLINICAL VARIABLES IN INTERFERON-BETA (IFN-B) TREATED RELAPSING-REMITTING MULTIPLE SCLEROSIS (RRMS) PATIENTS.

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Abstract Content: Introduction: RRMS is the most common form of Multiple Sclerosis (MS). It is an autoimmune demyelinating disease mainly affecting the Central Nervous System (CNS) (1). Pleiotrophin (PTN), a neuromodulatory (2) and immunomodulatory cytokine (3), is a good candidate for establishing a bidirectional communication between the CNS and the Immune System (IS). It is involved in other autoimmune diseases (4,5) and has been studied in an experimental model for MS (6), however, PTN concentration has not been explored in RRMS patients.

Methods: PTN serum levels were quantified by ELISA in 57 patients with RRMS (treated with Glatiramer acetate (GA), IFN-β, or non-treated) and in 18 controls. We also determined PTN mRNA expression in Peripheral Blood Mononuclear Cells (PBMC) by qPCR. Non-parametric analyses were performed. ROC curves were constructed to evaluate PTN as a possible biomarker. Written informed consent was obtained from all participants.

Results: PTN serum levels were significantly higher in patients when compared to controls (329.49±68.94 pg/mL and 139.61±67.52 pg/mL, respectively) ($p<0.05$). In IFN-β treated patients, PTN levels were higher after 8 years of disease duration, compared to 8 years duration or less ($p<0.05$). These patients showed a positive correlation between time of evolution ($r=0.484$, $p<0.05$) and time of IFN-β use ($r=0.423$, $p<0.05$) with PTN levels. In addition, we found a negative correlation between PTN concentration and the MS Severity Score ($r=-0.451$, $p<0.05$). There were no findings such as these in GA-treated and non-treated patients. PTN expression was not detected by qPCR in PBCM in any group. The ROC curve analysis had an AUC of 0.678, with a sensibility of 64.9% and specificity of 72.2% to identify patients and controls. However, in males PTN concentration had a better predictive power, with an AUC of 0.824, a sensibility of 70.8%, and a specificity of 77.8%. The AUC for females was considerably lower (0.564), meaning that hormones could be regulating PTN.

Conclusion: Our study provides first evidence that PTN is increased in patients with RRMS compared to controls, with a differential expression and correlation according to treatment, being significantly for IFN-β treatment. Future studies must be developed to confirm the role of PTN in RRMS, its possible use as a biomarker, and its participation in the connections between CNS and IS.

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Disclosure of Interest: None Declared

LT107

CCL18 INDUCES HEALTHY FIBROBLAST-LIKE SYNOVIOCYTES TO DEVELOP GENE EXPRESSION PROFILING SIMILAR TO FIBROBLAST-LIKE SYNOVIOCYTES OF RHEUMATOID ARTHRITIS

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Abstract Content:

Background: CC chemokine ligand 18 (CCL18) is a secretion chemokine that is produced by tumor associated macrophages in a variety of solid cancers, or by activated monocytes/macrophages and dendritic cells in nonneoplastic diseases. Our previous study demonstrated CCL18 significantly enriched in synovial fluid of patients with rheumatoid arthritis (RA), which may correlate with one-year radiographic progression through promoting migration of RA fibroblast-like synoviocytes (RA-FLS). Studies which reveal the biological regulation of CCL18 on FLS would increase the understanding of how the chemokine involves in autoimmune disease.

Objectives: To explore whether CCL18 can induce healthy FLS (HFLS) to develop gene expression profiling similar to RA-FLS, and determine the biological function of CCL18 on HFLS.

Methods: Primary RA-FLS were obtained from Parker-Pearson needle biopsy of synovial tissues and were cultured in vitro. Primary HFLS cells were purchased from Cell Applications Inc. Total RNA was isolated from HFLS treated with recombinant CCL18 (500ng/mL) or vehicle, and five cases of primary RA-FLS. RNA-Seq were performed using Illumina SBS technology. DESeq2 was implemented to assess differentiation expression genes (DEGs). Ingenuity pathway analysis (IPA) core analyses were conducted to identify the molecular activities of the DEGs along with literature reviewed.

Results: Compared with HFLS, 2698 genes were upregulated and 2317 genes downregulated in RA-FLS, while 249 upregulated genes and 311 downregulated gene in HFLS treated with CCL18 were determined when comparing with the untreated cells. Compare the above two datasets, it was found 117 upregulated DEGs and 148 downregulated DEGs in both RA-FLS and HFLS treated with CCL18, suggesting HFLS treated with CCL18 had similar gene expression profiling to RA-FLS. IPA core analyses were conducted focusing on these 265 common DEGs. Except unclassified categories, the first functional category of proteins encoded by these genes was enzyme, followed by transcription factor and transporter. Diseases & functions module in IPA showed these genes enriched in migration associated pathways including formation of cellular protrusions, formation of filopodia, migration of connective tissue cells and disruption of focal adhesions. According to Ingenuity in IPA and publications, 19 migration-associated genes were identified, including *ANXA2*, *CDH11* and *SUMO1* which were reported overexpression in RA-FLS and to promote cell migration. Besides, 10 genes encoded cytokines, growth factors, chemokines and pro-inflammatory proteins in extracellular space, such as *CCN2* and *CCL7*, both of which were reported overexpression in RA patient's serum and synovial fluid.

Conclusion: CCL18 induces HFLS to develop gene expression profiling similar to RA-FLS. CCL18 enriched in RA synovial fluid may promote migration of FLS though upregulation of migration-associated genes and maintain inflammation through upregulation of certain pro-inflammatory proteins.
Disclosure of Interest: None Declared

LT108
SALIVARY IL-32 IS ASSOCIATED WITH ORAL HYGIENE LACK DURING BEHCET DISEASE
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Abstract Content: Behcet disease (BD) is a chronic systemic inflammatory disorder that is considered as a vasculitis touching both veins and arteries of all sizes. The disease clinical expression varies among patients. However, aphtosis is a common feature. Oral microbiota has been incriminated in BD genesis. However, the implication of local immune response is still under investigation. In this study, we intended to explore the local versus systemic role of IL-32 and nitric oxide in the oral immune responses during BD. Sixty patients with BD (36 active and 24 inactive stage) and 25 control subjects were enrolled in this study. Freshly collected blood on heparinized tubes and spontaneously secreted saliva were used in the study. In the same time, oral health and hygiene were assessed through a previously prepared questionnaire. The samples were centrifuged and filtered then supernatants were conserved at -80. IL-32 was measured by ELISA (Biotechne) while nitric oxide was estimated by a modified Griess method in both samples types. Statistical analyses were measured by Mann Witney U test for group comparison while Spearman test was used for correlation analyses.
We observed a significant increase in IL-32 and nitric oxide levels during BD in comparison to controls ($p<0.05$). Both markers were increased in plasma and saliva ($p<0.05$). In plasma, both markers were increased during disease activity while in saliva, only nitric oxide levels were increased ($p<0.05$). In addition, nitric oxide levels were correlated with the number of mouth ulcers ($r=0.303$; $p=0.035$). In contrast, salivary IL-32 was not associated with aphtosis ($r=0.009$, $p=0.961$) but was strongly correlated with the dental plaque index ($r=0.819$, $p<0.0001$). We did not observe any correlation in control group ($p>0.05$).
Our results showed that Nitric oxide and IL-32 were increased in BD in comparison to control subjects. We observed a difference between local and systemic responses. Although both markers were associated with disease activity in plasma, in saliva, nitric oxide was associated with the genesis of oral mucosal lesions while IL-32 seems to play an important role in the excessive inflammatory responses to oral flora especially, plaque flora. Our results suggest different regulating mechanisms for the two considered molecules during BD.
Disclosure of Interest: None Declared

LT109
STAT4 REGULATES PATHOGENIC IL-21 AND IFN-G PRODUCTION IN T FOLLICULAR HELPER (TFH) CELLS IN MURINE AND HUMAN LUPUS
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Abstract Content: Follicular helper T cells (Tfh) cells shape the germinal center (GC) response by delivery of contact-dependent signals and cytokines, including IFN-γ and IL-21. The contribution of these discrete extrinsic signals to the mal-regulation of GC B cells in lupus is unclear. We found that in Tfh cells from young lupus-prone mice, the

transcription factors Bcl6 and T-bet are co-expressed, as they are in conventional Tfh cells following acute type 1 (viral) challenge, with robust IL-21 and IFN-γ production. In lupus, by contrast to acute viral challenge, GCs persist as disease progresses, and we observed that Bcl6 and T-bet expression declined in Tfh cells from chronic GCs during such progression, as they continued to robustly co-produce both IL-21 and IFN-γ with ongoing genesis of IgG2 autoantibody production necessary for immune complex glomerulonephritis. Transcriptional analysis of lupus-Tfh cells at different stages of disease revealed an evolving gene expression profile, with an increased STAT4 gene signature coincident with STAT4 phosphorylation that corresponded to disease progression. In a like manner to our murine findings, we found that in human lupus patients circulating Tfh cells secreted IL-21 and IFN-γ and that STAT4 phosphorylation was enhanced upon IFN-□ or IL-12 stimulation, coinciding with clinical disease activity. Furthermore, IFN-I blockade in lupus mice resulted in a decrease in IL-21 and IFN-γ production by Tfh cells. These data indicate that in the chronic inflammatory state characterizing lupus, STAT4 guides pathogenic cytokine production, providing a potential therapeutic target in patients with active disease.
Disclosure of Interest: None Declared

LT109b
THE T1D-ASSOCIATED LNCRNA LNC10 MODULATES THE TYPE I IFN SIGNALING AND ANTIVIRAL RESPONSE IN PANCREATIC BETA CELLS.
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Abstract Content: Type 1 diabetes (T1D) is a complex autoimmune disease in which genetic factors interact with environmental factors (e.g. viral infections) to trigger an autoimmune assault against insulin producing pancreatic β cells. The majority of T1D-associated SNPs lay into non-coding regions of the human genome and many have been predicted to affect the expression, secondary structure and function of long non-coding RNAs (lncRNAs). However, the molecular mechanisms by which these non-coding molecules contribute to T1D pathogenesis remain to be clarified.
Lnc10 is a lncRNA harboring a T1D-associated SNP that has been described to control *Ebi2* expression in immune cells. *Ebi2* is a trans-eQTL that regulates the IRF7-driven inflammatory network (iDIN), an antiviral gene network which is enriched with T1D-associated genes. Taking into account that the T1D-associated SNP is located in *Lnc10*, our hypothesis is that *Lnc10* might be dysregulated in T1D patients, influencing the regulation of the iDIN network at the pancreatic β cell level and provoking an exacerbated antiviral response, and eventually, β cell destruction.
Preliminary results of our group have demonstrated that diabetogenic stimuli, such as pro-inflammatory cytokines and viral infections, upregulate *Lnc10* expression in pancreatic β cells. *Lnc10* is located in the nucleus of β cells, both in basal and stimulated conditions, suggesting a potential role in transcriptional regulation. *Lnc10* overexpression in combination with polyinosinic:polycytidylic acid (PIC) transfection to mimic a viral infection, increased the expression of several antiviral genes of the iDIN pathway, including key genes in type I IFN signaling and antiviral response (*IRF7, STAT1, IFIT1, IFIT2* and *ISG15).* Interestingly, the type IFN signaling has been shown to play a crucial role in the initial stages of T1D pathogenesis in pancreatic β cells.
In conclusion, our results show that *Lnc10* is upregulated after a viral infection in pancreatic β cells. Upregulation of *Lnc10* in combination with a viral infection exacerbates the expression of several antiviral genes from the iDIN pathway. Further studies are required to elucidate the molecular mechanisms by which this lncRNA regulates the expression of iDIN genes and to determine its impact in virus-induced β cell dysfunction and T1D development in genetically susceptible individuals.
Disclosure of Interest: None Declared

Lightning Talk Session 2: Cytokine regulation II

LT110
TRANSFORMING GROWTH FACTOR BETA 1 (TGF-B1) EXPRESSION UPREGULATED FOLLOWING PROLOTHERAPY TREATMENT FOR CARTILAGE REGENERATION
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Abstract Content: Osteoarthritis is a highly prevalent disease associated with a shift in cytokine regulation within joints. Current treatments, such as corticosteroids, aim to reduce pain but do not slow disease progression. By understanding and targeting the inflammatory component of the disease, future therapies can more effectively treat patients. One promising therapy is proliferative therapy (prolotherapy), a complementary medical intervention with promising clinical evidence. Scientists hypothesize this treatment induces a localized inflammatory response leading to cartilage repair. However, the underlying mechanisms are not well understood. This present study investigates the molecular basis of prolotherapy by studying whether a common prolotherapy treatment, P2G, upregulates proliferation-enhancing cytokines in undifferentiated MC3T3-E1 preosteoblast cells as a model for chondroprogenitors. *In vitro* stimulation of chondroprogenitor cells was carried out with phenol-glucose-glycerin (P2G) prolotherapy treatment followed by qRT-PCR quantification to determine the mRNA expression of the genes BMP2, TGF-β1, AKT, and STAT1. We found that cells treated with P2G did not exhibit significant changes in AKT, STAT1, or BMP2 expression. However, cells increased expression of TGF-β1 approximately 2.3 fold by 30 hours after treatment initiation ($p<0.001$). Given the role of TGF-β1 in proliferation and stimulating chondrogenic collagen deposition, it follows that TGF-β1 may be involved

in P2G's mechanism in the chondroprogenitor response. The involvement of TGF-β1 could potentially slow or reverse cartilage degradation as chondroprogenitor cells proliferate and rebalance catabolic/anabolic roles to repair and maintain cartilage.
Disclosure of Interest: None Declared

LT111
INTEGRATED SINGLE-CELL ANALYSIS OF MULTICELLULAR IMMUNE DYNAMICS DURING HYPERACUTE HIV INFECTION

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Abstract Content: Development of effective vaccines and therapeutics is facilitated by understanding the earliest immune responses to infection. Here, we apply single-cell RNA-sequencing (scRNA-seq) and a novel gene module discovery-based framework to longitudinally profile peripheral multicellular immune responses in untreated hyperacute HIV infection.

We profiled >59,000 single cells from four individuals across multiple acute infection timepoints (including pre-infection), utilizing scRNA-seq data to identify transient immune responses with cell subset resolution. Onset of viremia induced interferon stimulated gene (ISG) responses integrated across multiple cell types, wherein monocytes notably contributed to the cytokine milieu, producing CXCL9 and CXCL10. Otherwise obscured in bulk analyses, we describe a second set of responses following ISG upregulation that align in time: pro-inflammatory T cell differentiation, prolonged monocyte MHC-II upregulation, and persistent NK cytolytic killing. Leveraging our temporal, cell-type resolved data, we nominate putative cell-cell signaling networks and their key cytokines during infection: IL-6/IL-8/IL-17 restrict CD8 T cell killing of HIV infected cells; TNF and IL-1β affect only CD4+ T cells, and IL-2/IL-15/IL-18 mediate persistent killing by NK cells and CD8 T cells. Two participants who later develop viremic control associated with elevated frequencies of proliferating cytotoxic cells, inclusive of a previously unappreciated NK cell subset, immediately following HIV detection.

Our study reveals both cooperative and cell subset specific immune responses during untreated hyperacute HIV infection with temporal resolution, nominating monocytes, NK cells, and pro-inflammatory signaling as perturbation targets for future vaccine studies. Moreover, our gene module discovery framework can be readily applied to other longitudinal studies in humans and other model organisms.

Disclosure of Interest: None Declared

LT112
SYSTEMATIC ANALYSIS OF KINOME INHIBITION FOR INTERFERON AND CYTOKINE GENE EXPRESSION

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Abstract Content:

Interferon and cytokine responses comprise complex intertwined signaling and effector pathways, many of which are driven by kinases' activities. Here, we employed chemical inhibitor (n=387) library targeting the kinome to investigate their effects on two different responses; one is transcriptional (IFN response), and the other is post-transcriptional (cytokine expression). The ISRE-linked transcriptional reporter was used in Huh-7 cell stimulated with IFN-α. The hierarchal clustering for multivariant analysis (Reduction and Selectivity) pointed to a cluster of 92 small molecules that selectively inhibit IFN response by targeting 40 individual kinases, involved in more than 12 biological pathways. The most notable pathways were inferred as PI3K/mTOR, cell cycle, JAK-STAT, protein tyrosine kinase, and several signaling pathways, previously unknown for involvement in IFN response. Representative kinase inhibitors from each group were selected, and it was found that the majority were able to reduce IFN-stimulated gene (ISG) expression at the mRNA level. Whereas, few inhibitors acted at the protein level without affecting mRNA expression.
The cytokine expression model employed a reporter system selective for 3'-untranslated region-AU-rich elements (ARE) derived from the TNF-α gene. The ARE sequence elements are important determinants of mRNA decay and translation and exist in many pro-inflammatory cytokine genes. Fifteen kinase inhibitors were found to reduce the TNF-ARE reporter activity. These inhibitors targeted fewer pathways largely MAPK pathway and cell cycle, indicating restricted kinase activity at the post-transcriptional level.

Among those novel players that affect ISG and ARE-dependent cytokine expression, is polo-like kinase 1 (PLK1). Accordingly, RNAseq experiments were performed with the PLK1 inhibitor, volasertib, showing reduction of many cytokine and chemokine ARE-mRNAs. PLK1 inhibition caused accelerated mRNA decay of cytokine mRNAs, and was associated with reduced phosphorylation of the mRNA decay-promoting protein, tristetraprolin (TTP/ZFP36). In contrast, ectopic expression of PLK1 caused an increased abundance of the phosphorylated TTP in normal cells. PLK1 effect was associated with the MAPK-MK2 pathway, a major regulator of ARE-mRNA stability, as evident from MK2 inhibition, in vitro phosphorylation, and knockout experiments. Moreover, mutational analysis demonstrated that TTP serine 186 is a target for the PLK1 effect. Treatment of mice with the PLK1 inhibitor reduced both

TTP/ZFP36 phosphorylation and cytokine expression in xenograft tissues.

To our knowledge, this is the first large and detailed screen of the kinome inhibitors with IFN and cytokine responses and a resource for the assessment of the kinome for potential therapeutic effects and off-target effects. The data also showed that PLK1 is an important regulator of TTP/ZFP36 phosphorylation in cytokine expression, and that kinase-mediated post-transcriptional process could be a target for therapeutic purposes.

Disclosure of Interest: None Declared

LT113

SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS NON-STRUCTURAL PROTEIN (SFTSV-NSS) ACTIVATES NFKB-DEPENDENT CYTOKINE STORM IN VITRO

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Abstract Content: Severe Fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by a tick-borne bunyangvirus of the family phenuiviridae, the SFTS virus (SFTSV). The syndrome is characterized by severe inflammatory process affecting the integrity of host tissues, such as liver, spleen, and lymph nodes and often associated with high morbidity and mortality rates (reaching 30%). Unfortunately, very little is known about the causative virus and its pathogenesis. As a result, there is currently no vaccine or efficient treatment for the syndrome.

Using different cell lines, DNA plasmids expressing various SFTSV viral proteins, and real virus infection, we identified both the viral protein responsible for the cytokine storm induction, and the transcription factor mediating the inflammatory process in the host.

In summary, the viral non-structural protein (SFTSV-NSs) is a potent activator of NFκB pathway, which induces the expression of cytokines at the transcriptional level, resulting in overly enhanced interlukin-8 (IL-8), interlukin-6 (IL-6), tumor necrosis factor α (TNF-α), and C-C motif chemokine ligand 2 (CCL2). Interestingly, interfering with and inhibiting NFκB resulted in significant decrease in the cytokines activation and alleviated the cytokine storm fundamentally. Since cytokine storm has been described clinically in SFTS patients, we propose that NFκB could be a therapeutic target in the future for treating SFTS.

Taken together, these findings present a new insight into the pathogenesis of lethal SFTS, and may promote not only the understanding of virus-host interactions but also the development of antiviral therapeutics.

Keywords: Cytokine storm, NFκB, SFTSV, Non-structural protein.

Disclosure of Interest: None Declared

LT115

THE CYTOKINE RESPONSE TO TOTAL KNEE ARTHROPLASTY IS ASSOCIATED WITH RISK OF PERSISTENT POSTOPERATIVE PAIN

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Abstract Content: Introduction: Persistent postoperative pain following total knee arthroplasty (TKA) can occur in as many as 20-40% of TKA patients[1]. There is growing evidence that cytokines and adipokines are associated with osteoarthritis (OA) severity, progression, and associated pain severity[2]. Specifically, tumor necrotic factor alpha (TNFα) and interleukin 6 (IL6) have been identified as correlating with increased pain in osteoarthritis patients[3,4]. In this study, we sought to determine if there are associations between blood and synovial fluid cytokine levels in end-stage OA patients during the TKA perioperative period and development of persistent postoperative pain.

Methods: 162 patients undergoing primary unilateral TKA were prospectively enrolled. Exclusion criteria included autoimmune/inflammatory disease, systemic steroid use within 6 months of surgery, active infection, a history of opioid use and/or chronic pain. Synovial fluid was collected pre-arthrotomy and plasma was collected prior to incision, in the recovery room, and on postoperative days (POD) 1 and 2. Numerical Rating Scale pain levels were recorded at rest and with movement preoperatively, on POD0, 1, 2 and 6 months post-operatively. Pain Catastrophizing Score (PCS) was recorded on POD2. Postoperative persistent pain was defined as ambulatory NRS ≥ 4 at 6 months. Cytokine levels were measured using the V-Plex Human Cytokine 30-Plex Panel (Mesoscale - Rockville, Maryland, USA).

Results: 15/162 (9.3%) of patients met criteria for postoperative persistent pain at 6 months following TKA for OA. Postoperative plasma levels of 4/31 cytokines studied were significantly different between persistent pain and non-persistent pain patients. IL10 levels were significantly lower (p=0.033) immediately postoperatively in the pain group. IL1B was significantly higher (p=0.0005) among persistent postoperative pain patients on POD1. VEGF was significantly lower (p=0.0239) on POD1. IL12_23p40 differentiated at both POD1 and POD2 (p=0.0199, p=0.0439) between groups. IL10 preoperative synovial fluid cytokine level was associated with development of postoperative persistent pain (p=0.0482).

Conclusion: This prospective cohort study describes variations in 4 cytokines in the perioperative period in patients who developed persistent pain after TKA, suggesting a distinct inflammatory profile in at risk patients. While baseline IL10 levels were lower in the synovial fluid of patients who developed persistent postoperative pain, differences in serum cytokine levels were only detected postoperatively in response to surgery. These results

support the hypothesis that pre- and post-operative cytokine pathways may influence long term postsurgical outcomes.

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Disclosure of Interest: None Declared

LT116

STAT3 REDUCES THE EXPRESSION OF THE MTOR INHIBITOR REDD1 IN A NON-CANONICAL FASHION

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Abstract Content: Interleukin 6 (IL-6) is a pleiotropic cytokine and an activator of Mammalian Target of Rapamycin (mTOR). In contrast, mTOR activity is negatively regulated by Regulated in Development and DNA Damage Responses 1 (REDD1). Expression of REDD1 is induced by cellular stressors such as glucocorticoids and DNA damaging agents. We show that the expression of basal as well as stress-induced REDD1 is reduced by IL-6. The reduction of REDD1 expression by IL-6 is independent of proteasomal or caspase-mediated degradation of REDD1 protein. Instead, induction of REDD1 mRNA is reduced by IL-6. The regulation of REDD1 expression by IL-6 is independent of Phosphatidylinositide-3-Kinase (PI3K) and Mitogen-Activated Protein Kinase (MAPK) signalling but depends on the expression and activation of Signal Transducer and Activator of Transcription 3 (STAT3). Furthermore, the reduction of basal REDD1 expression by IL-6 correlates with IL-6-induced activation of mTOR signalling. Inhibition of STAT3 activation blocks IL-6-induced mTOR activation.
The gene-repressory action of STAT3 is contrary to the canonical transcription-inducing function of STAT3, possibly reflecting a new non-canonical function of STAT3. We demonstrate an IL-6-dependent recruitment of STAT3 to the REDD1 promoter. Moreover, we determine the position of the STAT3 binding site within the REDD1 promoter. In summary, we present a novel STAT3-dependent mechanism of both IL-6-induced activation of mTOR and IL-6-dependent reversion of stress-induced inhibition of mTOR activity.

Disclosure of Interest: None Declared

LT117

TUMOR PROGRESSION LOCUS 2 (TPL2) PREVENTS IMMUNOPATHOLOGY DURING INFLUENZA INFECTIONS

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Abstract Content: Tpl2 (Tumor Progression Locus 2) is a serine/threonine kinase that transmits signals via several receptor families, including Toll Like Receptors (TLRs), cytokine receptors (TNF-alpha and IL-1 receptors), and Fc receptors, leading to the expression of inflammatory mediators. Our lab has previously shown that the *Tpl2-/-* mice are more susceptible to influenza infection, and the purpose of this study is to delineate the mechanisms responsible. *Tpl2-/-* mice ultimately clear the virus albeit with delayed kinetics. Despite eventual viral control, *Tpl2-/-* mice begin to exhibit severe clinical signs and require euthanasia. Therefore, we hypothesize that an overexuberant immune response rather than impaired viral control leads to severe pathology in *Tpl2-/-* mice in response to influenza infection. Histological examination of the lungs of influenza-infected *Tpl2-/-* mice showed increased alveolar septal necrosis, pleuritis, and more widespread lesions, which are signs of epithelial-endothelial barrier damage and inflammation. In order to assess potential cellular mediators of inflammation, the cellular profile of the lung was analyzed at 7 days post infection (dpi), when significant morbidity was observed in *Tpl2-/-* mice. An excess influx of inflammatory monocytes was observed in the lungs of *Tpl2-/-* mice, along with a corresponding increase in serum levels of MCP-1, the chemokine required for inflammatory monocyte recruitment. Because type I interferons promote inflammatory monocyte recruitment via the induction of MCP-1, ongoing studies are examining whether Tpl2 paradoxically limits inflammation during influenza infection by constraining the production of type I IFNs. This information will enhance our understanding of Tpl2 signaling and interferon regulation, which could provide insights into host-targeted therapies for treating highly pathogenic influenza which is typically associated with excess type I IFNs and robust inflammatory monocyte recruitment.

Disclosure of Interest: None Declared

LT118

IMMUNE REGULATOR LGP2 INHIBITS K63 POLYUBIQUITINATION

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Abstract Content: During RNA virus infection, type I interferon (IFN) production allows for the cell to achieve a robust antiviral response and it must be tightly regulated to prevent cytotoxic effects. IFN production is initiated when viral sensor proteins RIG-I, MDA5, and LGP2 bind to viral RNA, activating downstream E3 ubiquitin ligases TNF receptor-associated factors (TRAFs). Through the synthesis of K63-linked polyubiquitin chains, TRAFs recruit kinases which drive transcription of IFN. Overexpression of LGP2 has been shown to negatively regulate IFN production. The Horvath lab identified that LGP2 inhibits the production of K63 polyubiquitin chains on TRAF proteins, however the mechanism was not well understood. Experiments presented here demonstrate that LGP2 inhibits K63 polyubiquitination and downstream signaling without the need of a binding site on TRAF6. TRAF6 was unable to interact with its E2 conjugating enzyme Ubc13/Uev1a in the presence of LGP2. Using a different E3 ubiquitin ligase, LGP2 prevents K63 polyubiquitination by preventing

interaction between the E3 ligase and Ubc13/Uev1a. Using co-immunoprecipitation experiments, we determined that Ubc13/Uev1a interacts with LGP2 and this interaction is important in the inhibition of polyubiquitin chain formation. These findings establish a mechanism that LGP2 uses in order to inhibit K63 polyubiquitination of E3 ubiquitin ligases involved in the antiviral response.
Disclosure of Interest: None Declared

LT120
MODELING CELL-SPECIFIC DYNAMICS AND REGULATION OF THE COMMON GAMMA CHAIN CYTOKINES
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Abstract Content: Many receptor families exhibit both pleiotropy and redundancy in their regulation, with multiple ligands, receptors, and responding cell populations. Any intervention, therefore, has multiple effects, confounding intuition about how to precisely manipulate signaling for therapeutic purposes. The common γ-chain cytokine receptor dimerizes with complexes of the cytokines interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21 and their corresponding "private" receptors. These cytokines have existing uses and future potential as immune therapies due to their ability to regulate the abundance and function of specific immune cell populations. However, engineering cell specificity into a therapy is confounded by the complexity of the family across responsive cell types.

Here, we build a binding-reaction model for the ligand-receptor interactions of the common γ-chain receptor cytokines enabling quantitative predictions of response. We show that accounting for receptor-ligand trafficking is essential to accurately model cell response. This model accurately predicts ligand response across a wide panel of cell types under diverse experimental designs. Further, we can predict the effect and specificity of natural or engineered ligands across cell types. Using a panel of engineered, Fc-conjugated cytokines, we identify that reducing CD122/γ-chain affinity cannot overcome reductions in CD25 affinity when engineering regulatory T cell specificity. The orientation of Fc conjugation can also strongly affect affinity to each receptor and thus the signaling specificity. Finally, we show that tensor factorization is a uniquely powerful tool to visualize changes in the input-output behavior of the family across time, cell types, ligands, and concentration.

In total, these results present a more accurate model of ligand response validated across a panel of immune cell types and demonstrate an approach for generating interpretable guidelines to manipulate the cell type-specific targeting of engineered ligands. These techniques will in turn help to study and therapeutically manipulate many other complex receptor-ligand families. Lastly, we will outline a strategy for engineering single-cell variability in response due to family-wide heterogeneity in receptor abundance.
Disclosure of Interest: A. Farhat: None Declared, A. Weiner: None Declared, C. Posner Employee of: Visterra, Inc., Z. Kim: None Declared, B. Orcutt-Jahns: None Declared, S. Carlson Employee of: Visterra, Inc., A. Meyer Grant / Research support from: Visterra, Inc.

LT121
ABSENCE OF CCL5/CCR5 AXIS EXAGGERATES THROMBUS FORMATION THROUGH REDUCED UPA, TPA AND VEGF EXPRESSION IN MURINE DVT MODEL
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Abstract Content:
Introduction
Deep vein thrombosis (DVT) is multifactorial and often results from a combination of risk factors such as genetic conditions, obesity, drugs, pregnancy, aging, trauma, inflammation and malignancy. And DVT is a complex biological event, with endothelial injury, venous stasis and blood hypercoagulability. In this study, we examined the pathophysiological role of CCL5/CCR5 axis in the resolution of DVT on *Ccr5* knockout (KO) mice.
Methods
Eight-week-old C57BL/6 (WT) and KO mice underwent anesthesia and, via a laparotomy the femoral, the inferior vena cava (IVC) was exposed and ligated with 3-0 silk suture. At 1, 3, 5, 7, 10, 14 and 21 days after the IVC ligation, the blood flow in thrombosed IVCs were measured and mice were euthanized. After that thrombosed IVCs were harvested. The morphological study, immunohistochemical analyses, and double-color immunofluorescence analyses were performed. At 1, 3, 5, 10 and 14 days, the thrombus was separated from the vein wall and the intrathrombotic gene expressions were obtained by real-time RT-PCR.
Results
Immunohistochemically, in WT mice intrathrombotic CCR5-positive cells were detected whole of the observation period and increased gradually with time after IVC ligation. Concomitantly, the gene expression of *Ccr5* was detected in the thrombi. When KO mice were treated in the same manner, thrombus size was much larger than WT mice. Moreover, the blood flow of the IVC was more recovered in WT than in KO mice. And intrathrombotic *Plat*, *Plau* and *Vegf* mRNA expressions were significantly reduced in KO mice than WT mice. We explored the contribution of BM-derived $CCR5^+$ cells to thrombolysis by using BM chimeric mice generated from WT and KO mice. Both WT and KO mice transplanted with WT mouse-derived BM cells exhibited a higher thrombolytic activity than the recipients of KO mouse ones. Supportingly, recombinant murine (rm) CCL5 treatment enhanced gene expression of *Plau*, *Plat* and *Vegf* in WT-derived macrophages but not KO-derived ones. These observations indicated that CCR5-deficiency

impaired fibrinolytic activity and collagen production, both indispensable steps for thrombus resolution.

Conclusion

The absence of CCL5/CCR5 axis can have a detrimental role in the thrombus resolution by suppressing uPA, tPA and VEGF expression respectively. The CCL5/CCR5 axis can be a good molecular target for the DVT treatment.

Disclosure of Interest: None Declared

LT122

TRANSCRIPTIONAL REGULATION OF IL-21 EXPRESSION IN T FOLLICULAR HELPER CELLS

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Abstract Content: Introduction: T follicular helper (Tfh) cells play a key role in providing help to B cells during germinal center (GC) reactions. The generation and function of Tfh cells is regulated by multiple checkpoints including their early priming stage in T zones and throughout the effector stage of differentiation in GCs. Signaling pathways activated downstream of cytokine and costimulatory receptors as well as consequent activation of subset-specific transcriptional factors are essential steps for Tfh cell generation. While to date early signaling events for fate committed differentiation of Tfh cells are sufficiently studied, mechanisms allowing Tfh cells to maintain their commitment/programming are still unclear.

Methods: The regulation of IL-21 expression by STAT1/STAT3/Batf/IRF4 transcriptional complex in Tfh cells was assessed by FACS staining, quantitative Real-Time PCR, Chromatin Immunoprecipitation (ChIP) assay, luciferase reporter assay and retroviral transduction. For these assays FACS-sorted $CD4^{+}CD44^{hi}CXCR5^{hi}PD1^{hi}$ Tfh cells were utilized from wild-type, STAT1-, STAT3-, Batf-, and IRF4-deficient mice subjected to Ova immunization. Immunoblot analysis was performed to explore the mechanism whereby STAT1 along with STAT3, Batf, and IRF4 contributes to IL-21 production in Tfh cells.

Results: Here we report that signal transducer and activator of transcription 1 (STAT1) deficiency in CD4+ T cells leads to a diminished number of Tfh cells at day 7 and 14 days after antigen immunization, while at day 3-4, number of Tfh cells was comparable between STAT1-sufficient and STAT1 deficient mice, suggesting the role of STAT1 in Tfh lineage maintenance rather than in Tfh cell priming. In fact, in both mouse and human Tfh cells interleukin (IL)-21 mRNA and protein expression is dependent on the presence of STAT1. Functionally, STAT1 in cooperation with STAT3, Batf, and IRF4 triggers IL-21 production in Tfh cells by directly binding to and activation of the IL-21 gene locus. Moreover, utilizing gene knockout approach, we have determined that Batf is a crucial connector to form STAT1/STAT3/Batf/IRF4 active complex for triggering IL-21 transcription.

Conclusion: Our results thus indicate functional contribution of STAT1 towards regulation of IL-21 expression and Tfh lineage maturation/maintenance.

Disclosure of Interest: None Declared

LT123

GLUTEN INDUCES RNA METHYLATION CHANGES THAT REGULATE INTESTINAL INFLAMMATION VIA ALLELE-SPECIFIC XPO1 TRANSLATION

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Abstract Content: Celiac disease (CD) is an inflammatory intestinal disorder that develops in genetically susceptible individuals upon gluten ingestion. It is known that gluten triggers a proinflammatory response, but the underlying mechanisms remain unclear. CD-associated SNPs map mainly to noncoding regions and their effects are difficult to assess. Additionally, post-transcriptional RNA modifications, including m^6A, play crucial roles in the regulation of different autoimmune diseases.

Our hypothesis is that noncoding CD-associated SNPs can alter m^6A levels in adjacent motifs, affecting different cell processes and increasing CD predisposition. Along this line, SNP rs3087898, in the 5' UTR of the *XPO1* gene, is located close to an m^6A motif. We confirmed the methylation in this region, and observed that the RNA carrying the CD-risk allele shows higher methylation and binds the YTHDF1 reader more efficiently, promoting higher *XPO1* translation. Knocking down the m^6A-related machinery resulted in lower XPO1 protein levels; while gluten stimulations induced XPO1, METTL3 and YTHDF1 levels, both *in vitro* and *in vivo*. We also observed that gluten-induced activation of NFkB pathway, a hallmark of CD, could be reversed by inhibition of XPO1 or m^6A machinery and that the NFkB target IL8 cytokine and its murine homologs Cxcl1 and Mip2a increased upon gliadin stimulations via this m^6A-XPO1 pathway. Finally, we confirmed that rs3087898 SNP genotype influences XPO1 protein levels in human intestinal biopsy samples. In addition, CD patients show increased, gluten-dependent, *XPO1*, *METTL3* and *YTHDF1* expression and downstream IL8 inflammatory cytokine.

Altogether, we have demonstrated that intergenic SNPs associated with autoimmune disorders can alter m^6A levels and contribute to disease pathogenesis. Moreover, this is the first study linking gluten with alterations in m^6A-related mechanisms. We have observed that gluten induces the m^6A machinery resulting in an allele-specific increase of m^6A levels in the 5'UTR of *XPO1*, which in turn leads to augmented XPO1, NFkB activation and IL8 secretion in

patients harboring the risk allele, thus opening the door to new therapeutic approaches.
Disclosure of Interest: None Declared

LT124

INFLUENZA A/PR8 VIRUS INFECTION ATTENUATES OSM-INDUCED IL-33 CYTOKINE LEVELS AND TH2/M2 SKEWED LUNG INFLAMMATION IN MICE IN VIVO

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Abstract Content: Influenza A (Flu) infections typically induce innate immune mechanisms (including type 1 Interferons) and adaptive immunity with both B-cell and T-cell (typically Th1) responses. IL-33, a nuclear-localized "alarmin" cytokine with pro-inflammatory/immune effects generated by IL-33Receptor positive cells, has been suggested to be elevated in Flu models. IL-33 can be induced by the gp130 cytokine Oncostatin M (OSM), which we have previously shown to induce a Th2/M2-skewed inflammatory response (eosinopils, Arg1+Macrophages, IL-33, IL-4, eotaxin-2) in mouse lungs. Since IL-33 can influence both pathways of immune responses, and mediates the Th2/M2-skewed inflammation induced by OSM, we examined both OSM and IL-33 regulation by Influenza A (PR8 strain) lung infection of Naïve mice or of mice primed with Advector to overexpress OSM (AdOSM). C57Bl/6 female mice (n=5/group) infected intranasally with PR8 (1000pfu) showed time-dependent elevation of OSM protein (ELISA) detected in Broncoalveolar Lavage (BAL) (day 2, peaking day 4, still elevated at Day 7). Day 7 of mice infected with 250 or 1000pfu PR8 showed dose-dependent elevated OSM mRNA in total-lung-homogenates (TLhomogenates). However, mRNA levels of IL-33 in TLhomogenates were reduced by >50% in PR8-infected mice, and total IL-33 protein was also reduced by >50% as assessed by Western blots in PR8 infected mice. Although we detected small increases in IL-33 by ELISA in BAL (day 2 and 4) the total amount of IL-33 in lavage was a small fraction of IL-33 estimated in TLhomogenates (ELISA, corrected to mg/protein) which were reduced by >50% by PR8 infection, consistent with mRNA and Western blot data. Since this suggested active suppression of global lung IL-33 by PR8, we then assessed the effects of PR8 super-infection in mice with ongoing Th2/M2-skewed inflammatory environment induced by AdOSM administered 7 days prior. The effect of PR8 (250pfu, n>5 per group) was examined at day 2 and day 5 post-PR8, and showed, starting at Day 2 and most evident at day 5, that PR8 markedly reduced eosinophil and Arg1+Macrophage accumulation, IL-33, IL-4, and eotaxin-2 expression in lung. This was associated with increased levels of both IFNβ and IFNγ expression in total lungs of PR8/AdOSM treated animals. Both IFNβ and IFNγ suppressed OSM-induced IL-33 levels in mouse lung epithelial cells or fibroblasts in vitro, as assessed by Western blots of cell lysates. Collectively, the results show that IL-33 expression (either basal, or previously elevated by OSM-overexpression) is strongly suppressed in mouse lungs in vivo by PR8 infection, and this may be due to direct effects of Type 1 Interferons and/or IFNγ on IL-33 expressing cells. Thus, although influenza infection in lungs may induce OSM, influenza may also suppress OSM effects later in infection such as its regulation of IL-33. (Supported by CIHR).
Disclosure of Interest: None Declared

LT125

IL-6 PROMOTES TH17 CELL RESPONSES IN PATIENTS WITH T1R LEPROSY REACTIONS.

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Abstract Content: Background:
Type 1 reactions (T1R), an inflammatory condition, are represented as inflammation of local skin patches in leprosy patients. Approximately, 30-40% leprosy patients have undergone *T1R* during the course of MDT. Previously, we have reported that IL-23 is involved in Th17 cells differentiation and IL-6 in non-reactions (NR) leprosy patients. Subsequently, recent finding by our group on immunopathology of leprosy reactions showed that IL-6 induces Th17 cell differentiation together with TGF-β in leprosy reactions. Here, we thus asked the next question whether IL-6 or IL-23 induced Th17 cells are different in nature?
Methods & Materials
A total of 56 newly diagnosed and untreated non reaction and T1R reactions patients were recruited. 48 hours PBMCs cultures were established with different combination of recombinant IL-6, IL-23 and TGF-β with or without *Mycobacterium leprae* sonicated antigen (MLSA). Subsequently PBMCs cultures were blocked with either antagonized sIL6R or sIL23R antibodies. Real Time PCR was further used for gene expression analysis of IL-17A, IL-17F, IL-6R and IL-23R. Different phenotypes of Th17 cells were studied by flowcytometry and culture supernatant was estimated for cytokine ELISA.
Results:
In this study, leprosy reactions showed high percentage of IL-17A producing $CD4^+$ $IL6R^+$ T cells as compared to stable leprosy patients ($p<0.001$). On the other hand T1R leprosy reactions showed significantly low ($p<0.001$) IL-17A producing $CD4^+$ $IL23R^+$ Th17 cells as compared to NR leprosy patients in 48 hours *MLSA* stimulated cultures . Furthermore, recombinant IL-6, IL-23 and TGF-β significantly ($p<0.001$) promoted IL-17A in $CD4^+IL6^+$ T cells. Subsequently, IL-6R and IL-23R blocking experiments showed significantly ($p<0.01$) down regulated IL-17A in T1R reaction as compared to NR leprosy patients.

Conclusion:
This study for the first time establishes that pathogenic Th17 cells produce IL-17 via IL-6R pathway in leprosy T1R reactions. Thus, present approaches that specifically target Th17 cells and/or the cytokines that promote their development, such as IL-6, TGF-β and IL-23A may provide

more focused treatment strategies for the management of *M. leprae* and its reaction. Hence, therapeutic approaches that aim to re-establish homeostasis by decreasing the production of IL-17 by Th17 cells may prove effective in the control of leprosy reaction and during its emergency.
Disclosure of Interest: None Declared

LT126
INTERLEUKIN-7 IS A POTENT INDUCER OF HIV-SUPPRESSIVE CHEMOKINES
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Abstract Content: Although interleukin-7 (IL-7) may promote T-cell reconstitution in HIV-1 infection, its effects on HIV-1 replication are still uncertain. In this study, we investigated the ability of IL-7 to induce the production of chemokines that may play a role in the endogenous control of HIV-1 replication in infected hosts. Treatment with IL-7 induced peripheral blood mononuclear cells (PBMC) from healthy donors to secrete significant amounts of the CCR5 ligands CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES, as well as other antiviral chemokines, both in the presence and absence of concomitant T-cell receptor-mediated stimulation. Dose-response experiments showed that supra-homeostatic concentrations of IL-7 are required for induction of HIV-suppressive chemokines. Neutralization of CCL3, CCL4, and CCL5 increased HIV-1 replication in IL-7-treated cells starting on day 3 post infection. Although T cells were the main chemokine source in IL-7-stimulated PBMC, depletion of monocytes significantly reduced chemokine production levels, suggesting that cross-talk between T cells and monocytes is critical for this effect. We also investigated the signaling pathways involved in the mechanism of IL-7-mediated chemokine induction, showing that STATs play an important role. These results delineate potential mechanisms whereby IL-7 may facilitate HIV-1 control in infected individuals.
Disclosure of Interest: None Declared

LT127
DISTINCT ANTIVIRAL AND IMMUNE RESOLUTION FUNCTIONS OF ZINC-FINGER ANTIVIRAL PROTEIN ISOFORMS ZAP-S AND ZAP-L
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Abstract Content: Interferons are rapidly induced to combat the virus. After virus clearance, interferons need to return to basal levels as they can cause tissue damage. We identified zinc-finger antiviral protein (ZAP) as a negative regulator of interferon, that binds to the 3'UTR of both type I and III interferon mRNAs. Previous studies have shown that ZAP is a major antiviral restriction factor of alphaviruses and HIV. However, we have uncovered two distinct functions of ZAP which are determined by alternative splicing. Of the two major alternatively spliced ZAP isoforms, the constitutively expressed long isoform (ZAP-L) acts as the host's major alphavirus restriction factor through direct binding of vRNA and recruitment of the exosome machinery for vRNA decay. Conversely, the short isoform (ZAP-S), which contains identical RNA-binding domains but is only expressed late after virus and interferon sensing, binds to and mediates the degradation of several interferon mRNAs, thus acting as a negative feedback regulator of the interferon response. While ZAP-S and ZAP-L are expressed from the *ZC3HAV1* gene and harbor identical RNA-binding domains, we uncovered differences in their intracellular localization and mRNA splicing are the major determinants for their specificity toward host versus viral RNA. ZAP-S is diffusely cytoplasmic, whereas ZAP-L forms perinuclear foci and co-localizes with membranes of modified endosomes – used as replication organelles by the alphavirus Sindbis virus – which is mediated by the C-terminal prenylation motif in ZAP-L. Through this, ZAP-L restricts viral replication early during infection, while ZAP-S curbs interferon responses at a later stage. Through mRNA splicing assays, we identified ZAP-S mRNA is produced by a weaker intronic non-canonical polyadenylation signal (ncPAS). The selection of this ncPAS to produce ZAP-S mRNA is interferon dependent. We also identified the cleavage stimulation factor subunit 2 (CSTF2) as the effector protein that preferentially induces alternative polyadenylation to produce ZAP-S mRNA. Together, our study provides the molecular mechanisms underlying mRNA splicing and distinct subcellular localization of ZAP isoforms, which determine their distinct specificity to host and viral target RNA.
Disclosure of Interest: None Declared

LT128
GUT DYSBIOSIS EXISTS IN PULMONARY NONTUBERCULOUS MYCOBACTERIA INFECTION
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Abstract Content: Background: Pulmonary **nontuberculous mycobacteria** infection increases in recent decades. However, the clinical relevance of positive sputum for **nontuberculous mycobacteria** is far around 30-50 %. indicating its probable host susceptibility. The cytokine response of peripheral mononuclear cells has been reportedly decreased but the nature remains unclear. The role of gut dysbiosis of microbiota could be responsible for it but lacks of study in Pulmonary **nontuberculous mycobacteria** infection.

Methods: Patients with Pulmonary **nontuberculous mycobacteria** infection and healthy controls were enrolled. The cytokine response of peripheral mononuclear cells and gut microbiota as well as stool toll like receptor (TLR) ligands were checked and analyzed.
Results: We stimulated the participant's PBMCs and found that cytokines of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) were lower in patients that that in the controls. In regard to gut microbiota, there is dysbiosis with decreasing Shannon index in the patients with pNTM comparing with their controls. The TLR2 ligand was decreased in pNTM patients than the controls.
Conclusions: We preliminarily found that the cytokines of PBMC response, stool TLR2 ligand and microbiota diversity of gut were decreased in patients withPulmonary **nontuberculous mycobacteria** infection. The gut dysbiosis might play a role in immunosuppression for pathogenesis of Pulmonary **nontuberculous mycobacteria** infection. The mechanism required further investigation.
Disclosure of Interest: None Declared

LT129

SERINE 727 PHOSPHORYLATION OF STAT1 WORSENS A SEVERE NEUROLOGICAL DISEASE INDUCED BY COMBINED CHRONIC PRODUCTION OF IFN-A AND IL-6

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Abstract Content: In the central nervous system (CNS), cellular production and signaling of cytokines is tightly regulated. Not surprisingly, an increase in cytokine signature has been documented as a key feature of a number of neurological disorders including Aicardi-Goutières syndrome, multiple sclerosis and neuromyelitis optica. Cytokine-induced disease features are closely modeled through the use of transgenic mice with CNS-targeted production of single cytokines such as IFN-α (GFAP-IFN mice) and IL-6 (GFAP-IL6 mice) where clinical and pathological phenotypes reflect the actions of the particular cytokine produced. A key finding in GFAP-IFN and GFAP-IL6 mouse models is the activation of transcription factor STAT1 by serine 727 phosphorylation (pS-STAT1). However, GFAP-IFN or GFAP-IL6 mice that express S727A mutant STAT1 are indistinguishable from GFAP-IFN and GFAP-IL6 controls, suggesting a minimal biological impact for pS-STAT1 in response to either IFN-α or IL-6 in the living CNS. Here we studied the contribution of pS-STAT1 in a mouse model of combined IFN-α or IL-6 production in the CNS.
To study the actions of IFN-α and IL-6 in combination, we interbred GFAP-IFN mice and GFAP-IL6 mice to obtain double transgenic GFAP-IFN-IL6 mice. Compared with single transgenic animals, GFAP-IFN-IL6 mice developed a more pronounced and accelerated disease that was characterised by severe ataxia and tremors. In the cerebellum of these mice, gross leukocyte infiltrates, destruction of the Purkinje cell layer and increased microgliosis and astrogliosis was observed. This was characterised by an exaggerated IFN-α response and increased levels of STAT1 activation by tyrosine 701 and serine 727 phosphorylation but not STAT3 activation in comparison to single transgenic controls. To study further the role of STAT1 transactivation in IFN-α and IL-6 signalling together, we interbred the GFAP-IFN-IL6 mice with mice that express a S727A mutant STAT1. In the absence of pS-STAT1, mice were partially protected against the IFN-α and IL-6 driven neurological disease, with decreased expression of inflammatory genes. This indicates that serine 727 phosphorylation regulates STAT1 transcriptional activity, and enhances the pathogenic actions of IFN-α and IL-6 together in the CNS.
Overall, our findings suggest that serine phosphorylation of STAT1 is a potent modulator of neuroinflammation. Consequently, defining its modulatory role in these diseases will advance current understandings of complex cytokine signaling and the pathogenesis of neuroinflammatory diseases.
Disclosure of Interest: None Declared

LT130

EFFECTS OF PROBIOTIC SUPPLEMENTATION ON CYTOKINES BALANCE AFTER A MARATHON RACE.

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Abstract Content: Background
Previous studies have demonstrated several changes in the cytokines production after strenuous exercise. Cytokines greatly influence the immune response and metabolism of athletes who often suffer immunomodulation after prolonged exercise. Probiotic supplementation has been used to mitigate the effects of strenuous exercise on the immune system. Thus, the present study aimed to evaluate the effect of daily probiotic supplementation on IL-2, IL-4, IL-10 and TNF-α concentration and IL2/IL-4 and TNF-α/IL-10 ratios of runners submitted to an official Marathon race.
Methods
Runners (n:30) were supplemented for thirty days with probiotics $10x10^9$ CFU of *Lactobacillus Acidophilus* and *Bifidobacterium Lactis* or placebo double-blinded. At baseline (30d before), 24 hours before (1d before), one hour after (1h after), and five days after an official marathon (5d after), blood were collected for analyses. The plasma concentration of IL-2, IL-4, IL-10, and TNF-α was analyzed using the Simultaneous analysis of multiple cytokines (Luminex technology with magnetic Beads). Data normality verified using the Shapiro-Wilk's test, and the Anova Two-Way with a significance level of $p \leq 5\%$ was applied.
Results
In both groups, IL-2 decreased at all moments compared to the baseline. At probiotic group, the IL-2 decreased 1h after and 5d after compared to 24h before. Compared to the baseline, IL-4 concentration decreased 1h after and 5d after on placebo group, while significantly decreased 5d after on

probiotic group. IL-10 increased 2.700% and 2.100% on placebo and probiotic, respectively, 1h after the Marathon race compared to 1d before and return to baseline levels 5d after in both groups. TNF-α decreased at all moments compare to 30d before in both groups. No statistical differences were found between groups for Th1/Th2 balance. However, 5d after the race, there was a change in IL-2/IL-4 favoring the immune response in the probiotic group compared to 30d before. The IL-10/TNF-α ratio was lower at 1d before and 5d after compared to 30d before in both groups. After the marathon race, the IL-10/TNF-α balance was higher compared to all moments demonstrating higher IL-10 concentrations.

Conclusions

The supplementation of $10x10^9$ CFU of *Lactobacillus Acidophilus* and *Bifidobacterium Lactis* for thirty days did not modify the plasma concentration of IL-2, IL-4, IL-10 and TNF-α and Th1/Th2 balance compared to placebo. Specific changes in the probiotic group were found 5d after the marathon race favoring the Th1 response. Strenuous physical exercise has a potential to increase momently the IL-10/TNF-α balance. Several studies demonstrate benefits of probiotic supplementation. Further investigations are necessary to verify the efficacy of these specifics strains and dose to immune system.

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Disclosure of Interest: None Declared

LT132

FEMALE T-LYMPHOCYTES INHIBIT CSF1-MICROGLIA INDUCED PAIN HYPERSENSITIVITY

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Abstract Content: Activation of spinal cord microglia is an important contributor to peripheral nerve injury-induced mechanical hypersensitivity, a hallmark of neuropathic pain. We previously reported that nerve injury induces *de novo* primary sensory neuron expression of colony-stimulating factor 1 (CSF1), a cytokine that activates spinal cord microglia, and that upregulation of CSF1 is required for the associated mechanical hypersensitivity. Here we report that intrathecal (i.t) injection of CSF1 is sufficient to induce mechanical hypersensitivity in male but not female mice. Interestingly, i.t. injection of CSF1 not only promotes proliferation, but also leads to microglia activation and loss of morphology complexity in males, but not females. Transcriptional profiling of spinal cord microglia after i.t. CSF1 demonstrated that male, but not female microglia, robustly upregulate immune activation genes involved in cytokine production, chemotaxis and cell adhesion and overall acquire a phenotype previously identified as 'disease associated microglia'. In addition, male microglia downregulate many of their tissue supportive functions. To identify the mechanisms that underlie these sex specific differences in the microglia response to CSF1, we examined the potential contribution of the adaptive immune system. Although we never detected infiltration of lymphocytes in the spinal cord after i.t. CSF1, we did find a robust expansion of lymphocyte populations, in particular T-lymphocytes, in spinal cord meninges. Furthermore, i.t. CSF1 readily provoked mechanical hypersensitivity in T-lymphocyte depleted female mice. Ongoing work is identifying the role of specific T-lymphocyte subsets in CSF1 induced pain hypersensitivity. In conclusion, our data reveals that CSF1 activates microglia in a sex specific manner and that the adaptive immune system is involved in this process.

Disclosure of Interest: None Declared

LT133

CHANGES IN CYTOKINE PRODUCTION BY IMMUNE CELLS AND ADIPOSE TISSUE DURING COLD ADAPTATION

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Abstract Content: Maintaining energy homeostasis at reduced temperatures is essential for the survival of the organisms. We determined the effect of cold stress and cold adaptation on the immune system in a rat model, as well as on the cytokine production in different types of adipose tissue.

Various factors participate in the process of thermoregulation, a crucial step involves the binding of norepinephrine (NE) to adrenergic receptors, and in long-term adaptation, thyroid hormones play an important role. To decipher neuro-immune interaction at the reduced temperature, we monitored changes in the presence and activation of individual immune cell populations isolated from peripheral blood, spleen and peritoneal cavity at various time points of cold adaptation and correlated them with the expression of enzymes crucial for NE and thyroid hormone (triiodothyronine – T3) activity. Significant changes in immune response to various stimuli, including cytokine production, in particular IL-1β, IL-4, IL-6, IL-10 and TNF-α, indicated switch to anti-inflammatory environment and response skewed towards Th2 immunity in cold adapted animals.

Surprisingly, we observed change in IL-17 production that was associated with γδT-cell population. Adaptation to the reduced temperature was also accompanied by changes in cytokine production in brown and white adipose tissue. We suggest that cellular metabolism and activity of uncoupling proteins in immune cells are responsible for observed changes. However, further studies of neuro-immune crosstalk that occur during cold adaptation are necessary. Understanding mechanisms of cold adaptation and subsequent metabolic changes can help in the development of therapeutic approaches for obesity and related metabolic disorders.

Disclosure of Interest: None Declared

LT134

CHEMOKINE FRACTALKINE (CX3CL1) MEDIATES NEURAL PRECURSOR CELL FUNCTION FOR ENHANCED OLIGODENDROCYTE PRODUCTION

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Abstract Content:

Introduction: Oligodendrocytes produce myelin, an essential component of the central nervous system. In the adult brain, oligodendrocytes are generated via a 2-step process: neural precursor cells (NPCs) in the subventricular zone (SVZ) niche are committed to oligodendrocyte precursor cells (OPCs), which then differentiate into oligodendrocytes. Formation of oligodendrocytes from their multipotent progenitors is regulated by signals present in the NPC niche. The chemokine fractalkine (CX3CL1), which is secreted by neurons in the NPC niche and signals via its sole receptor (CX3CR1), increases oligodendrocyte formation from cortical NPCs during embryonic development (Voronova et al., Neuron, 2017). CX3CR1 is also expressed in postnatal and adult NPCs and OPCs (reviewed in Watson et al., Neurosci Lett 2020). However, the role of fractalkine signalling in postnatal NPCs and OPCs is not currently known.

Methods: We utilized single cell RNA fluorescent *in situ* hybridization (RNA scope) to detect CX3CR1 expression in postnatal brain. We also injected fractalkine directly conjugated to fluorophore Alexa-647 (CX3CL1-647) directly into the lateral ventricle of adult brain to identify which cell types bind fractalkine *in vivo*. We then infused fractalkine into the lateral ventricle of adult NPC lineage tracing mice ($NestinCre^{ERT2}$;$RosaYFP^{STOP/+}$) to assess the role of fractalkine signaling in *de novo* oligodendrocyte genesis. To test the direct effect of fractalkine on NPCs, we generated NPCs or OPCs from murine postnatal SVZ neural stem cell neurospheres, in which the only cells that propagate are precursors while other cells such as microglia, neurons and astrocytes do not survive. Microglia-free NPC or OPC monolayer cultures were incubated in the presence of soluble fractalkine or CX3CL1 or CX3CR1 function blocking antibodies and analyzed for differences in proliferation, differentiation and survival.

Results: Here we provide evidence that in addition to microglia, postnatal SVZ NPCs express CX3CR1 and bind fractalkine *in vitro* and *in vivo*. When FKN is added to microglia-free NPC cultures, it enhances their differentiation into OPCs and oligodendrocytes without affecting NPC proliferation or survival. Inhibition of fractalkine signalling in microglia-free OPC cultures with function-blocking antibodies inhibits oligodendrocyte differentiation. Finally, infusion of fractalkine into lateral ventricle of adult NPC lineage tracing mice enhances OPC and oligodendrocyte genesis from Nestin-positive SVZ NPCs *in vivo*.

Conclusions: In summary, we demonstrate fractalkine signalling directly mediates postnatal SVZ NPC and OPC function. Our results raise the possibility that immunological chemokines, such as fractalkine, may i) play an important role in neural precursor function; ii) be used for engagement of NPCs for enhanced oligodendrocyte regeneration; and iii) be involved in novel cell-to-cell interactions between NPCs and microglia, both of which express chemokine receptors, such as CX3CR1. Our future studies will address the ability of exogenous fractalkine to enhance remyelination and modify precursor-microglia crosstalk in mouse models of Multiple Sclerosis.

Disclosure of Interest: None Declared

LT135

INTERLEUKIN-10 DEFICIENCY EXACERBATES BRAIN IL-6 AND LPS-INDUCED TAU PATHOLOGY

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Abstract Content: Neurodegenerative diseases, such as Alzheimer's disease, are associated with accumulation of microtubule associated protein tau (MAPT or tau). The presence of hyperphosphorylated tau (p-tau) has been shown to strongly correlate with cognitive decline and neuroinflammation. Though anti-inflammatory cytokines are key players in regulating immune responses, the direct effects of certain prominent anti-inflammatory cytokines in tau pathology is still unclear. One of the most established anti-inflammatory cytokines is interleukin-10 (IL-10) which plays a role in downregulating inflammatory microglia responses. We hypothesized that IL-10 plays a role in regulating the pro-inflammatory pathway that induces tau phosphorylation within the brain.

Using lipopolysaccharide (LPS, 3 mg/kg b.w.; i.p.; single dose) to induce inflammation in C57BL/6J mice, we compared the effects of systemic inflammation on endogenous mouse tau in the brains of *Il10*-deficient ($Il10^{-/-}$) mice versus wildtype controls ($Il10^{+/+}$). First, basal levels of tau phosphorylation measured through western blot with AT8, AT180, and PHF1 antibodies were no different in $Il10^{-/-}$ and $Il10^{+/+}$ mice. Following LPS administration, tau phosphorylation increased in both groups with significant multi-fold increases of p-tau in $Il10^{-/-}$ hippocampal (Hp) lysates compared to $Il10^{+/+}$ controls. Interestingly, total tau levels were significantly reduced in $Il10^{-/-}$ mice after LPS administration indicating loss of neuronal tau. Quantification of AT8+ and AT180+ area through IHC confirmed significantly increased phosphorylated tau epitopes in the dentate gyrus of $Il10^{-/-}$ mice. Additionally, we observed a small but statistically significant decrease in NeuN+ area with notable detection of TUNEL positive staining in the CA3 region of $Il10^{-/-}$ LPS mice compared to the control group.

Microglia cell morphology and inflammatory cytokines within the perfused brain tissue was examined. Quantitative comparison of microglia morphology showed that $Il10^{-/-}$ microglia had significantly reduce branching compared to $Il10^{+/+}$ microglia suggesting an activated cell type. In both groups, LPS administration elevated active p38 MAPK, which is pro-inflammatory and shown to phosphorylate tau. In $Il10^{-/-}$ mice, phospho-p38 elevation was nearly two-fold significantly higher than the $Il10^{+/+}$ group. Interestingly, statistically significant lower amounts of Total p38 were observed in $Il10^{-/-}$ Veh group compared to $Il10^{+/+}$ Veh controls. After LPS, the $Il10^{+/+}$ group had elevated

levels of IL-6, IL-1b, and TNFa with concomitant increase in IL-10 within cortical brain lysates. Furthermore, *Il10*$^{-/-}$ mice had significantly enhanced levels of IL-6 and TNFa compared to *Il10*$^{+/+}$ mice after 24 hours of LPS administration. IL-6 was particularly untethered in the IL-10 deficient mice compared to the IL-10 positive group. To evaluate the potential role of this cytokine to promote tau phosphorylation in neurons, we administered recombinant IL-6 directly to primary neuronal mouse cells, *in vitro*, and observed a pattern of tau phosphorylation and total tau loss compatible with *in vivo* observations. These data support the significance of IL-10 in modulating the pro-inflammatory pathway that induces microglia activation and tau phosphorylation.
Disclosure of Interest: None Declared

LT136
SALT GENERATES ANTI-INFLAMMATORY TH17 CELLS BUT AMPLIFIES THEIR PATHOGENICITY IN PRO-INFLAMMATORY CYTOKINE MICROENVIRONMENTS
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Abstract Content: T helper cells integrate signals from their microenvironment to acquire distinct specialization programs for efficient clearance of diverse pathogens or for immunotolerance. Ionic signals have recently been demonstrated to affect T cell polarization and function. Sodium chloride (NaCl) was proposed to accumulate in peripheral tissues upon dietary intake and to promote autoimmunity via the Th17 cell axis. Here we demonstrate that high NaCl conditions induced a stable, pathogen-specific, anti-inflammatory Th17 cell fate in human T cells in vitro. The p38/MAPK pathway, involving NFAT5 and SGK1, regulated FoxP3 and interleukin (IL)-17A-expression in high-NaCl conditions. The NaCl-induced acquisition of an anti-inflammatory Th17 cell fate was confirmed in vivo in an experimental autoimmune encephalomyelitis (EAE) mouse model, which demonstrated strongly reduced disease symptoms upon transfer of T cells polarized in high NaCl conditions. However, NaCl was coopted to promote murine and human Th17 cell pathogenicity, if T cell stimulation occurred in a pro-inflammatory and TGF-b-low cytokine microenvironment. Taken together, our findings reveal a context-dependent, dichotomous role for NaCl in shaping Th17 cell pathogenicity. NaCl might therefore prove beneficial for the treatment of chronic inflammatory diseases in combination with cytokine-blocking drugs.
Disclosure of Interest: None Declared

Lightning Talk Session 2: Innate Immunity II

LT137
THE GASDERMIN-D PORE ACTS AS A CONDUIT FOR IL-1B SECRETION
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Abstract Content: The cytokine IL-1β is an important driver of fever and a key factor in the host response against infection. Interestingly, IL-1 β differs from other common cytokines (TNF-□, IL-8) as it is produced as a pro-form and requires catalytical processing by caspase-1 yielding its mature form; but also since is released in an unconventional manner, rather than being released over the conventional route via ER and Golgi. It was shown that caspase-1 also processes the protein gasdermin-D (GSDMD) and that the N-terminal GSDMD fragment that is generated by this cleavage forms pores in the plasma membrane, thereby causing a caspase-1 dependent form of necrotic cell death, known as pyroptosis. Since IL-1β secretion also depends on GSDMD we investigated the role of GSDMD in unconventional secretion of IL-1β. Here we show that gasdermin-D is required for IL-1□ secretion by macrophages, dendritic cells and partially in neutrophils, and that secretion is a cell-lysis-independent event. Liposome transport assays in vitro further demonstrate that gasdermin-D pores are large enough to allow the direct release of IL-1□. Moreover, IL-18 and other small soluble cytosolic proteins can also be released in a lysis-independent but gasdermin-D-dependent mode, while proteins larger than 25 kDa or artificially enlarged IL-1□ (tagged with eGFP) remain trapped in the cytosol, suggesting that the gasdermin-D pores allow passive the release of cytosolic proteins in a size-dependent manner.
Disclosure of Interest: None Declared

LT138
TOLL-LIKE RECEPTOR 4-MEDIATED INFLAMMATION TRIGGERED BY EXTRACELLULAR IFI16 IS ENHANCED BY LIPOPOLYSACCHARIDE BINDING
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Abstract Content:
Background: Since its discovery in the early 90s, a cornucopia of biological activities has been attributed to the IFI16 protein, including cell cycle regulation, tumor suppression, apoptosis, DNA damage signaling, virus sensing, and virus restriction. In addition, aberrant IFI16 expression and release in the extracellular space has been reported in a series of inflammatory conditions. The current hypothesis is that overexpression of the IFI16 protein occurs in tissue compartments where it is not physiologically expressed during inflammation. The ensuing release of the IFI16 protein into the extracellular space may allow it to behave like a damage-associated molecular pattern (DAMP) that signals through the Toll-like receptor 4 (TLR4) triggering inflammation by itself or through interaction with exogenous molecules, e.g. lipopolysaccharide (LPS).
Methods: Pull down assays and ELISA were used to characterize IFI16 binding activity to LPS. The human monocytic cell line THP-1 and the renal carcinoma cell line 786-O were used as target cells to define IFI16-induced

proinflammatory activity. Co-immunoprecipitation (co-IP), surface plasmon resonance (SPR), and silencing experiments were used to define IFI16 signaling.
Results: We show that the IFI16 HINB domain binds to the lipid A moiety of either high or weak TLR4 agonist LPS variants. Treatment of THP-1 or 786-O cells with IFI16 led to increased production of proinflammatory cytokines, which was further enhanced when IFI16 was pre-complexed with sub-toxic doses of high TLR4 agonist LPS but not low agonists. Silencing of TLR4/MD-2 or MyD88 abolished cytokine production. These findings alongside with other in vitro binding experiments indicate that IFI16 interacts and signals through TLR4.
Conclusions: Collectively, our data provide compelling evidence that: i) IFI16 is a DAMP that triggers inflammation through the TLR4/MD2-MyD88 pathway; and ii) its activity is strongly enhanced upon binding to LPS variants regarded as full TLR4 activators. These data strengthen the notion that extracellular IFI16 functions as DAMP and point to new pathogenic mechanisms involving the crosstalk between IFI16 and subtoxic doses of LPS.
Disclosure of Interest: None Declared

LT139

BRAIN HISTOLOGY AND PATHOBIOLOGY OF WEST NILE VIRUS INFECTION IN THE COLLABORATIVE CROSS MOUSE MODEL

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Abstract Content: West Nile Virus (WNV) is an emerging neuroinvasive flavivirus. During human infection, WNV activates host innate antiviral immunity through the RIG-I-like receptor pathway, but evades the actions of interferon (IFN) and can cause serious encephalitis disease with a 6% case lethality. WNV infection immunopathology has been well characterized in C57BL/6J mice, but understanding the full impact of host genetics on the variety of disease outcomes and susceptibility is limited with this inbred model because of the lack of genetic variability. We used the Collaborative Cross (CC) mouse model to evaluate the pathobiology and innate immune regulation that underlies WNV disease progression and neuroinvasion. By using a standardized histology scoring system to evaluate viral localization and immunopathology of WNV infection in brain tissue isolates from CC lines, we evaluated correlates of innate immune action and IFN actions in 101 genetically diverse CC mouse lines. Our studies reveal that WNV infection in the CC captures the diversity in disease susceptibility and outcomes observed in humans, with 72% of the evaluated lines demonstrating brain histopathologies during the course of infection. We also observed lines with unique disease-course histopathologies, which may provide a new modeling resource for the study of alternative WNV infection disease courses. Overall, the severity and number of lines exhibiting brain histopathology increased over the studied time course, with the greatest histopathological disease severity in the largest number of lines occurring at day 12 post-infection. Meningitis, perivascular inflammation, and parenchymal inflammation were the most prevalent types of brain histopathological phenotypes. We found that brain histopathology associated with other WNV disease measures, such as weight loss and brain viral load, and may associate with gene expression of IFIT, IFN-beta, and IL-12 in the brain. Using the histological phenotype information, we performed genetic mapping of quantitative trait loci (QTL) and discovered genomic regions of interest that were associated with WNV-infection induced brain histopathologies. Together our findings show that the CC model of WNV is a relevant and powerful mouse model for mechanistic studies of WNV infection and neuropathology.
Disclosure of Interest: None Declared

LT140

OBESITY-RELATED AND LDL-MEDIATED LOSS OF NK CELL DEGRANULATION AND CYTOTOXOCITY IS IN PART MEDIATED BY NFKB INHIBITION

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Abstract Content: The global obesity epidemic increases cardiovascular disease (CVD) risk worldwide. In the U.S., obesity is most prevalent among African-American (AA) women, contributing to CVD disparities. Natural Killer (NK) cells have been reported as important in CVD development and display impaired function in obese individuals. However, little is known about the potential drivers of obesity-related NK cell dysfunction especially in at-risk groups like AA women. Thus we explored links between NK cells and obesity and aimed to identify potentially significant biological pathways for both chronic disease outcomes and prevention. First, we compared the NK cell profile of lean AA women (BMI<25) and AA women with obesity (BMI>35) (n=15 lean/14 obese, age-matched). While we saw no significant differences in overall CD3-/CD56+ NK cell proportions we found a significant decrease in CD56dim/CD16hi cytotoxic NK cells and a trending increase in CD56hi/CD16low proliferating NK cells among those with obesity. CD3-/CD56+ isolated NK cells of 7 women in each group were further characterized. NK cells isolated from AA women with obesity displayed less degranulation towards K562 cells. Decreased intracellular levels of Granzyme B were also found. In a second step, we used an *ex vivo* approach to determine possible mediating variables. Primary naïve NK cells of a healthy donor were treated with study participants' serum overnight (n=60, 93% female, age 60.8 years, all AA). Degranulation and intracellular cytokine levels were detected after exposure to K562 cells and the results subjected to multivariable regression analysis against clinical parameters. Interestingly, after adjusting for ASCVD and BMI, serum LDL levels negatively associated with CD107a (b=-0.23, p=0.07), IFNg (b=-0.35, p=0.005),

GM-CSF (b=-0.38, p=0.002), perforin (b=-0.30, p=0.02), and Granzyme B (b=-0.39, p=0.002), indicating loss in function and cytotoxicity with increasing LDL levels. To determine if LDL could be a driver of obesity-related NK cell function loss, naïve isolated NK cells were treated with LDL. In accordance with the *ex vivo* data significant decreases in CD107a, IFNg, and perforin were detected, with additional downregulation of TNFa. Analysis of the secreted cytokines of LDL-treated NK cells revealed a decrease in IFNg and TNFa. By using proteomics of LDL-treated naïve NK cells (n=3), we determined that NFkb was significantly decreased in its expression. It is noteworthy that NFkb pathway is important in NK cell cytotoxicity and therefore critical for NK cell function. Together, our data suggest that obesity as a significant risk factor of CVD acts on NK cells function and cytotoxicity via LDL by regulating NFkb signaling pathways. Our findings suggest crucial signaling mechanisms in NK cell mediated cytotoxicity that may be most affected by obesity and hyperlipidemia as CVD risk factors.

Disclosure of Interest: None Declared

LT141

CD86-BASED ANALYSIS ENABLES IDENTIFICATION OF HEMATOPOIETIC PROGENITORS UNDER BIOLOGICAL STRESSES WHICH UPREGULATE INTERFERON

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Abstract Content: Systemic infection and inflammation skew hematopoiesis and break balances between blood cell populations. Examining the impact of infection/inflammation in hematopoiesis could be helpful to understand the pathology. Sca-1 has long been used as an essential cell surface marker to distinguish hematopoietic stem progenitor cells (HSPCs; Sca-1$^+$) from Sca-1$^-$ erythrocyte/megakaryocyte- or myeloid-progenitors such as MEPs, GMP and CMPs. It has been recently reported that interferon (IFN), which could be upregulated during viral and bacterial infection, make the identification of hematopoietic progenitors difficult by inducing Sca-1 expression in the Sca-1$^-$ downstream progenitors.

In this study, we confirmed IFN-dependent Sca-1 upregulation in downstream progenitors after lipopolysaccharide administration or induction of systemic infection with a cecal ligation puncture model. IFN-mediated Sca-1 upregulation in downstream progenitors overestimated apparent number of HSPCs and underestimated the number of downstream progenitors. To solve this issue, we screened a panel of cell surface molecules and newly identified CD86 as an alternative marker for Sca-1. Under steady-states, the CD86-expressing cells mostly overlapped with the cells expressing Sca-1. Contrary to Sca-1, however, CD86 expression was nearly unchanged under infection/inflammation. At an early phase of infection, CD86-based analysis demonstrated the intact stemness of HSCs and activation of erythropoiesis, which were not observed by using Sca-1-based analysis.

Collectively, CD86 appears to be an alternative useful marker for Sca-1, which allows us to analyze "*bona fide*" hematopoietic responses under biological stresses such as infection and inflammation.

Disclosure of Interest: None Declared

LT142

A DISTINCTIVE THERAPEUTIC CANDIDATE TO RESTRICT INFLUENZA VIRUS-CAUSED LUNG INFECTION: INTERFERON-LAMBDA-LOADED SURFACTANT NANOPARTICLE

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Abstract Content: Rationale: We verified that interferon (IFN)-λ gene expression and secreted protein were more highly induced after influenza A virus (IAV) infection and also propose that IFN-λ is the primary interferon to mediate an anti-viral defense *in vivo* respiratory epithelium. We found that inhaled delivery of IFN-λs induced antiviral innate immune responses in respiratory tract and surfactant-based nanoparticle might be a useful material for IFN-λ delivery to *in vivo* lung.

Material and Methods: C57BL/6 mice (N=30) were exposed to whole body mainstream by the SCIREQ "InExpose" system and surfactant-based nanoparticle (diameter 100nM) including recombinant IFN-$\lambda_{2/3}$ (10 ug), surfactant (1mg/ml), protamine (2.5ug/ml) was inoculated into mice by inhaled delivery.

Results: IAV-infected mice were observed to have most significant loss of body weight, severe pathologic findings, viral replication and highest viral titer in their lung at 7 days after infection. IAV mRNA levels, nucleoprotein and viral titer were completely lower in the lung of IAV-infected mice which were inoculated with IFN-λ-loaded nanoparticles at the same time with IAV infection. In addition, the lung inflammation was significantly improved and body weight was maintained until 7 days after infection in IAV-infected mice with IFN-λ-loaded nanoparticles. IAV-infected mice with administration of with IFN-λ-loaded nanoparticles exhibited an even greater increase of IFN-stimulated genes (ISG) in their lung and more induction of ISG after inoculation of IFN-λ-loaded nanoparticles could last longer compared the IAV-infected mice with single treatment with IFN-λ.

Conclusion: The current data show that inhaled delivery of IFN-λs mediated more potent antiviral immune responses and restrict IAV replication in the respiratory tract. Surfactant-based nanoparticle can deliver IFN-λ more efficiently to in vivo lung and IFN-λ-loaded surfactant nanoparticle might be effective therapeutics to limit IAV-caused lung infection.

Disclosure of Interest: None Declared

LT144

DELETION OF TRANSFERRIN RECEPTOR 2 (TFR2) ENHANCES INFLAMMATION AND MACROPHAGE ACTIVATION

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Abstract Content:
Rheumatoid arthritis is an autoimmune disease characterized by disordered innate immunity, chronic inflammation and progressive joint destruction with frequent synovial iron deposition. Transferrin receptor 2 (Tfr2) is a focal point in the regulation of systemic iron levels and bone mass. Loss of Tfr2 function results in low hepcidin expression and iron overload. As neither the role of iron nor Tfr2 has been explored in arthritis, here, we aimed to investigate whether Tfr2 deletion and/or iron overload impacts on the pathogenesis of inflammatory arthritis.
We analyzed iron overloaded Tfr2-deficient mice (*Tfr2*$^{-/-}$) and their wild-type (*Tfr2*$^{+/+}$) littermate controls as well as mice lacking Tfr2 only in macrophages that are not iron overloaded for their capacity to develop K/BxN serum transfer arthritis (STA). Arthritis severity was assessed by clinical scores of paw swelling and hind paws were collected for micro-CT, histomorphometry, quantitative PCR and flow cytometry.
Tfr2$^{-/-}$ mice developed more pronounced joint swelling with enhanced mRNA expression of inflammatory markers (*Il1b*, *Inos and Ifng*), synovial hypertrophy and bone erosion as compared to *Tfr2*$^{+/+}$ mice. Furthermore, the infiltration of myeloid cells including GR1^{+} neutrophils and F4/80^{+} macrophages/monocytes at day 7 was increased in the joints of *Tfr2*$^{-/-}$ mice, suggesting a role of Tfr2 and/or iron overload in arthritis progression. To elucidate whether Tfr2 has a direct role in the pathogenesis of arthritis or whether the effects are merely mediated via the systemic iron overload, we induced STA in mice with a conditional deletion of Tfr2 in the myeloid lineage that have normal iron loading. *Tfr2*$^{fl/fl}$*-LysMCre+* showed increased disease development with enhanced synovial hypertrophy and bone erosion in the arthritic joints compared to *Cre-* control littermates.
Since iron controls inflammatory responses and macrophages sense and regulate iron availability, we hypothesized that Tfr2-deficiency in macrophages could modulate their activation and polarization into a pro-inflammatory phenotype that contributes to arthritis progression. *Tfr2*$^{-/-}$ macrophages showed a differential transcriptional response to IFN-γ stimulation by increasing the expression of *Il1b*, *Il6, Inos*, *Cxcl10* compared to *Tfr2*$^{+/+}$ macrophages. Furthermore, the deletion of Tfr2 in macrophages led to enhanced and prolonged IFN-γ-induced STAT1 activation, while it downregulated STAT3 activation likely via increasing *Socs3* expression.
Taken together, these findings suggest a protective role of Tfr2 in the progression of arthritis and that it is involved in repressing macrophage pro-inflammatory activation.
Disclosure of Interest: None Declared

LT145
REGULATION OF INTERFERON STIMULATED GENES AT HOMEOSTASIS

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Abstract Content: Introduction
Interferon stimulated genes (ISGs) are a collection of genes important in the early innate immune response. The regulation of ISGs has been extensively studied in cells exposed to significant interferon stimulation, but less is known about ISG regulation in homeostatic regimes. Most previous homeostatic ISG studies have focused on a small number of ISGs in a single cell type. Our goal in this study was to characterize homeostatic ISG regulation across a broad array of ISGs and potential regulation factors and across multiple cell types.
Methods
We collected 33 expression datasets from the GEO database across four cell types: CD4, pulmonary epithelial, fibroblast
and macrophage cells. We sought to construct ISG-regulator modules that identified subsets of ISGs with expression levels that correlated with expression levels of subsets of regulator genes. We used a linear regression model to construct such modules and to quantify the degree to which ISG expression levels was predicted by regulator expression levels.
Results
Our results show regulation of 100s of ISGs at homeostasis through the ISGF3 molecule and also suggest additional regulation through IRF7 and IRF8 associated pathways. We find that at homeostasis roughly 50% of ISGs have expression levels significantly correlated with ISGF3 expression. We find that ISG expression levels varied in their correlation with ISGF3 across cell types, with epithelial and macrophage cells showing more correlation than CD4 and fibroblast cells. Our analysis also provides a novel approach for decomposing and quantifying ISG regulation.
Reference:
Leviyang S., Strawn N., Griva I. (2020) Regulation of interferon stimulated gene expression levels at homeostasis. Cytokine.
Disclosure of Interest: None Declared

LT146
TUBERCULOSIS IMPACTS IMMUNE-METABOLIC PATHWAYS RESULTING IN PERTURBED IL-1 CYTOKINE RESPONSES

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Abstract Content: Tuberculosis (TB) remains a major public health problem. Host-directed therapeutics are proposed as novel treatment strategies, with TNF, IL1, and IFN cytokine pathways all strongly implicated in differential disease outcome. However, the successful development of host-directed therapeutics still requires a comprehensive

understanding of how *Mycobacterium tuberculosis* (*M.tb*) infection impacts immune responses.
To address this challenge, we applied standardised immunomonitoring tools to compare induced immune responses between individuals with latent *M.tb* infection (LTBI) and active TB disease. This revealed distinct responses between TB and LTBI groups at transcriptomic, proteomic and metabolomic levels. Specifically we observed dysregulated induced IL-1 responses in TB patients, which could be explained through differences in upstream TNFα and type I interferon, and downstream granzyme B responses. At baseline, we identified pregnane steroids and the PPARγ pathway as novel immune-metabolic drivers of IL-1ra secretion.
These findings improve our knowledge of how *M.tb* alters immune responses, and may support development of improved diagnostic, prophylactic and therapeutic tools.
Disclosure of Interest: None Declared

LT147

COMPARISON OF GEOGRAPHICALLY DISTINCT ZIKA VIRUS STRAINS REVEALS DIFFERENTIAL ACTIVATION OF HUMAN HOST INNATE IMMUNITY DIRECTING INNATE IMMUNE POLARIZATION

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Abstract Content: Recently, Asian lineage-derived Zika virus (ZIKV) strains have emerged, causing outbreaks across the Pacific Ocean resulting in new disease manifestations, including fetal infection with congenital ZIKV syndrome and adult infection presenting Guillain-Barré syndrome. We hypothesize that changes in viral sequence contribute to phenotypic differences in virus replication and host cell interactions between geographically distinct ZIKV strains that underlie differential disease. We therefore conducted de novo viral sequence and comparison analyses to evaluate how viral sequences of several geographically distinct ZIKV strains link with virologic differences and features of innate immune activation in human cells *in vitro*. Endemic African lineage-derived ZIKV/Dakar/1984/ArD41519, endemic Asian lineage-derived ZIKV/Malaysia/1966/P6740, and emerging Asian lineage-derived ZIKV/French Polynesia/2013/H-PF-2013, ZIKV/Puerto Rico/2015/PRVABC59, and ZIKV/São Paulo/2015/SPH2015 strains responsible for the Pacific and South American ZIKV outbreaks were assessed. De novo viral sequence determination and alignment revealed several amino acid substitutions in each viral genome compared to pre-emergent ZIKV/Malaysia/1966/P6740. One-step virus growth analysis revealed profound differences in replication kinetics between strains, whereas measurement of host cell IRF3 and NF-κB activation identified distinct virus-specific innate immune activation profiles. Among these, ZIKV/French Polynesia/2013/H-PF-2013 exhibited faster replication kinetics and more robust innate immune activation compared to ZIKV/São Paulo/2015/SPH2015 and ZIKV/Puerto Rico/2015/PRVABC59 linked with slower replication kinetics and weaker innate immune activation. Moreover, examination of innate immune activation by ZIKV/Dakar/1984/ArD41519 and ZIKV/Malaysia/1966/P6740 demonstrated differential and selective activation of the IRF3 and NF-κB signaling axes within infected cells, revealing strain-specific innate immune polarization. Our observations show that viral amino acid sequence differences link with ZIKV replication kinetics and detection by host cells wherein early virus detection and innate immune activation serve as critical restriction points to control viral replication. Distinct ZIKV-induced IRF3 and NF-κB activation demonstrates the concept of virus-directed innate immune polarization leading to antiviral versus inflammatory signaling to drive distinct immune responses linked with ZIKV infection and disease.
Disclosure of Interest: None Declared

LT148

POST-TRANSCRIPTIONAL REGULATION OF ANTIVIRAL GENE EXPRESSION BY N6-METHYLADENOSINE

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Abstract Content: Type I interferons (IFN) drive the induction of hundreds of IFN-stimulated genes (ISGs). The expression of these genes must be carefully regulated to allow for both efficient production of antiviral effectors and controlled shut-off of inflammatory factors to avoid tissue damage and autoimmunity. The RNA base modification N6-methyladenosine (m^6A) has emerged as an important post-transcriptional regulator of RNA expression. Indeed, m^6A has been shown to regulate both viral infection and the expression of IFN-beta, but its role in the type I IFN response has not been explored. To investigate the role of m^6A in the type I IFN response, we mapped m^6A in the IFN-induced transcriptome using meRIP-seq, and found that the transcripts of many ISGs are m^6A-modified. We then perturbed the expression of the m^6A deposition enzymes, METTL3 and METTL14 (METTL3/14) and found that the protein expression of certain m^6A-modifed ISGs is enhanced by METTL3/14, however their mRNA levels were unaffected. Polysome profiling determined that METTL3/14 regulates the translation of these m^6A-modified ISGs. To identify the full complement of METTL3/14-regulated ISGs, we performed ribosome profiling and quantitative mass spectrometry. These data revealed a class of ISGs whose protein expression is enhanced by m^6A, including many genes with known antiviral functions. Indeed, we found that METTL3/14 augments the antiviral effects of type I IFN during vesicular stomatitis virus infection. Together, these studies identify m^6A as a post-transcriptional control of ISG translation during the type I IFN response, which promotes an antiviral cellular state.
Disclosure of Interest: None Declared

LT149

SYSTEMATIC ANALYSIS OF CELL-INTRINSIC INNATE IMMUNE ANTAGONISM BY RNA VIRUSES

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Abstract Content: Emerging and re-emerging RNA viruses such as Zika virus (ZIKV), yellow fever virus (YFV), SARS-CoV-2, and endemic RNA viruses such as hepatitis E virus (HEV) remain global health problems. Most of these viruses have devised mechanisms to evade antiviral defenses, thereby creating a host cellular environment that is more conducive to viral replication. In particular, proteases encoded by RNA viruses have been shown to inactivate different components of antiviral signaling pathways, blunting the cell's ability to mount type I and III interferon (IFN) dependent defenses. Examples of this antagonism in well-characterized RNA viruses include the hepatitis C virus NS3/4A protease and ZIKV NS2B3 protease, which cleave the innate immune adaptor proteins MAVS and STING respectively. In this work, we have developed a platform to interrogate the putative innate immune antagonizing activities of several poorly-characterized human viruses: HEV, Usutu virus (USUV), and Powassan virus (POWV). Utilizing a live-cell STAT1 reporter in cells expressing a viral protease of interest, we are able to determine the necessity of type I and type III IFN signaling in the host cells' ability to mount an antiviral defense. Subsequent biochemical analyses allow us to quantify protein and transcript levels of innate immunity proteins before and during protease expression to pinpoint the stage of innate immune signaling that is antagonized by the virus. Preliminary data suggests that HEV can dampen multiple innate immunity pathways at an intermediate level not seen in other viral families, which opens up exciting new avenues of investigation. Collectively, our work provides mechanistic insights into how these emerging and endemic human pathogens evade innate immunity and may also reveal new targets for antiviral therapy.

Disclosure of Interest: None Declared

LT150

IDENTIFICATION OF NOVEL LIPOPHILIC LIGANDS OF SIGLEC RECEPTORS THAT MODULATE INNATE IMMUNITY

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Abstract Content: Sialic acid-binding immunoglobulin-like lectins (Siglecs) are a family of cell-surface receptors that bind to sialic acid at terminal glycan residues. In this study, we found that Siglec5 and 14 recognize *Trichophyton,* a pathogenic fungal species. Biochemical approaches revealed the chemical structures of the ligands, which were identified as alkanes and triacylglycerols. This unexpected finding prompted us to further search for novel endogenous lipid ligands. Although Siglec5 weakly recognized several endogenous lipids, cardiolipin and 5-palmitic acid-hydroxy stearic acid (5-PAHSA) showed potent ligand activity. The hydrophobic stretch in the N-terminus region of Siglec5 was found to be required for efficient recognition of these lipophilic ligands. Notably, this hydrophobic stretch is dispensable for sialic acid recognition. Siglec5 is an inhibitory receptor, which negatively regulates cell activation upon ligand binding. The lipophilic ligands suppressed IL-8 production in human monocytic cells expressing Siglec5. Siglec14 and 5 have high sequence identity in the extracellular region and thus, Siglec14 also recognizes the lipophilic ligands. In contrast to Siglec5, Siglec14 transduces activating signals upon ligand recognition. Indeed, the lipophilic ligands induced IL-8 production in human monocytic immune cells expressing Siglec14. Collectively, our study demonstrates that Siglec5 and 14 have the potential to recognize lipophilic ligands and modulate innate immune responses. To our knowledge, this is the first study to report the binding of Siglecs to lipid ligands, which will expand our understanding of the biological function and importance of Siglecs.

Disclosure of Interest: None Declared

LT151

REPORTER NANOPARTICLE FOR REAL-TIME INFLAMMASOME MONITORING DURING A DISEASE-PROGRESSION

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Abstract Content: Inflammasome activation is critical to variety of chronic inflammatory diseases like Colitis, Non-Alcoholic-Steatohepatitis, Alzheimer's. This innate immune response induced by microbial or sterile invasion activates caspase-1 enzyme, an important hallmark of inflammasome activation. Active caspase-1 further cleaves pro-IL-1b and pro-IL18, converting them to their active forms which are released outside the cell, resulting in enhanced immune response and pyroptosis. Currently available techniques fail to provide temporal kinetics of inflammasome activation and cytokine release during disease progression, presenting a window of opportunity for the development of inflammasome monitoring tools. To address this gap, we have developed a polymer-nanoparticle encapsulated caspase-1 activatable probe that will emit signal specifically at inflamed sites upon active-caspase-1 cleavage. This biocompatible platform enhances the probe's residence time in circulation by preventing its opsonization and allowing its sustained release over time. Preliminary observations show that the probe is cleaved specifically by recombinant active-caspase-1 and can be encapsulated stably inside polymer nanoparticle. We have also successfully demonstrated its *in-vitro* testing in inflammasome-activated-bone-marrow-derived-macrophages. We further plan to translate this study in relevant animal models. This entire system will help in the precise estimation of caspase-1 activation and cytokine release kinetics during disease progression, hence ensuring early detection of pathogenic inflammation and its timely treatment.

Disclosure of Interest: None Declared

LT152

CLEARANCE OF PEGYLATED INTERFERON BY KUPFFER CELLS DETERMINES INNATE IMMUNE ACTIVATION AND RESPONSE TO TREATMENT OF CHRONIC HEPATITIS B

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Abstract Content: Approximately 257 million people worldwide are chronically infected with the hepatitis B virus (HBV). About 900,000 people die from HBV-related liver failure and/or hepatocellular carcinoma each year, which makes HBV more deadly than HIV, malaria and tuberculosis combined. Pegylated interferon-alpha (PegIFNα) is the sole curative treatment for chronic hepatitis B virus (HBV) infection, but it is unknown why only a minority of patients respond to PegIFNα. Cure of chronic HBV infection is defined as HBsAg loss from the blood.

Here, we investigated the mechanisms that contribute to a patient's response to PegIFNα therapy. Using sequential blood and liver samples, we found that patients with early natural killer (NK) cell activation after PegIFNα injection experienced greater liver inflammation, lysis of HBV-infected hepatocytes and HBsAg decline than those without. The level of NK cell activation depended on PegIFNα pharmacokinetics, because serum levels of PEG and IFNα were 80-fold and 125-fold higher in patients with NK cell responses than in patients without NK cell responses at 6 hours and 24 hours after the first PegIFNα injection, respectively. The PegIFNα level in the blood was determined by differential Kupffer cell-mediated clearance of PegIFNα/PEG-antibody complexes as revealed by immunohistochemistry of liver biopsies, taken prior to and 6 hours after PegIFNα injection.

These results indicate that NK cell activation and NK cell-mediated lysis of HBV-infected hepatocytes plays a pivotal role in the clinical response to PegIFNα therapy, and that PEG clearance by Kupffer cells impairs therapeutic activity during PegIFNα treatment. We postulate that repeated exposure to PEG, which is widely used in dairy products (such as shampoo, cosmetics, laxative) might induce the production of anti-PEG antibody prior to PegIFNα treatment and accelerate PEG clearance by Kupffer cells. Accelerated PEG clearance by Kupffer cells needs to be considered beyond the HBV field to improve the efficacy of a wide variety of pegylated drugs, that are now used and being developed for multiple chronic diseases and cancer.
Disclosure of Interest: None Declared

LT153

HIV LATENT CELLS HAVE ACQUIRED RESISTANCE TO CELL-INTRINSIC INNATE IMMUNITY

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Abstract Content: The persistence of latent cells that silently harbor HIV proviruses despite antiretroviral therapy (ART) is a major barrier to an HIV cure. HIV infection triggers cell-intrinsic immune defenses which can block viral replication in acutely infected cells. These innate immune defenses are mediated by pathogen recognition receptors that sense viral products and activate type 1 interferon (IFN) production, leading to induction of hundreds of interferon-stimulated genes (ISGs), many with antiviral activity. Despite these defenses, some acutely infected cells become latent and silently persist for years in vivo, suggesting they have escaped IFN-mediated immunity. Using a variety of primary and cell line models of HIV latency, we evaluated innate immune activation and IFN signaling in latently infected cells. We found that HIV latent T cell lines have functional viral RNA sensing pathways that drive IRF3 activation and IFN production. However, unlike uninfected cells, latent cells respond poorly to type 1 IFN treatment and fail to induce specific antiviral ISGs. RNA sequencing analysis of latent vs uninfected cells stimulated with IFN revealed a network of dysregulated ISGs that we hypothesize may be important for restricting latency. Furthermore, HIV infected primary CD4+ T cells suppressed with ART demonstrate dampened responses to IFN stimulation compared to uninfected cells. These findings suggest that reservoir cells have intrinsic defects in IFN signaling, which may be selected for during initial infection and/or actively maintained throughout the transition to latency. We are further investigating the mechanism of IFN signaling dysregulation in latent cells. Our work reveals a previously undescribed role for type 1 IFN in regulating HIV latency, which may be exploited to design curative therapies aimed at eradicating the reservoir.
Disclosure of Interest: None Declared

LT154

VANCOMYCIN INTERMEDIATE STRAINS OF STAPHYLOCOCCUS AUREUS HAVE DAMPENED INDUCTION OF TYPE I INTERFERON PRODUCTION

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Abstract Content: *Staphylococcus aureus* is a leading cause of bacterial pneumonia and we have shown previously that type I interferon (IFN) contributes to pathogenesis of *S. aureus* pneumonia. In this study, we screened 75 *S. aureus* strains for their ability to induce type I & III IFN. Both cytokine pathways were differentially induced by various *S. aureus* strains independently of their isolation sites or methicillin resistance profiles. These induction patterns persisted over time and type I & III IFN generation differentially correlated with TNF-α production. Investigation of one strain, strain 126, showed a significant defect in type I IFN induction that persisted over several time points. The lack of induction was not due to differential

phagocytosis, subcellular location or changes in endosomal acidification. Strain 126 had a decreased rate of autolysis and increased resistances to lysostaphin degradation and host cell mediated killing. This strain displayed decreased virulence in a murine model of acute pneumonia compared to USA300 (current epidemic strain and commonly used in research) and had reduced capacity to induce multiple cytokines. We observed this isolate to be a vancomycin intermediate *S. aureus* (VISA) strain. The relationship between reduced type I IFN induction levels and decreased autolysis was found to be shared by other VISA strains and studied using an isogenic pair of wild type and VISA mutant strains. We conclude that the reduction in type I IFN induction was largely the result of inherent cellular properties of VISA strains whose reduced autolysis prevents the release of pathogen-associated molecular patterns. Overall, this study demonstrates the heterogeneity of IFN induction by *S. aureus* and uncovered a unique property of VISA strains in their inability to induce type I IFN production.
Disclosure of Interest: None Declared

LT156

HOST CYTOKINE DYSREGULATION AND ENHANCED RESPIRATORY DISEASE ELICITED BY VACCINE-INFLUENZA MISMATCH.

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Abstract Content: Influenza A virus outbreaks in humans and pigs are mitigated through vaccination. Pigs vaccinated with adjuvanted whole-inactivated virus vaccine (WIV) that are later infected with a mismatched influenza strain of the same HA subtype can develop vaccine associated enhanced respiratory disease (VAERD). VAERD occurs in the presence of high titer non-neutralizing antibodies, with similar viral loads between VAERD-affected and unvaccinated animals. To elucidate the mechanisms underlying the severe pulmonary pathology, we examined host gene alterations in WIV vaccinated pigs affected by VAERD compared to naïve infected pigs. qPCR arrays and whole-genome RNA-seq analyses from pulmonary alveolar macrophages and lung tissue were utilized to assess the transcriptomic response. ELISA and MAGPIX® Luminex™ platforms were used to analyze responses at the protein level. Pigs with VAERD had dysregulation in many anti-viral or pro-inflammatory cytokine genes compared to naïve challenged pigs, including a diminished type-I interferon response (IFN-a). Genes associated with helper T-cell driven inflammation (IL-17A, IL-22 and IL-23) were higher at the RNA and protein level in VAERD animals compared to naïve challenged pigs. Additionally, RNA-seq analysis identified genes involved with wound repair and tissue remodeling were upregulated in VAERD pigs. These results revealed that cytokines associated with helper T-cell responses were upregulated with VAERD and may provide possible mechanisms that lead to the severe pulmonary immunopathology; whereas wound repair and tissue remodeling gene upregulation may be linked to the resolution of the VAERD lesions. These underlying mechanisms may provide targets for preventing and/or treating VAERD.
Disclosure of Interest: None Declared

LT157

INNATE IMMUNE SIGNALING DRIVES PATHOGENIC EVENTS LEADING TO AUTOIMMUNE DIABETES

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Abstract Content: Introduction: Type 1 diabetes (T1D) is a chronic autoimmune disease, characterized by the immune-mediated destruction of insulin-producing b cells of pancreatic islets. Essential components of the innate immune antiviral response, including type I interferon (IFN) and IFN receptor (IFNAR)-mediated signaling pathways, are candidates for determining susceptibility to human T1D.
Methods: We previously generated *Ifnar1*$^{-/-}$ LEW.1WR1 rats that have a significant reduction in diabetes frequency following Kilham rat virus (KRV) infection. To delineate the impact of IFNAR1 loss on adaptive immune responses in KRV-induced diabetes, we examined immune cell populations in the spleen during the preinsulitic stage. We used approaches such as flow cytometry, bulk RNA sequencing, and single-cell RNA-sequencing to identify major leukocyte subsets which are altered in spleen preceding insulitis in both WT and *Ifnar1*$^{-/-}$ rats after viral infection.
Results: Examination of splenic T cells and natural killer (NK) cells before the onset of insulitis and diabetes revealed a significant decrease in CD8+ T cells and NK cells in *Ifnar1*$^{-/-}$ rats compared to WT rats. In addition, splenic regulatory T cells were diminished in WT but not *Ifnar1*$^{-/-}$ rats. Conversely, splenic neutrophils were increased in KRV-infected *Ifnar1*$^{-/-}$ rats compared to the infected WT rats. We also compared gene expression changes in spleens from virus-infected WT and *Ifnar1*$^{-/-}$ rats using bulk RNA-seq at the prediabetic stage. RNA-seq revealed diminished interferon-stimulated genes and inflammatory gene expression in spleens from *Ifnar1*$^{-/-}$ rats relative to WT rats. Single cell RNA-seq of spleens confirmed a decrease of NK cells in KRV-infected *Ifnar1*$^{-/-}$ rats compared to WT rats.
Conclusions: Collectively, these findings identify novel innate immune players in the KRV-induced diabetes in rats and firmly establish that disruption of IFNAR signaling dynamically alters immune cell populations in a manner which is protective against the development of autoimmune diabetes.
Disclosure of Interest: None Declared

LT158

ANTIVIRAL EFFECT OF THE RIG-I INDUCED PATHWAY OF APOPTOSIS (RIPA) IS BOOSTED BY ITS ABILITY TO TRIGGER DEGRADATION OF THE DEUBIQUITINASE, OTULIN

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Abstract Content: Infection of mammalian cells by many RNA viruses trigger the RIG-I pathway which activates IRF3. IRF3 exerts its antiviral effects by inducing IFN synthesis and another antiviral pathway, called RIG-I induced pathway of apoptosis (RIPA). RIPA requires linear polyubiquitination of IRF3 by the enzyme complex, LUBAC; ubiquitinated IRF3 binds to Bax and translocates it to mitochondria causing the release of Cytochrome C, activation of caspases and apoptosis of the infected cell. Here, we report that Otulin, the deubiquitinase that removes linear polyubiquitin chains, inhibits RIPA by binding to LUBAC and deubiquitinating IRF3. Ablation of Otulin expression enhanced RIPA and its overexpression inhibited RIPA. We discovered that to overcome Otulin-mediated inhibition, RIPA actively degrades Otulin. This degradation required sequential actions of RIPA-activated Caspase 3 and proteasomes. Caspase 3 cleaved Otulin first at D31 and then at D322; the D31A mutant was not cleaved at all. The caspase-cleaved fragment was totally degraded by proteasomes, which required its K48-linked ubiquitination. The E3-ubiquitin ligase, HOIP, a component of LUBAC, ubiquitinated Otulin at K64 and K197 to trigger proteasome-mediated degradation. To assess the impact of Otulin degradation on RIPA-mediated antiviral action, we expressed in Otulin-ablated cells, a non-degradable mutant of Otulin, in which D31, K64 and K197 had been mutated. In these cells, RIPA was less active and replication of Sendai virus was more pronounced. Thus, our study has revealed an important positive feedback loop of RIPA.
Disclosure of Interest: None Declared

LT159

A SYNTHETIC REXINOID REDUCES LPS-ACTIVATED NLRP3 ACTIVATION IN BONE MARROW DERIVED M1 MACROPHAGES.

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Abstract Content: The NLR family pyrin domain containing 3 (NLRP3) inflammasome is a critical mediator of inflammation in response to pathogens, tissue damage, and inflammatory diseases. These large multi-protein complexes form rapidly in response to danger and pathogen-associated signals, serving as scaffolds to promote maturation of caspase-1, a cysteine protease that processes inactive pro-IL-1 beta and pro-IL-18 to their active pro-inflammatory cytokines, IL-1 beta and IL-18, respectively.
Retinoid X receptors (RXRs) are nuclear receptors and play essential roles for macrophage biology. Rexinoids are synthetic molecules that bind and activate RXRs. We found that our rexinoid drug inhibited LPS-induced M1 macrophage inflammation in vitro and were interested to know whether this effect was mediated via NLRP3 inflammasome complex. For this, bone marrow derived macrophages (BMDM) from tibia and femurs of mice were differentiated by granulocyte-macrophage colony-stimulating factor (GMCSF) to skew them towards M1 phenotype. The rexinoid drug suppressed expression of NLRP3 protein and hindered the processing of IL-1 beta and Caspase-1 proteins in LPS+ATP-primed M1 macrophages. Secreted IL-1 beta and IL-18 mRNAs and proteins were also significantly ($p<0.05$) decreased in LPS+ATP-stimulated M1 macrophages when treated with either the rexinoid drug or NLRP3 siRNA.
These data suggest that the rexinoid drug can reduce NLRP3-mediated activations in M1 macrophages. Future studies will explore models of macrophage mediated inflammatory diseases.
Disclosure of Interest: None Declared

LT160

MURINE AND HUMAN MACROPHAGES MEDIATE RECOGNITION OF THE INTRAERYTHROCYTIC APICOMPLEXAN PARASITE BABESIA DIVERGENS.

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Abstract Content: *Babesia divergens*, is a natural pathogen of cattle, and one of the species responsible for human babesiosis, causing death unless treated promptly. This worldwide emerging disease, with many clinical features that are like those of malaria, is transmitted by ticks and transfusion and is increasing in frequency and geographic range. Thus, babesiosis has a significant impact on human and animal health.
During its asexual life cycle, *B. divergens* infects and reproduces within red blood cells (RBCs) and builds a complex population of intraerythrocytic (IE) and free infective merozoites. The invasion by the free merozoites and posterior destruction of the RBCs is a critical point in the progress and prognosis of the disease.
Macrophages are innate immune cells responsible of recognition and elimination of pathogens at early stages of infection. Macrophage polarization is known to be important in parasitic infections. Cytokines and chemokines produced by macrophages are crucial for establishment of immune response against pathogens and disease prognosis.
Thus, in this study we describe an *in vitro* response of both human and murine bone-marrow differentiated macrophages (BMDM) exposed to mock treatment, uninfected human red blood cells (RBC), *B. divergens*-

infected RBCs or to free merozoites in order to characterize the possible reaction against the parasite during infection.
Results show a strong expression of IFN-β mRNA and some proinflammatory cytokines such as IL-6, TNF-α, IL-1β in GM-CSF derived BMDM, in comparation with M-CSF BMDM, in response to the presence of free merozoite. Notably, as compared to the free merozoite, a weak proinflammatory response was induced in BMDM against *B. divergens*-infected RBCs in both murine and human BMDM. This result suggests that macrophages cannot efficiently recognize the *B. divergens* parasite when is within its host cell.
Disclosure of Interest: None Declared

LT160c

CONGENITAL DEFICIENCY IDENTIFIES CRITICAL ROLE OF ISG15 IN SKIN HOMEOSTASIS

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Abstract Content: Ulcerating skin lesions are a newly described manifestation of human ISG15 deficiency, a type I interferonopathy. However, it is unclear whether their etiology can be completely explained by chronic inflammation in dermal cells. We describe two siblings with chronic recurrent skin ulcers that healed with scar formation upon corticosteroid treatment. Both had a homozygous nonsense mutation in ISG15, which led to a truncated ISG15 protein that was unstable *in vitro*. We created immortalized dermal fibroblasts, HaCaT keratinocytes, and hiPSC-derived vascular endothelial cells with targeted deletions of ISG15. Transcriptomic and proteomic analyses revealed the expected hyperinflammatory phenotype, but also dysregulated expression of molecules critical for connective tissue and epidermis integrity, such as reduced collagens and adhesion molecules, but increased matrix metalloproteases. $ISG15^{-/-}$ cells exhibited elevated ROS production and reduced ROS scavenger expression. As opposed to hyperinflammation, defective collagen and integrin synthesis could not be rescued by conjugation deficient ISG15. A 3D model of epidermis formation showed markedly reduced desmosome density, loose architecture, and reduced collagen synthesis, all of which could be reversed by treatment with the Jak/Stat inhibitor ruxolitinib plus doxycycline/TGF-β1. These results reveal regulation of connective tissue and epidermis homeostasis as a novel function of ISG15 that likely requires its conjugation to yet unidentified targets.
Disclosure of Interest: None Declared

LT160d

NHR-49/PPAR-ALPHA AND HLH-30/TFEB PROMOTE C. ELEGANS HOST DEFENSE VIA A FLAVIN-CONTAINING MONOOXYGENASE

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Abstract Content: During bacterial infection, the host is confronted with multiple overlapping signals that are integrated at the organismal level to produce defensive host responses. How multiple infection signals are sensed by the host and how they elicit the transcription of host defense genes is much less understood at the whole-animal level than at the cellular level. The model organism *Caenorhabditis elegans* is known to mount transcriptional defense responses against intestinal bacterial infections that elicit overlapping starvation and infection responses, but the regulation of such responses is not well understood. Direct comparison of *C. elegans* that were starved or infected with *Staphylococcus aureus* revealed a large infection-specific transcriptional signature. This signature was almost completely abrogated by deletion of transcription factor *hlh-30/TFEB,* except for six genes including a flavin-containing monooxygenase (FMO) gene, *fmo-2/FMO5*. Deletion of *fmo-2/FMO5* severely compromised infection survival, thus identifying the first FMO with innate immunity functions in animals. Moreover, the mechanism of *fmo-2/FMO5* induction required the nuclear hormone receptor, NHR-49/PPAR-α, which induced *fmo-2/FMO5* and host defense cell non-autonomously. These findings for the first time reveal an infection-specific host response to *S. aureus,* identify HLH-30/TFEB as its main regulator, reveal that FMOs are important innate immunity effectors in animals, and identify the mechanism of FMO regulation through NHR-49/PPAR-α in *C. elegans,* with important implications for innate host defense in higher organisms.
Disclosure of Interest: None Declared

LT160e

CELL-INTRINSIC COMPLEMENT PATHWAY ENHANCES TLR4-DEPENDENT PRO-INFLAMMATORY CYTOKINE PRODUCTION AND TLR4 ENDOCYTOSIS VIA INHIBITION OF CAMP

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Abstract Content: Hyper-production of inflammatory cytokines by cells of macrophage-monocyte lineage is associated with increased severity of disease and mortality in sepsis patients. Sepsis is a deleterious immune response to infection that leads to organ failure and is the cause of over 10 million deaths annually. Currently, septic patients are treated with palliative care due to the lack of effective immune therapeutics, thus there is an urgent need to understand the cellular pathways within human macrophages that contribute to this pro-inflammatory response. The complement pathway and toll-like receptor (TLR) signaling are two cell-intrinsic innate immune pathways within macrophages/monocytes that contribute to the production of proinflammatory cytokines during sepsis. We have recently reported that membrane-bound complement receptor C3aR is required for expression of

pro-inflammatory cytokines downstream of toll-like receptor-4 (TLR4) activation during exposure to Gram-negative bacterial lipid lipopolysaccharide (LPS), in primary murine and human macrophages. Moreover, we have shown that C3aR-dependent expression of pro-inflammatory mediators is required for protection against multiple Gram-negative bacterial pathogens, and that *c3ar* is over-expressed in the blood of septic patients, suggesting clinically relevant implications for the crosstalk between C3aR and TLR4. Currently, it is not known how C3aR is enhancing TLR4 signaling and cytokine expression within macrophages. In this study, we find that C3aR is functioning as an inhibitory G protein-coupled receptor (G_i) within macrophages to inhibit production of cyclic-AMP (cAMP) during LPS stimulation. cAMP regulates intracellular signaling via multiple target proteins, including two ubiquitously expressed cAMP receptor enzymes: protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac). We found that C3aR inhibited activation of PKA, but not Epac, and this inactivation of PKA correlated with decreased expression of both NFκB (IL-6, IL-1*β*) and IRF3 (IFN*β* and Rsad2) regulated genes during LPS stimulation. It has been previously shown that increased cAMP and PKA activation results in dysregulated TLR4 trafficking and decreased inflammation in macrophages. Here we find that inhibition of C3aR signaling results in altered TLR4 endocytosis at 60-120 minutes post-LPS stimulation, similar to what was previously seen with pharmacological activation of PKA. Together, these data suggest that C3aR activation is dampening intracellular cAMP production and PKA activation, resulting in altered TLR4 endocytosis and inhibition of NFκB- and IRF3-regulated pro-inflammatory cytokine expression in macrophages during LPS stimulation. This study provides the first mechanistic insight underlying the molecular crosstalk between complement pathway and TLR signaling macrophages, and is pivotal in understanding the relationship between microbe-sensing TLRs and the complement pathway, two pro-inflammatory signaling pathways that have been implicated in regulating sepsis mortality and outcome. In future studies, it will be imperative to understand if this pathway can be therapeutically targeted in septic patients.
Disclosure of Interest: None Declared

LT160f
VIRUS-MEDIATED LOSS OF MCL-1 PROMOTES GSDME-DEPENDENT INFLAMMATORY CELL DEATH IN BARRIER EPITHELIAL CELLS
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Abstract Content: Pattern recognition receptors (PRRs) and intracellular guard proteins are critical components of the innate immune system. While PRRs recognize microbial products, guard proteins often detect virulence factor activities by the surveillance of homeostatic processes within cells. In contrast to PRRs, which are well-known for their roles in many types of infections, the role of guard proteins in most infectious contexts remains less well understood. In this study, we demonstrate that inhibition of host-protein synthesis during viral infection is sensed as a virulence strategy and initiates pyroptosis in primary human epithelial cells. We identify the highly labile Bcl-2 family member Mcl-1 as a sensor of translation shutdown. Mcl-1 depletion during infections with multiple distinct viruses stimulates caspase-dependent cleavage of gasdermin E, resulting in pyroptosis and the release of pre-formed IL-1α. Moreover, direct inhibition of Mcl-1, in the absence of infection, was sufficient to engage this pyroptotic pathway. Thus, Mcl-1 can act as a guard protein that senses the dysregulation of host-cell translation during viral infections of barrier epithelia.
Disclosure of Interest: None Declared

Lightning Talk Session 3: Cancer

LT162
PLASMA CYTOKINES PREDICT LYMPHEDEMA DEVELOPMENT IN BREAST CANCER PATIENTS 12 MONTHS BEFORE CLINICAL PRESENTATION
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Abstract Content: BACKGROUND: Lymphedema (LE) is a dreaded complication of cancer treatment, afflicting at least 40% of breast cancer survivors. Arm, trunk, and breast swelling, along with pain, depression, skin fibrosis, and cellulitis susceptibility, severely reduce quality of life. Clinically, LE is diagnosed once limb swelling exceeds 5% over baseline volume, but there is growing evidence of a “latent,” pre-swelling form of LE. LE-associated swelling can appear during chemotherapy, surgery, and/or radiation treatment, or suddenly appear years later. There is currently no cure for LE, but early palliative treatment has been shown to significantly improve outcomes, by stalling or reversing LE swelling. Standard-of-care, palliative LE treatment includes manual lymphatic drainage (specialized massage) to remove excess fluid, compression bandaging to prevent fluid buildup, pneumatic compression therapy for home use, and meticulous skin care to prevent infections. Lymphatic microsurgeries are growing in popularity to improve LE outcomes, but outcomes for these procedures are optimal only for treatment of early-stage LE. Clearly, there is a need for a tool to detect LE at the earliest, possibly “latent,” stage possible.
METHODS: Data were collected as part of an ongoing, prospective, longitudinal surveillance of 100 breast cancer patients undergoing neoadjuvant chemotherapy, axillary lymph node dissection (ALND), and radiation treatment (RT) (ClinicalTrials.gov, NCT02959726). Milliplex cytokine/chemokine levels were determined from plasma

samples that were collected from patients before ALND, after ALND but before RT, and at 6, 12, and 24 months after RT. Perometry, a method to measure arm volumes using an optoelectronic device, was performed at each of the time points, and LE was clinically diagnosed when cancer-affected-side arm volume exceeded contralateral arm volume by 5%. Data from the first 12 study subjects were analyzed for correlation between median cytokine/chemokine levels for the groups with or without clinical LE before ALND and at 12 months post-RT (Wilicoxon nonparametric test), as well as linear regression analysis of perometer/% relative limb volume change (% RVC) values and picograms/milliliter plasma cytokine/chemokine levels at 12 months post-RT.
RESULTS: Levels of several cytokines/chemokines (G-CSF, GM-CSF, fractalkine, IL-12p40, IL-15, and IL-2) were significantly elevated in plasma samples, taken at 12 months post-RT, from study subjects who developed LE at 12 months post-RT. R-squared values for correlation between plasma levels of these six cytokines/chemokines and % arm swelling were 0.53, 0.76, 0.67, 0.63, 0.50, and 0.60, respectively. Moreover, values of these cytokines/chemokines were also significantly elevated before ALND—over 12 months before clinical diagnosis of LE, in the group that developed LE by 12 months post-RT.
CONCLUSIONS: Plasma cytokine/chemokine levels of G-CSF, GM-CSF, fractalkine, IL-12p40, IL-15, and IL-2 could be used as a tool to identify breast cancer patients who are at high risk to develop LE within 12 months post-RT. This screening tool could enable early initiation of LE treatment ("pre-hab"), and greatly improve patient outcomes.
Disclosure of Interest: None Declared

LT163

IL-1B DRIVES GLIOBLASTOMA PROGRESSION IN A SEX-SPECIFIC MANNER

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Abstract Content:
Glioblastoma (GBM), the most common primary malignant brain tumor, remains uniformly lethal due to therapeutic resistance by profound immunosuppression. Males constitute 60% of GBM patients and have a worse disease outcome. However, the contribution of the differences in anti-tumor immunity to GBM sexual dimorphism has not been elucidated. We previously demonstrated that myeloid-derived suppressor cells (MDSCs) expand in GBM patients and are associated with poor prognosis. Using multiple syngeneic mouse GBM models, we investigated whether MDSC subsets promoted tumorigenesis in a sex-specific manner. Our results demonstrated that monocytic MDSCs (mMDSCs) accumulate in tumors of male mice, while female tumor-bearing mice had a two-fold increase in circulating granulocytic MDSC (gMDSC) frequency. Consistently, male GBM patients had greater immunosuppressive myeloid cell infiltration, and female patients with a high gMDSC gene signature had a worse outcome. In line with the differential MDSC subset association, targeting gMDSCs with anti-Ly6G neutralizing antibodies significantly extended the lifespan of female mice without affecting males. However, mMDSCs were protected from the anti-Ly6C depletion strategy due to their systemic and local proliferation, as indicated by ex vivo Ki-67 staining. Drug-prediction using the differential gene expression profiles of MDSC subsets and subsequent pre-clinical testing established that mMDSCs can be targeted by chemotherapies in males. In contrast, IL-1β, which was expressed at higher levels by female murine gMDSCs and correlated with poor prognosis of female patients, was identified as a drug candidate to target gMDSC activity. Neutralization of this cytokine provided a female-specific survival advantage by reducing circulating gMDSC frequency. This effect was accompanied by a decline in the number of tumor-infiltrating microglia and tumor cell proliferation rate. In vitro assessment of IL-1β inhibition resulted in reduced viability and expression of activation markers by primary microglia. These findings highlight a gMDSC-microglia communication axis mediated by IL-1β signaling as a driver of GBM in females and indicate that MDSC subset variation and the IL-1β signaling axis represent opportunities for improved immunotherapy efficacy while accounting for sex as a biological variable.
Disclosure of Interest: None Declared

LT164

HETERODIMERIC IL-15 THERAPY PROMOTES INTRATUMORAL LYMPHOCYTE AND DENDRITIC CELL ACCUMULATION BY A CYTOKINE NETWORK INVOLVING XCL1, IFN-GAMMA, CXCL9 AND CXCL10, RESULTING IN CONTROL OF PRIMARY AND METASTATIC TUMOR

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Abstract Content: Immunotherapy has emerged as a valuable strategy for the treatment of several type of cancers. The presence of tumor-infiltrating lymphocytes is considered an important predictive biomarker for clinical benefit in response to immunotherapies. Interleukin-15 (IL-

15) promotes growth and activation of cytotoxic CD8⁺T and natural killer (NK) cells. Bioactive IL-15 is produced in the body as a heterodimeric cytokine, comprising the IL-15 and IL-15 receptor alpha chains that are together termed heterodimeric IL-15 (hetIL-15). Several preclinical models have supported the anti-tumor activity of hetIL-15, and based on these results, it has advanced to clinical trials. The objectives of this study were to explore how hetIL-15 promotes lymphocyte entry into the tumor and to characterize the interactions between tumor-infiltrating lymphocytes (TILs) and other cell types through the analysis of cytokines and chemokines affected by hetIL-15 treatment. hetIL-15 treatment was effective in controlling primary tumor growth in murine cancer models, including the MC38 colon carcinoma, TC-1 cervical carcinoma, B16 melanoma models and orthotopic breast cancer models. In breast cancer models, hetIL-15 also reduced significantly metastatic disease. hetIL-15 directly affects NK, CD8⁺ and CD4⁺ T cells within the tumor promoting their proliferation (Ki67), survival (Bcl-2) and cytotoxic commitment, with high levels of IFN-γ production and upregulation of GzmA, GzmB and perforin. Activated lymphocytes secrete XCL1 in response to hetIL-15 treatment, a chemokine responsible for the enhanced intratumoral influx of cross-presenting dendritic cells (cDC1). Such cells expressing XCR-1, IRF-8 and CD103 were detected in tumors of hetIL-15-treated animals. In addition to macrophages and monocyte-derived DC, cDC1 produce CXCL9 and CXCL10 in response to hetIL-15. Importantly, hetIL-15-driven CXCL9 and CXCL10 secretion by myeloid cells required exposure to IFN-γ, indicating a positive feedback loop whereby the hetIL-15-dependent increase in intratumoral XCL-1 and IFN-γ–producing lymphocytes enhances cDC1 recruitment and amplifies the CXCL9/10-directed intratumoral infiltration by CXCR3⁺ effector lymphocytes. The role of cDC1 in hetIL-15 anti-cancer efficacy is currently being investigated in Batf3 knock-out mice, lacking cDC1.

Overall, our results show that hetIL-15 orchestrates a multipronged immune response that includes leucocyte expansion and trafficking to the tumor, lymphoid-myeloid cell interactions and enhanced cytotoxic responses through the induction of a favorable cytokine milieu. hetIL-15 therapy may be a general method to enhance T and cDC1 cell entry in tumors, increasing the success rate of immunotherapeutic interventions.

Disclosure of Interest: None Declared

LT166

PD-L1 ENHANCES RESISTANCE TO DNA DAMAGE BY REGULATING DOUBLE-EDGED RESPONSES TO TYPE I INTERFERON IN CANCER CELLS

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Abstract Content: Programmed death ligand 1 (PD-L1) on cancer cells binds to PD-1 on activated T cells, suppressing anti-cancer immunity. In this study, we investigated immune-independent functions of PD-L1, which inhibit the efficacy of radiation or chemotherapy by regulating type I interferon (IFN-I) responses in a cancer cell-intrinsic manner. As elucidated in previous studies of ourselves and others, acute stimulation with high doses of IFNβ enhances therapeutic responses, whereas chronic stimulation with low doses of IFNβ increases resistance to DNA damage by up-regulating a set of IFN-related DNA damage resistance signature (IRDS) genes.

We find that the expression of PD-L1 and IRDS genes, including OAS1, OAS2, Mx1, IFIT1, and IFIT3, is concurrently elevated in a subset of cancer cells of various origin, including breast, colon, lung, and kidney. Using an *in vitro* model in which only cancer cells are present, we found that lung cancer cells expressing high levels of PD-L1 and IRDS proteins are highly resistant to cisplatin or ionizing radiation, and that knock-down of PD-L1 re-sensitizes these cells. Since IRDS genes are induced by IFN-I, we examined cancer-cell intrinsic IFN-I responses in the cancer cells, finding that the cGAS-STING-IFNβ pathway is constitutively activated. When we knocked down cGAS or the IFN-I receptor (IFNAR1), the expression of PD-L1 as well as IRDS proteins was substantially decreased, suggesting that cancer cell-intrinsic chronic IFN-I responses are responsible for high levels of PD-L1 and IRDS expression in the cancer cells. Interestingly, knock-down of PD-L1, which sensitizes the cancer cells to DNA damage, substantially decreased IRDS expression, indicating that PD-L1 makes cancer cells resistant to DNA damage by enhancing chronic IFN-I responses, which induce IRDS expression. On the other hand, acute responses to high levels of IFN-I are themselves cytotoxic, and also a new synthesis of IFN-I in cancer cells increases the cytotoxicity of radiation or chemotherapeutic agents. We found that PD-L1 inhibits the acute IFN-I responses, as shown by our observation that PD-L1 knock-down dramatically increases the phosphorylation of STAT1 and STAT2 in response to high doses of IFN-I. Taken together, this study reveals that the resistance of a subset of cancer cells to DNA damage is due to the ability of PD-L1 to regulate IFN-I responses by sustaining chronic IFN-I responses that induce IRDS expression and by inhibiting cytotoxic acute IFN-I responses.

Immune checkpoint blockade, including anti-PD-L1, is a landmark advance for treating advanced cancers but is not effective in patients who have poor pre-existing immunity. This study elucidating the immune-independent roles of PD-L1 in cancer therapy will help to direct therapeutic strategies using PD-L1 inhibitors to improve their response rates, regardless of patients' pre-existing immune status.

Disclosure of Interest: None Declared

LT167

THE AIM2 DNA SENSOR PROMOTES STAT3-DRIVEN TUMORIGENESIS INDEPENDENT OF INFLAMMASOMES VIA EPITHELIAL CELL MIGRATION

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Abstract Content: The link between chronic gastric inflammation – caused by over-activation of the immune system – and gastric cancer (GC) is well defined, however the identity of key regulators of the immune system that promote GC is not well understood. The pattern recognition receptor (PRR) absent in melanoma 2 (AIM2) is a cytosolic DNA sensor which contributes to the pathogenesis of numerous autoimmune and chronic inflammatory diseases in the context of inflammasome complexes. AIM2 is also linked with cancers, although its roles have been reported to be tissue-specific and often ill-defined. Considering the role of AIM2 in innate immune responses, we investigated whether it contributed to the pathogenesis of GC.

AIM2 expression at the mRNA and protein levels was significantly upregulated in tumours of GC patients, specifically in the epithelium, and strongly correlated with patient survival. Using the well-established *gp130*$^{F/F}$ mouse model of GC, we also observed high expression of AIM2 in tumour-bearing mice compared to their wild-type counterparts. The coupling of the *gp130*$^{F/F}$ mouse model with mice lacking AIM2 significantly decreased tumour burden, thus supporting the pro-tumourigenic role of AIM2 in GC. Interestingly, our data showed that while gastric tumourigenesis was suppressed, inflammation or activation of the inflammasome was unaffected, suggesting the existence of a novel inflammasome-independent pro-tumourigenic mechanism for AIM2 in GC.

Specifically, we identified a decrease in the migration of AIM2-deficient primary mouse gastric epithelial cells and, conversely, an increase in migration of AIM2-overexpressing human GC MKN-1 cells, measured by *in vitro* 3D migration transwell and spheroid assays. This which was accompanied by cellular morphological differences, alterations in actin cytoskeleton polymerisation, increased filopodia formation and differences in adhesion gene expression. These data support changes in epithelial cell migration and motility as the underlying mechanism of the pro-tumourigenic role of AIM2 in GC. A strategy to identify potential AIM2-interacting partners that mediate the pro-migratory function of AIM2 will also be discussed.

Taken together, this work uncovers a novel, inflammasome-independent pathogenic mechanism for AIM2 in GC 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.
Disclosure of Interest: None Declared

LT168
A NEW MECHANISM FOR PROINFLAMMATORY CYTOKINE-PROMOTED EXTRACELLULAR MATRIX REMODELING IN BREAST CANCER
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Abstract Content: Invasive ductal carcinoma (IDC) is a serious problem for patients as it metastasizes, decreasing 5-year patient survival from >95% to ~27%. The breast tumor microenvironment (TME) is often saturated with proinflammatory cytokines, such as oncostatin M (OSM), which are secreted from tumor-activated neutrophils and macrophages and whose expression is correlated with increased breast cancer metastasis. Remodeling the extracellular matrix (ECM) of the TME plays an important role in promoting invasive and metastatic potential of IDC. Specifically, the reorganization and alignment of collagen fibers in stromal ECM leads to increased tumor cell motility, which promotes metastasis. Lysyl oxidase like-2 (LOXL2) is a protein that catalyzes ECM remodeling by crosslinking of collagen I in the ECM. We propose a novel mechanism whereby OSM induces LOXL2 expression, mediating stromal ECM remodeling of the breast TME. Our studies demonstrate that IDC patients with high LOXL2 and OSM co-expression have a worse prognosis than those with high levels of each, individually, and LOXL2 expression is positively correlated to OSM/ OSM receptor (OSMR) expression in IDC patients. Furthermore, human IDC cells treated with OSM results in a significant increase in secreted, enzymatically active LOXL2. OSM-induced LOXL2 promotes significantly increased ECM collagen I fiber crosslinking and alignment. Taken together, these results provide a new paradigm through which proinflammatory cytokine OSM promotes tumor progression, by promoting ECM remodeling in the TME.
Disclosure of Interest: None Declared

LT169
IL33 CYTOKINE SIGNALLING IN GASTROINTESTINAL CANCERS - A THERAPY TARGET?
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Abstract Content: Cytokine-mediated inflammation is a driver of gastric and colonic tumorigenesis. IL1-family cytokine Interleukin 33 (IL33) regulates inflammatory responses and antibodies targeting IL33 or its receptor ST2 are in clinical trials against various inflammatory and allergy diseases. Only recently, a role of IL33 in cancer started to emerge. Depending on the cancer stage or type IL33 can provoke either pro- or anti-tumoral responses [1].
To evaluate the potential of targeting IL33 signalling to abrogate gastric and colonic cancer growth, we employ *gp130*$^{Y757F/\ Y757F}$ (FF) gastric cancer mice and the 6xAOM sporadic colon cancer model as well subcutaneously engrafted MC38 colon cancer models. IL33 signalling inhibition was achieved via genetic deficiency (*St2*$^{-/-}$), while recombinant IL33 was administered to hyper activate the IL33-signalling pathway.
We found, that IL33 cytokine and ST2 receptor are overexpressed in human gastric cancers and high ST2 expression predicts poor survival for gastric cancer patients. In accordance, IL33 and ST2 are highly elevated in our FF mouse tumors. Deficiency of IL33 signalling (ST2$^{-/-}$) diminishes gastric tumour growth, and is associated with a decrease in tumour-adjacent pro-tumoral mast cells and

tumour-infiltrating macrophages (TAM) as well as reduced angiogenesis. Furthermore, ST2-deficiency potentiates the anti-tumor effects of oxaliplatin chemotherapy in the FF mouse model. Mechanistically, we show that tumour-produced IL33 can activate mast cells, which in turn recruit pro-tumoral and pro-angiogenic macrophages to the tumour through release of chemo-attractants like Ccl2, Ccl3 and Ccl7 [2].

In the colon cancer setting, ST2-deficiency led to increased tumour burden in 6xAOM sporadic colorectal cancer. Reciprocal bone marrow chimera experiments indicated that the radio-resistant non-hematopoietic cell compartment contributes to the increased tumour growth. Indeed, we found St2 expression in the mesenchymal cells of the tumor microenvironment. Loss of IL33 signalling in the non-haematopoietic radio-resistant compartment coincided with a strong reduction of an IFNy gene expression signature. Importantly, IL33 cytokine administration reduced colon cancer allograft growth associated with tumour-infiltrating IFNy-positive T cells [3].

Our data demonstrates the opposing roles of IL33 signalling in the growth of gastric and colonic cancers and identifies cellular mediators of these divergent tumor responses. Further studies are warranted to stratify IL33-signalling sensitive GI cancers and to verify the potential of anti-IL33/ST2 antibodies against gastric cancer and activating IL33 cytokine administration in colon cancers as novel therapy strategies.

[1] Eissmann MF, *et al.* IL33 and Mast Cells—The Key Regulators of Immune Responses in Gastrointestinal Cancers? Front. Immunol. 2020;11:1389. doi: 10.3389/fimmu.2020.01389

[2] Eissmann MF, *et al*. IL-33-mediated mast cell activation promotes gastric cancer through macrophage mobilization. *Nature Communications* 2019 Jun; 21;10(1):2735

[3] Eissmann MF, *et al*. Interleukin 33 Signaling Restrains Sporadic Colon Cancer in an Interferon-y-Dependent Manner. *Cancer Immunol Res* 2018 April; 6(4):409-421.

Disclosure of Interest: None Declared

LT171

CURRENT UNDERSTANDINGS ON HOW AN INFLAMMATORY TUMOR MICROENVIRONMENT PROMOTES INVASION AND EARLY STAGE BREAST CANCER METASTASIS

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Abstract Content: The propensity of primary breast cancer to invade and metastasize has been partially explained by high levels of endogenous inflammation. However, it is not fully understood how inflammatory mediators, expressed either by the primary breast cancer cells or cells of the tumor microenvironment (TME), specifically contribute to metastasis. Our lab has been studying how oncostatin M (OSM), an interleukin-6 (IL-6)-family inflammatory cytokine, promotes the development of early metastatic properties *distinct* from IL-6 and other family members. Human breast tissue microarray analysis shows that OSM is expressed at highest levels in the precancerous epithelial cells of ductal carcinoma *in situ* (DCIS), suggesting a role for autocrine-produced OSM in invasive potential. Tumor-associated neutrophils (TANs) release large amounts of OSM when they come into contact with breast cancer cells, and this paracrine-produced OSM induces invasive capacity. Employing human breast and mouse mammary orthotopic xenograft and syngeneic mouse models, OSM promotes metastasis to bone, lung, and other organs. Furthermore, OSM increases circulating tumor cell (CTC) numbers, which are reduced in an OSM knockout background, demonstrating the importance of both paracrine- and autocrine-produced OSM in this process. When supported by *in vitro* results, where OSM remains active when it binds to extracellular matrix (ECM) proteins and induces tumor cell-produced protease secretion and other ECM-modifying enzymes, our findings suggest a role for OSM in early stage metastasis. Finally, the importance of OSM relative to IL-6 in these processes will be discussed, and as anti-IL-6 cancer therapies (siltuximab) are failing in clinical trials, our results suggest that OSM may be a more rational target. Collectively, these data suggest that OSM, whether TME- or autocrine-produced, is a critical factor driving breast cancer invasion and tumor cell dissemination with subsequent metastasis.

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Disclosure of Interest: None Declared

LT173

COMPLETE REGRESSION OF MURINE BREAST TUMORS AND LONG-TERM ANTI-TUMOR IMMUNITY BY HETIL-15 MONOTHERAPY IS MEDIATED THROUGH THE INTERACTION OF T, NK, CDC1 CELLS AND A NOVEL POPULATION OF DENDRITIC CELLS

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Abstract Content: Background and Hypotheses: IL-15 is a cytokine which stimulates the proliferation and cytotoxic function of $CD8^+$ T and NK cells. We have produced the native heterodimeric IL-15 (hetIL-15) of human, macaque or mouse origin. The objectives of the study were to explore the mechanisms of anti-tumor effects of hetIL-15 after locoregional administration in orthotopic breast cancer models and to characterize the interactions between tumor-infiltrating lymphoid and myeloid cells.

Study Design and Methods: We studied the therapeutic efficacy of hetIL-15 in the murine EO771 orthotopic breast cancer model in syngeneic C57BL/6 mice. The effects of hetIL-15 on immune cells were analyzed by flow cytometry, immunohistochemistry (IHC) and gene expression profiling. We also assessed the metabolic profile of tumor infiltrating T cells.

Results and Conclusions: hetIL-15 peritumoral administration resulted in complete regression in 40% of the treated animals and increased survival. Subsequent rechallenges with the same cell line failed to generate tumors. Therefore, locoregional hetIL-15 administration induced long term immunological memory. $CD8^+$ T and NK cells were found increased in hetIL-15 treated tumors and showed enhanced activation and proliferation. Transcriptomic analysis confirmed the activated state of the T and NK cells, whereas metabolic flux analysis of the tumor infiltrated CD8+T cells from treated mice confirmed a rise in oxygen consumption rate (OCR) with substantial increase of spare respiratory capacity. In addition, peritumoral hetIL-15 administration resulted in an increase of infiltrating, conventional type 1 dendritic cells (cDC1s), suggesting mechanisms of DC-lymphocyte interactions in the tumor. We also observed a new population of intratumoral DCs found only in the hetIL-15 treated animals. The cDC1s and the new DC population were inversely correlated with the tumor size. Phenotypic profile of the new DC population showed expression of several cDC1 specific markers, including CD103 and IRF8. RNAseq analysis of the sorted DC populations derived from the treated tumors, verified unique clustering of this new DC population.

Therefore, locoregional administration of hetIL-15 results in complete eradication of EO771 primary breast cancer tumors, prolonged survival and long-lasting specific anti-tumor immunity. The anti-cancer activity of hetIL-15 in primary EO771 tumors is orchestrated by the interplay of NK, CD8+T cells, cDC1 and a novel subset of antigen presenting cells with a distinct phenotypic profile.

Disclosure of Interest: None Declared

LT175

IL-35 SIGNALING PROMOTES REGULATORY B CELL GENERATION AND TUMOR ACCUMULATION BY ENHANCING STAT1/STAT3 AXIS

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Abstract Content: IL-35 signaling promotes regulatory B cell generation and tumor accumulation by enhancing STAT1/STAT3 axis

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Abstract

Transcription factors guided by interaction of signals through cytokine and toll-like receptors are critical for B cell differentiation. We recently showed IL-35, a novel immune-regulatory cytokine produced by B cells suppress anti-tumor T cell response and promotes pathogenesis of pancreatic cancer. Previous studies have shown that STAT3/STAT4 heterodimer is linked to IL-35 downstream signaling in T cells. However, the exact role of IL-35 and its downstream signaling pathways in B cells that can induce immune suppressive Breg cell differentiation has not been fully understood. In this study, we showed that IL-35 induced STAT1/STAT3 signaling is essential for generation of Breg cells from naïve B precursors and their tumor accumulation. Increased IL-35 and IL-10 production and altered suppressive activity were found in pancreatic cancer educated B cells. IL-35 strongly induces *p35, EBi3, IL-10* and *CD1d* gene expression in pancreatic cancer educated naïve B cells as well as promotes their tumor infiltration. Chromatin immunoprecipitation confirms Breg cell suppressive genes *p35*, *EBi3*, *IL-10* and *CD1d* are the direct target of STAT1/STAT3 in IL-35 dependent manner. Our results demonstrate that IL-35, via STAT1/STAT3, induces generation of immunosuppressive Breg phenotype and maintenance in tumor microenvironment. In sum, these studies define new insight into the specific role of STAT1/STAT3 signaling particularly in the Breg cell lineage differentiation and identify STAT1/STAT3 axis as a novel target for treatment of B cell dependent deadly cancers like pancreatic cancer.

Disclosure of Interest: None Declared

LT176

CHARACTERIZATION OF IL-15 DEFICIENT THYMOCYTES IN THE MODEL OF SPONTANEOUS LEUKEMIA

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Abstract Content: Leukemia is a diverse group of hematopoietic malignancies that cause significant morbidity and mortality in children and adults. T cell acute lymphoblastic leukemia (T-ALL) is an aggressive tumor that accounts for 10-15% of pediatric ALL. Even though intensive chemotherapy can cure 80% of pediatric cases, chemo-resistant and relapse cases have poor prognosis. Treatment of such cases requires the development of new approaches through better understanding of the molecular mechanisms of leukemogenesis.

We reported that NOD.Scid (NS) mice lacking either interleukin-15 NOD.Scid.Il15-/- or IL-15 receptor alpha chain NOD.Scid.Il15ra-/- spontaneously develop T cell leukemia with 100% penetrance by 30 weeks of age. Leukemic cell

lines established from NOD.Scid.Il15-/- mice express the T-ALL marker TdT, display constitutive NOTCH1 activation and are sensitive to NOTCH inhibition. Depletion of NK cells did not promote the development of leukemia in NOD.Scid mice, indicating NK-independent role for endogenous IL-15 in preventing the survival and outgrowth of leukemic cell precursors. Analysis of thymi from 4 wks-old NOD.Scid.Il15-/- mice show discernible changes in the phenotype of DN thymocytes compared to those of control mice. Whereas NOD.Scid mice showed developmental arrest at the DN3 stage, substantial numbers of DN4, DP and SP cells were observed in NOD.Scid.Il15-/- thymi.
These findings strongly suggest that IL-15 signaling within the thymus controls the emergence of leukemogenic precursors from developing thymocytes.
Disclosure of Interest: None Declared

LT177

NEWCASTLE DISEASE VIRUS (NDV) ONCOLYTIC ACTIVITY IN HUMAN GLIOMA TUMORS IS DEPENDENT ON TYPE I IFN GENE CLUSTER CODELETION.

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Abstract Content: Glioblastoma (GBM) is the most devastating and least treatable primary brain tumor in adults with a median overall survival of 15 months. Some of the most common and relevant mutations in gliomas are the deletions at Ch9p21, which involves the genetic loss of the *CDKN2A*. We have evaluated the genetic loss of the *type I IFN* locus in 1018 glioma tumors and the NDV oncolytic effect in six GBM cancer stem cells (CSCs). Human GBM *CDKN2A* (p16) deletion represents 57% homozygous deletions and 19% hemizygous detentions. Next to it, complete loss of the *type I Interferon (IFN)* gene cluster, represent 1/3 of all the tumors that have lost CDKN2A. Newcastle disease virus (NDV), an avian paramyxovirus, has been proposed for the treatment of different tumors, including GBM, due to its oncolytic and immunostimulatory properties. *IFN* cluster deletion implies the loss of an efficient antiviral response. The secretion of *type I IFNs* that triggers an antiviral response blocking virus replication. GBM susceptibility to NDV is dependent on the loss of the *type I IFN*. A recombinant NDV bearing the NS1 gene (rNDV-NS1), a strong antagonist of the *IFN* response, could exert higher oncolytic activity in the type I IFN-competent CSCs. GBM patient stratification, evaluating type-I *IFN* competence, could predict NDV and other oncolytic therapies.
Disclosure of Interest: None Declared

LT178

INTRACELLULAR EXPRESSION OF IFN-λ4 LEADS TO ER STRESS, ENHANCED IRF1 SIGNALING AND DECREASED PROLIFERATION IN HEPATIC CELLS THAT MIGHT PROTECT HCV+ PATIENTS FROM LIVER CIRRHOSIS

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Abstract Content: Several genetic variants in the human *IFNL3/IFNL4* locus have been associated with reduced hepatic fibrosis despite poor clearance of HCV infection. We expanded this analysis to cirrhosis, a more advanced stage of fibrosis. In 2931 individuals with chronic HCV, the *IFNL4* genotype that generates IFN-l4 was associated with protection from cirrhosis (OR=0.65, p=0.012, adjusted for age and sex). The *IFNL4* genotype affects the production of IFN-l4 and, additionally, may affect expression levels of IFN-l3, making it difficult to delineate the individual contribution of these IFNs. To address this, we established HepG2-based cell models engineered to inducibly express either IFN-l3 or IFN-l4. Using RNA-seq based expression profile generated in these hepatic cell lines, we explored the global transcriptome of liver tumors (n=373) from The Cancer Genome Atlas to identify transcription factor networks affected by these IFNs in the liver. Several networks, including of the IRF1, were upregulated by IFN-l4 more strongly than by IFN-l3. Intracellular expression of IFN-l4 but not of IFN-l3 also led to potent IRF1-dependent antiproliferative effects. Live cell imaging revealed that IFN-

l4 was poorly secreted, mainly accumulated in lysosomes, and caused apoptosis, suggesting increased ER-stress via the misfolded protein response. Knockdown of DNA damage-inducible transcript 3 (*DDIT3*), an ER-stress response effector, significantly attenuated the antiproliferative effects of IFN-l4. This novel interplay of enhanced IRF1 signaling coupled with intracellular accumulation and induction of ER stress by IFN-l4 may have complex consequences on liver homeostasis during chronic HCV infection but also mediate anti-cirrhotic phenotypes.
Disclosure of Interest: None Declared

LT179

COMBINING PD-1/PD-L1 BLOCKADE AND RANKL INHIBITORS TO TREAT BREAST CANCERS UNRESPONSIVE TO STANDARD THERAPY

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Abstract Content: Background: In the past decade, immunotherapy using immune checkpoint inhibitors (especially targeting the PD-1/PD-L1 axis) has been demonstrated as a promising strategy for the treatment of cancers that do not respond to classical chemoradiotherapy. Given that cancer cells have the potential to express many immunosuppressive molecules other than PD-L1, the combination of immune checkpoint inhibitors with other drugs thwarting tumor immunosuppressive microenvironment could represent a promising strategy. Among these immunosuppressive molecules, RANKL, a member of the TNF superfamily, which mainly affects the immune system and bone remodeling, has been shown to be a key factor promoting the progression of breast cancer. In addition, RANKL induces the formation of tolerogenic dendritic cells and Treg cells, which promotes immunotolerance to the tumor.
The aim of this research project is to study the impact of several RANKL inhibitors on triple negative breast cancer and to analyze the efficiency of their association with anti-PD-1/PD-L1 agents.
Material and methods: We studied RANKL and PD-L1 expression in several murine and human breast cancer cell lines by immunohistochemistry. The secretion of RANKL was analyzed by ELISA. Inhibitors of RANKL were then tested *in vitro*. We selected several RANKL inhibitors: anti-RANKL antibody, RANK-Fc, Isoliquiritigenin and Gallocatechin gallate. The efficacy of these inhibitors was indirectly evaluated with the murine macrophage RAW264.7 cell line which undergoes, in the presence of RANKL, an osteoclast differentiation (TRAP and Cathepsin K expression). The efficacy of RANKL inhibitors was then evaluated, in this model, by RT-qPCR. Apoptosis, proliferation and mitochondrial respiration of the cancer cell lines in the presence of the inhibitors were also analyzed. RANKL inhibitors were then tested *in vivo* in a mice model. Tumor growth was followed and the infiltration of tumor microenvironment by different immune cell populations were analyzed by flow cytometry.
Results: RANKL/PD-L1 expression profile on specimens from each breast cancer subtypes showed that both immunosuppressive molecules are expressed by all breast cancers with a significantly more intense immunoreactivity for triple negative breast cancers. Most of the cell lines expressed both proteins. We found that RANKL is secreted in their extracellular environment. RANKL inhibitors are efficient *in vitro* and are currently been tested *in vivo*. First results highlight a decreased tumor growth in the presence of RANKL inhibitors. Moreover, an increase in TCD4+ and TCD8+ cells and a decrease in granulocytes and M2 macrophages have been observed in the different immune cell populations infiltrating the tumor.
Conclusions: Several other murine triple negative breast cancer cell lines will also be sub-cutaneously injected in mice and the efficacy of both RANKL and PD-L1 inhibitors will be evaluated (separately or in combination). The infiltration of tumor microenvironment by different immune cell populations, the presence of metastasis and the tumor growth will be analyzed.
Disclosure of Interest: None Declared

LT180

DONOR IFNL4 GENOTYPE PREDICTS NON-RELAPSE MORTALITY AFTER UNRELATED DONOR MYELOABLATIVE HEMATOPOIETIC CELL TRANSPLANTATION FOR ACUTE LEUKEMIA

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Abstract Content: Background. *IFNL4* genotype regulates the immune response by controlling the production of IFN-λ4, a type III interferon. We evaluated the association between donor and recipient *IFNL4* genotype with survival in patients with acute leukemia who received myeloablative 10/10 HLA-matched hematopoietic cell transplant (HCT).
Methods. We conducted a two-stage cohort study: in a discovery set of 404 patients we genotyped *IFNL4* polymorphisms (rs368234815, rs12979860, and rs117648444) with TaqMan assays; and in an independent validation of 1245 patients from the DISCOVeRY-BMT study we used existing Illumina array genotype data. Pre-HCT blood samples and outcome data were provided by the Center for International Blood and Marrow Transplant Research.
Findings. *IFNL4-positive* genotype of donor but not recipient was associated with increased risk of non-relapse

mortality (NRM; HR-per allele=1.60, 95% CI=1.23-2.10, p=0.0005, and HR=1.22, 95% CI=1.05-1.40, p=0.007, in the discovery and validation, respectively). The effect was driven by excess risk of deaths from infections and graft-*versus*-host disease (HR-combined sets=1.54, 95% CI=1.16-2.04, p=0.003, and 1.46, 95% CI=1.11-1.94, p=0.008), respectively. An association between donor *IFNL4-positive* genotype and patient post-HCT overall survival (OS) was also noted (HR-combined sets=1.11, 95% CI=1.02-1.22, p=0.02). Single-cell RNA-sequencing analysis identified *IFNL4* as the main interferon transcript expressed in bone marrow cells, detected in 0.67% of all cells and 83.1% of all interferon-expressing cells.
Interpretation. Prioritizing HCT donors with *IFNL4*-Null genotype may decrease the risk of NRM and improve OS without significantly limiting the candidate donor pool.
Disclosure of Interest: None Declared

LT180b
ERK IS INVOLVED IN STEROID-LIKE CUCURBITACIN D-INDUCED ANTITUMOR EFFECTS ON ATL CELLS
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Abstract Content: Adult T cell leukemia (ATL) is an aggressive andmalignant blood disease. We previously reported that steroid-structured cucurbitacin D (CuD) induces apoptosis in ATL cells from patients and cell lines. In this study, we investigated the effects of mitogen-activated protein kinase (MAPK) signaling inhibitors on CuD-induced cell death in peripheral blood lymphocytes (PBLs) isolated from ATL/ acute lymphoblastic leukemia (ALL) patients and ATL cell lines (MT-1 and MT-4). Cell surface markers were examined using flow cytometry. Serum cytokine levels were estimated using LEGENDplex. Cell proliferation was assessed using the Cell Titer-Glo luminescent cell viability assay. Protein expression was determined by western blotting. PBLs from patients highly expressed CD4 and CD5. Serum from the patient contained high levels of interleukin (IL)-8, IL-10, IL-18, and IFN-γ compared to the healthy donor. CuD-induced cell death was enhanced by the mitogen-activated protein kinase kinase (MEK)1/2 inhibitor U0126. However, a c-Jun N-terminal kinase (JNK) inhibitor prevented CuD-induced cell death. Immunoblot analyses revealed that CuD reduced the phosphorylation of ERK, p38, and JNK, and co-treatment with CuD and U0126 did not affect the phosphorylation of ERK. MEK1/2 and p38 inhibitors enhanced CuD-induced cell death, and U0126 enhanced the CuD-induced de-phosphorylation of ERK in MT-1 and MT-4 cells. We conclude that CuD reduces ERK activation, resulting in enhanced antitumor effects on ATL/ALL cells.
Disclosure of Interest: None Declared

Lightning Talk Session 3: Innate Immunity III

LT181
A NETWORK OF CYTOPLASMIC AND NUCLEAR LONG NON-CODING RNAS REGULATES HUMAN MACROPHAGE IMMUNITY
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Abstract Content: The activity of mammalian phagocytes is controlled at several levels by regulatory RNAs, which ensure the correct timing of soluble inflammatory mediator production. While the fine-tuning activities of microRNAs in this process are well-understood, the mechanistic implications of long non-coding RNAs (lncRNAs) are only beginning to emerge. We previously determined the global association of TLR-inducible lncRNAs with macrophage protein machines by combining subcellular fractionation with density gradient centrifugation and RNA-seq and found that lncRNAs in LPS-activated macrophages are often localized in the cytoplasm and interact with ribosomes. Here, we selected from the non-ribosome-associated lncRNAs the top 10 LPS-inducible transcripts in blood-derived and tissue-resident human macrophages for a small-scale CRISPR interference screen. Loss-of-function phenotypes of the tested lncRNAs ranged from imbalances in the TLR-NFκB and JAK-STAT signalling pathways to changes in macrophage differentiation programs. Mechanistically, LPS-inducible lncRNAs interacted with cytoplasmic TLR signalling components, alternative splice factors and membraneless nuclear organelles to regulate pro-inflammatory macrophage activity. Our data suggest that alongside potentially translated novel LPS-responsive RNAs, true long non-coding RNAs constitute an important pillar of the cytoplasmic and nuclear networks involved in the adequate activation of human macrophage immunity.
Disclosure of Interest: None Declared

LT182
GLUTEN CONSUMPTION MAINTAINS VIRAL INDUCED TYPE I INTERFERON PATHWAY THROUGH AN INCREASE OF M6A LEVELS
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Abstract Content:
Celiac disease (CeD) is a complex autoimmune disorder in which gliadin from gluten is the known triggering agent and where both the innate and the adaptive immune responses are implicated. While adaptive immune mechanisms have been deeply studied, the role of innate immunity in the development of the disease remains mainly unclear. Among other environmental factors, enteroviral infections have been linked to the risk of developing the disease. The type I interferon (IFN-I) pathway, which is upregulated in CeD patients, is activated upon viral infections, being an important innate immune response mechanism. It has been described that m6A methylated RNAs are able to control the

innate immune response to infections by targeting IFN-Is and have also been involved in the development of autoimmunity.
We hypothesized that gluten consumption can reactivate the IFN-I pathway in patients that have been previously infected by enteric virus, and that this may be regulated by an m6A-dependent mechanism. To test this hypothesis, we used polyinosinic-polycytidylic acid (PIC) treatment as a viral mimic in combination with gluten stimulations in HCT15 intestinal cells. As expected, PIC treatment induced the IRF7-IFNB pathway, together with downregulation of m6A eraser ALKBH5 and an overall increase in m6A levels after 24h. We then combined PIC and gliadin stimulations to evaluate the effect of gluten on the m6A machinery and IFN-I pathway after a mimic viral infection. We observed a synergistic effect, since both ALKBH5 decrease and IRF7 induction were augmented in cells treated with both agents. METTL3 expression was also increased by gliadin stimulation after PIC treatment. In addition, ALKBH5 silencing and METTL3 overexpression increased IRF7 expression and downstream genes STAT1 and CXCL10. Methylation of IRF7 mRNA was confirmed by m6-RIP-qPCR, suggesting that the induction observed after the combination of viral mimic and gliadin stimulation might be, at least partially, mediated by an m6A increase. Likewise, we observed that total m6A levels together with some m6A machinery genes are higher in biopsies from CeD patients. Besides, IRF7, STAT1 and CXCL10 are constitutively upregulated in celiac patients.
In conclusion, our results suggest that in the context of viral infection, gliadin consumption may lead to an autoimmune response by the alteration of m6A machinery and the induction of innate immunity pathways.
Disclosure of Interest: None Declared

LT183

ROLE OF MICRORNA-24-3P IN THE REGULATION OF HERPES SIMPLEX VIRUS-1 INDUCED STING SIGNALLING

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Abstract Content: The type I interferon system is the first line of cellular antiviral innate immune response; virus infection is recognized by various pattern recognition receptors in the infected cell and it activates the interferon system to inhibit virus replication. However, viruses have evolved various mechanisms to reduce the cellular immune response and enhance viral replication. For Herpes Simplex Virus-1, the cGAS/STING pathway is the principal route of interferon induction; we report here a new mechanism by which HSV1 evades this process by inducing a microRNA that inhibits STING expression. Bioinformatics analysis revealed that a cellular miRNA, miR-24-3p, can target the 3'UTR of STING mRNA and experimental manipulation of its cellular level confirmed that it regulates STING expression. HSV1 stimulated miR-24 synthesis through activation of the Erk1/2 and JNK MAP kinases; consequently, in the infected cell, miR-24-3p level was higher and STING level was lower. The physiological relevance of this phenomenon was confirmed by inhibiting the action of Erk1/2 and JNK kinases: in the presence of MAPK inhibitors, mir-24-3p was not induced, STING level was unaltered and HSV1 replicated less efficiently. *In Vivo* mouse studies revealed reduced HSV-1 replication and alleviated disease symptoms upon silencing of miR-24-3p in mice brain. Silencing of miR-24 enhanced IFN-β driven antiviral response against HSV-1 infection in mice. Thus, our experimental findings identified a new pathway by which HSV-1 can evade antiviral immune responses by inhibiting STING mRNA translation.
Disclosure of Interest: None Declared

LT184

ADAPTIVE CHANGES IN HIV-1 ENVELOPE RESULTING FROM IN VIVO ADAPTATION OF SHIVS CONFERS RESISTANCE TO INTERFERON

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Abstract Content: HIV-1 does not persistently infect macaques due to restriction by several type-I interferon (IFN)-induced host factors. To overcome these restrictions chimeric SIV/HIV-1 viruses (SHIVs), which encode the SIV antagonists of the known restriction factors and HIV-1 Envelope glycoprotein (Env), are used to infect macaques to model HIV-1 infection. Existing SHIV/macaque models typically employ SHIVs that encode HIV-1 sequences from viruses amplified in culture that were further adapted in macaques (adapted SHIVs). Development of SHIVs encoding HIV-1 variants derived directly from infected humans without adaptation (unadapted SHIVs) has been challenging as these SHIVs replicate poorly in macaque cells. Our prior studies demonstrated that in contrast to the adapted SHIVs, the unadapted SHIVs have lower replication kinetics in macaque lymphocytes and are sensitive to IFN. The HIV-1 *envelope* gene was defined as the major determinant of both replication and sensitivity to the IFN response in macaque lymphocytes. However, these studies did not directly examine the determinants of IFN adaptation in SHIVs that were adapted through serial macaque-passage.
In order to identify the viral determinant(s) of macaque-passaged SHIVs that confer resistance to IFN *in vivo,* we took advantage of our previous observations, which showed that the serial macaque-passage of a subtype C SHIV encoding a recently transmitted strain of HIV-1 led to a more IFN-resistant virus population. We generated SHIV infectious molecular clones (IMCs) encoding the parental subtype C *env* and representative *env* clones from the sequential macaque-passaged viruses. Importantly, these IMCs are isogenic in the SIV portion of the genome and differ only in HIV-1 *env* sequences. We found that the unpassaged, parental SHIV IMC is potently inhibited by IFN (mean IC_{50} range 1.9 to 54.6 U/ml), whereas the SHIV IMCs encoding macaque-passaged *envs* are resistant to IFN inhibition (mean IC_{50} >5000 U/ml), consistent with the studies of the adapted SHIV quasispecies. In addition, we

found that SHIV IMCs encoding macaque-passaged *envs* have high replication capacity and most, but not all, have more virion Env content. Next, we took a gain-of-function approach and generated chimeras that introduce portions of *env* gene from IFN-resistant SHIV IMC into the parental IFN-sensitive SHIV IMC. Using this approach, we mapped the determinant of IFN resistance and high replication capacity to the gp120 subunit of HIV-1 Env.
In conclusion, our results indicate that the adaptive changes in the gp120 subunit of HIV-1 *env* resulting from the *in vivo* adaptation of SHIVs via serial macaque-passage are sufficient to increase resistance to IFN replication capacity, and virion Env content.
Disclosure of Interest: None Declared

LT185
RAPID REMODELING OF POISED CHROMATIN LANDSCAPES AND TRANSCRIPTION FACTOR REPURPOSING FACILITATE GENE INDUCTION IN NATURAL KILLER CELLS
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Abstract Content: Innate immune responses rely on rapid and precise orchestration of gene regulation mediated by accessibility of regulatory regions to key transcription factors (TFs). Enhancers, whose competence, but not potency, is established during cell lineage acquisition, are promptly engaged to mount acute responses to pathogens. However, the extent to which acute activation alters DNA-accessibility and the roles of distinct classes of TFs in this process are unsatisfactorily understood. Here, we investigated epigenomic modifications and transcriptomic changes occurring upon activation of natural killer (NK) cells. In addition to developmentally primed enhancers, rapid, high-level gene induction was regulated by *de novo* chromatin accessibility and enhancer remodeling. Activation resulted in redeployment of a lineage-specific TF to *de novo* enhancers, independent of its DNA sequence-specific motif recognition. Thus, in NK cells, acute signaling shapes enhancer function through the combinatorial usage and repurposing of both lineage-determining and signal-regulated TFs to ensure a rapid innate immune response.

Disclosure of Interest: None Declared

LT186
MITOCHONDRIAL SLC25A13 INTERACTS WITH NLRP3 AND REGULATES INFLAMMASOME ACTIVITY
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Abstract Content: The NLRP3 protein is a key initiator of inflammation in humans. NLRP3 becomes activated by a multitude of danger signals, including microbial infection, metabolic dysfunction, and cell-internalized particulates. Upon activation, NLRP3 nucleates formation of a multiprotein complex called the inflammasome, in which caspase-1 activity mediates processing of the pro-inflammatory cytokines IL-1β and IL-18 and induces pyroptosis. This process drives a pro-inflammatory form of cell death that serves to release the mature cytokines along with other inflammatory mediators, thereby initiating the inflammatory response. While multiple regulators of the NLRP3 inflammasome have been described, specific ligands of NLRP3 and its mechanism of activation remain largely unknown. We performed a proteomics screen using co-immunoprecipitation (IP) and mass spectrometry to identify cellular proteins that bind NLRP3. Using this screen, we identified multiple NLRP3 interactors, including a family of solute carrier (SLC) proteins that localize to the inner mitochondrial membrane. Co-IP experiments verified that four of these proteins, namely SLC25A1, a citrate transporter, SLC25A6, an ADP/ATP translocase, SLC25A11, a 2-oxoglutarate/malate carrier, and SLC25A13, a calcium-dependent glutamate transporter, specifically interact with NLRP3. Because of its linkage with known metabolic disease, we further assessed the role of SCL25A13 in inflammasome function. When ectopically expressed, SLC25A13 induced IL-1β release in cells, while CRISPR/Cas9 knockout of SLC25A13 from THP-1 macrophages partially abrogated NLRP3-dependent pyroptosis, IL-1β release, and ASC speck formation. This regulatory activity of SLC25A13 was dependent on its calcium-sensing N-terminal domain. Importantly, this regulation was specific to NLRP3 and did not affect the AIM2 inflammasome or other cell death pathways. Altogether, our studies reveal a novel role for SLC25A13 in regulation of the NLRP3 inflammasome and inflammatory cytokine release.
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Disclosure of Interest: None Declared

LT187
IMPAIRED TYPE I INTERFERON RESPONSE TO ZIKA INFECTION IN HUMAN NEURAL PROGENITOR CELLS DUE TO LIMITED RIG-I SIGNALING
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Abstract Content: Intrapartum infection with Zika virus (ZIKV) has been linked to devastating fetal microcephaly and other abnormalities of brain development. Neural progenitor cells (NPCs) appear to be the major tropic cell of ZIKV in the central nervous system (CNS), and because persistence of ZIKV in brain tissue is a hallmark of congenital infection, we hypothesized that an impaired innate immune response might underlie the susceptibility of NPCs to ZIKV. Consistent with this, we found that during *in vitro* infection NPCs support higher ZIKV RNA copy number than do differentiated cells but do not induce IFN-β. To explore the underlying mechanisms for this differential response we first examined expression of several PRRs and found that compared to differentiated cells NPCs express lower levels of retinoic acid-induced gene I (RIG-I) the major pathogen recognition receptor detecting ZIKV and other flaviviruses. We performed fluorescence-activated cell

sorting and single-cell RNA sequencing to characterize the innate immune response of identified cell types including NPCs, post-mitotic neurons and astrocytes. We found that astrocytes are a primary source of IFN-β production and response during ZIKV infection. In contrast, NPCs exhibit blunted response to IFN- with lower induction of interferon-stimulated genes (ISGs), including RIG-I. These findings suggest that limited signaling via RIG-I in NPCs underlies an attenuated innate immune response and increased permissiveness for ZIKV for impaired viral control in fetal ZIKV infection. Moreover, our findings demonstrate that significant heterogeneity exists in the innate immune response to viral infection across cell types in the developing brain. Given the importance of innate immunity and type I interferon actions in processes ranging from viral infection to degenerative disease of the CNS, these findings have important implications for the development of targeted therapeutic interventions for ZIKV and other neurologic diseases.

Disclosure of Interest: None Declared

LT188

MANGANESE ENHANCES KU70-STING MEDIATED IFN-LAMBDA 1 INDUCTION BY INCREASING IN PHOSPHORYLATION OF TBK1

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Abstract Content: Transition metals are essential for a variety of physiological processes including reproduction, neuronal function, and antioxidant defenses, but little is known for their roles in innate immunity. Here, we found that, among the transition metals (Cr^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}), only Mn^{2+} significantly enhanced Ku70-STING DNA sensing pathway in HEK cells, THP-1 cells and human primary macrophages. In the presence of Mn^{2+} at the concentration of 25 mM, DNA-mediated induction of IFN-λ1, IFN-α, and IFN-β was increased by 7.1-, 15.8- and 9.3-folds, respectively. To further characterize the gene regulation profile by Mn^{2+} treatment, a microarray analysis was performed. The gene expression was compared between presence and absence of Mn^{2+} treatment. We found that the expression of 351 genes was ≥2-fold greater with Mn^{2+} and DNA treatments than with DNA stimulation alone. Using DAVID, a functional annotation bioinformatics tool, we further identified that most of those genes were involved in biological processes such as innate immune response, inflammatory cytokines or cytokine-mediated signaling pathway. Western blot analysis revealed that, in the presence of DNA, Mn^{2+} treatment greatly enhanced the activation of STING-TBK1-IRFs signaling pathway. And Mn^{2+} treatment alone induces phosphorylation of TBK1. Finally, we found that Mn^{2+} protected human primary macrophages from HSV-1 infection by increasing in IFN induction. Our finding sheds a light on a potential beneficial role of Mn on the application of DNA-based therapeutic or preventive reagents. (Funded by NCI Contract No. HHSN261200800001E)

Disclosure of Interest: None Declared

LT189

PML-DEPENDENT MEMORY OF TYPE I IFN TREATMENT PROMOTES A REPRESSIVE FORM OF HERPES SIMPLEX VIRUS LATENCY

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Abstract Content: Herpes simplex virus-1 (HSV-1) establishes a lifelong latent infection of neurons. HSV-1 latency is characterized by the association of viral lytic promoters with repressive heterochromatin. At the individual genome level, HSV-1 latency is heterogenous, which likely also results in populations of latent genomes with distinct types of heterochromatin. Although latent viral genomes have been shown to associate with different subnuclear structures, including promyelocytic leukemia-nuclear bodies (PML-NBs), how this nuclear distribution arises and its impact on the ability of a latent genome to reactivate following various neuronal stresses remain unknown. We hypothesized that inflammatory conditions during initial infection could impact the nature of HSV latency. We specifically investigated the contribution of type I IFN to regulating the nature of HSV-1 latency using a primary neuronal model system. Surprisingly, we found that treatment of primary neurons with type I IFN solely at the time of infection resulted in a form of HSV latency that was more restricted for reactivation. We also show that primary murine sympathetic and sensory neurons are largely devoid of PML-NBs. However, treatment with type I IFN infection induces the formation of PML-NBs, which persist even following cessation of ISG expression. A proportion of latent HSV-1 genomes are stably entrapped within PML-NBs throughout a latent infection of IFN pre-treated sympathetic neurons. Furthermore, depletion of PML prior to infection rescues the ability of the virus to reactivate only in IFN-treated neurons, therefore indicating that IFN-induced PML-NBs directly contribute to a deeper form of latency that is resistant to reactivation. This study demonstrates how type I IFN-treatment during de novo infection alters subnuclear positioning of viral genomes and ultimately the ability of a latent genome to reactivate.

Disclosure of Interest: None Declared

LT190

COULD A WORLD WAR II DRUG BE REPURPOSED TO LIMIT VIRAL HYPERINFLAMMATION?

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Abstract Content: Severe influenza A virus (IAV) infections are associated with damaging hyperinflammation that can lead to mortality. The repurposing of drugs with existing and studied pharmacokinetic and safety profiles is an attractive strategy to develop new therapeutics for severe and pathogenic IAV infections. We have previously demonstrated that the NLRP3 inflammasome plays a

temporal role during severe IAV infection with early protective responses, however, subsequent dysregulation leads to excessive inflammation, contributing to disease severity. While therapeutic treatment with the potent NLRP3 inhibitor MCC950 provided protection later in infection, it renders mice susceptible early in infection.
We have identified two existing drugs which target P2X7 receptor signalling and dampen NLRP3 inflammasome responses during severe IAV infection. Our study demonstrates that the anti-inflammatory drugs Probenecid and AZ11645373 are effective at dampening hyperinflammation and severe influenza disease providing potentially new therapeutic strategies for treating severe or pathogenic IAV infections. Intranasal therapeutic treatment of mice displaying severe influenza disease reduced pro-inflammatory cytokine production, cellular infiltrates in the lung and provided protection against disease. Importantly, Probenecid and AZ11645737 could be administered at either early or late stage of disease and provide therapeutic efficacy. Probenecid has been used in humans since World War II to extend the life of penicillin and is currently used to treat Gout. Furthermore, AZ11645373 has been clinically trialled for treating inflammatory diseases such as arthritis. We propose that drugs such as Probenecid and AZ11645737 which are less potent than those that directly target NLRP3 such as MCC950, are effective as they dampen NLRP3 responses to a level that is not detrimental or damaging, but sufficient to provide protection
Understating the kinetics and mechanisms involved in this 'switch' from early protective to late damaging NLRP3 responses during IAV infection is of therapeutic benefit. Myeloid-derived cells, such as neutrophils and Ly6C+ inflammatory macrophages are present in very low numbers in the airways in the absence of infection and accumulate peaking on day 3 following severe HKx31 infection (mice require ethical euthanasia by day 4). We have identified that following IAV infection, infiltrating myeloid-derived cells such as neutrophils and Ly6C+ inflammatory macrophages express significant NLRP3. We are currently examining the roles of epithelial cells and infiltrating myeloid cells, in mediating excessive NLRP3 responses may differ and hypothesise that cellular targeting with anti-P2X7 receptor drugs *in vivo* could potentially improve anti-NLRP3 efficacy.
Disclosure of Interest: None Declared

LT191

OM85BV PROTECTS AGAINST RESPIRATORY VIRAL INFECTION IN HIGH-RISK INFANTS BY MODULATING INNATE IMMUNITY

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Abstract Content: Background: Lower respiratory infections (LRI) in infancy are a major cause of morbidity. In a recent clinical trial, we demonstrated that treatment of infants with the microbial-derived immunomodulator OM85BV provided significant protection against LRI[1]. The focus of this study is on the mechanisms-of-action of OM85BV. We hypothesized that OM85BV may modulate innate immune functions central to anti-microbial defence.

Aims: To elucidate the effect of OM85BV treatment on innate immune function in a high-risk infant cohort during their first winter using gene co-expression network analysis.

Methods: OM85BV treatment (or placebo) was carried out across the 5-month winter period; PBMC were collected post-treatment and stimulated *in vitro* with PolyI:C and LPS. RNAseq profiling was performed to identify gene co-expression networks underlying immune responses. Cytokines were measured via Luminex.
Results:
The OM85BV-treatment and placebo groups mount robust responses to PolyI:C (3468 differentially expressed genes [DEG] and 3191 DEG respectively (FC>1.5)); and LPS (1930 and 1982 DEG respectively (FC>1.5)). In both groups, the PolyI:C DEG were enriched with type I/III interferons, and the LPS DEGs were enriched with cytokine signalling (IL23, IL27 and IL12) and IFNg. Network analysis revealed that both OM85BV-treatment and placebo groups activate a large antiviral module containing type I and type III interferon signalling in response to PolyI:C. In the LPS response, both groups upregulated a cytokine signalling module containing a number of proinflammatory genes (IL6, IL8, CCL3, IL1b). This module was less complex and contained fewer genes in the OM85BV group (n=263) compared to Placebo (n=881). TNF was contained within the large interferon module in Placebo. The OM85 group contained an additional small innate immunity module that contained TNF and was downregulated in response to LPS. IL6 protein was significantly lower in OM85BV ($p<0.05$) with a trend towards lower TNF protein ($p=0.055$).

Conclusion: OM85BV treatment during infancy tunes maturation of mechanisms that regulate innate immune responses to PolyI:C and LPS such that proinflammatory signals are selectively attenuated without constraining interferon-associated networks central to first-line antimicrobial defence.
[1]PMID:31185221
Disclosure of Interest: None Declared

LT192

LYMPHOID-MYELOID CELL FATE DETERMINATION BY ENDORIBONUCLEASES REGNASE-1 AND -3

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Abstract Content: Hematopoietic stem and progenitor cells (HSPCs) give rise to all blood lineage cells through complex lineage-determining mechanisms. Recent work shows that multipotent progenitor (MPP) and even hematopoietic stem cell (HSC) clones are heterogeneous and possess differentiation preferences toward either myeloid or lymphoid lineages, suggesting the existence of lineage bias. Such lineage bias plays an important role in stressed hematopoiesis (e.g. inflammatory conditions), which

enhances myelopoiesis at the expense of lymphopoiesis. However, it is unclear how the HSPC lineage commitment is regulated under native and stressed hematopoiesis.
Here we show that Regnase-1 (also known as Zc3h12a) and its family member, Regnase-3/Zc3h12c, govern early-stage lymphopoiesis by regulating mRNA decay in HSPCs. Lack of Regnase-1 and Regnase-3 (DKO) in mice resulted in a severe loss of B lymphocytes but not myeloid lineage cells. In contrast, a single deficiency of Regnase-1 or Regnase-3 did not show impairment in lymphopoiesis. Competitive reconstitution of control and DKO fetal liver cells resulted in a profound reduction of DKO lymphoid progenitors including lymphoid-primed multipotent progenitor (LMPP). However, DKO cells showed a mild increase in myeloid and megakaryocyte/erythroid (Meg/E) lineages. Nevertheless, proliferative activity and cell viability of HSC and LMPP were comparable between control and DKO. Single cell RNA-seq (scRNA-seq) analysis of Lineage$^-$Sca1$^+$c-Kit$^+$ (LSK) fraction revealed that DKO cells had a skewing of lineage biases toward myeloid and Meg/E cells, not only in lineage composition but in gene expression profiles. In addition, bulk RNA-seq analysis of LMPP showed that DKO cells showed higher expression of sets of myeloid- as well as Meg/E-related genes, compared to the counterpart. From the data of scRNA-seq and bulk RNA-seq, we identified *Nfkbiz* as a novel promoter of myelopoiesis. Crosslinking and immunoprecipitation (CLIP)-seq analyses revealed that Regnase-1 and Regnase-3 can interact shared stem loop structures present in mRNA 3' untranslated regions. *Nfkbiz* was turned out to be a target mRNA both for Regnase-1 and Regnase-3 for degradation. Interestingly, ablation of Regnase-1 and Regnase-3 in cultured LMPPs resulted in a rapid increase of myeloid-related genes including *Nfkbiz*, accompanied by subsequent myeloid differentiation. Overexpression of *Nfkbiz* in lymphoid progenitor cells showed a marked induction of myeloid lineage cells and a severe reduction in B cell differentiation , indicating that the Regnase-1/-3-*Nfkbiz* axis acts as a rheostat for regulating myeloid- and lymphoid-lineage bias in LMPPs. Finally, inflammatory conditions by LPS challenge decreased the expression of Regnase-1/3 in HSPCs, consistent with the enhancement of myelopoiesis. Thus, Regnase-1/-3 may contribute to emergency myelopoiesis through the alteration in their protein expressions. Taken together, the post-transcriptional mechanism mediated by Regnase-1 and Regnase-3 is a novel regulatory phase for the lymphoid-myeloid cell fate decision, acting at the interface between HSPCs and inflammation.
Disclosure of Interest: None Declared

LT193

VIRAL PAMP SIGNALING DIRECTS RIG-I-DEPENDENT DEATH OF TUMOR CELLS

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Abstract Content: Most malignant cells are poorly immunogenic and fail to elicit an effective antitumor response. However, viral infection of cancer cells can trigger intracellular innate immune defenses that impart cell death signaling, known as "oncolytic signaling." Our lab has identified a viral pathogen associated molecular pattern (PAMP) RNA motif from hepatitis C virus (HCV) that when delivered to cells in a liposomal formulation engages RIG-I to induce innate immune signaling. We found that PAMP RNA can direct RIG-I dependent signaling to drive cell death in tumor cells, indicating that targeting the RIG-I pathway with PAMP has applications for oncolytic destruction of tumors. Preliminary structure-function assays reveal that RIG-I binds to a novel protein TRIM16, and that TRIM16 is required for this oncolytic phenotype. Therefore, we hypothesize that TRIM16 mediates a non-canonical RIG-I dependent cell death pathway. In-vitro studies using CRISPR knockout cell lines confirmed that this cell death signaling pathway requires RIG-I and the downstream adaptor protein MAVS. Overexpression assays show that TRIM16 does not affect IFN-beta expression nor does IFN-beta affect TRIM16 expression, suggesting that TRIM16 is not involved in innate immune signaling or response but instead imparts a novel and non-canonical RIG-I mediated pathway of cell death. We are currently working to identify the structural components of RIG-I and TRIM16 required for cell death signaling and are developing an in-vivo model to study PAMP oncolytic actions. In addition, we are developing TRIM16 conditional knockout mice to interrogate the actions of TRIM16 in vivo. This research will reveal the role of TRIM16 and the novel RIG-I mediated cell death signaling pathway toward design of anti-tumor therapeutics leveraging viral PAMP actions.
Disclosure of Interest: None Declared

LT194

TLRS 1/2, 2/6, 4 AND 7/8 ENGAGE THE ALTERNATIVE NLRP3 INFLAMMASOME PATHWAY TO INDUCE INTERLEUKIN-1B RELEASE FROM PRIMARY HUMAN MONOCYTES.

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Abstract Content:
Introduction
Interleukin-1β (IL-1β) is expressed as a pro-form that requires cleavage to an active form by caspase-1 as a part of the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome. This process usually requires two signals; toll-like receptor (TLR) activation to transcribe pro-IL-1β and NLRP3, alongside a second signal to activate the inflammasome, such as K^+ efflux. In addition, caspase-1 cleaves gasdermin D (GSDMD) leading to the formation of membrane pores that permit the release of IL-1β and induce pyroptosis[1,2]. However, an alternative pathway has been described in BLaER1 cells, a human cell line which shares some similarity to primary human monocytes. Upon TLR4 stimulation, IL-1β was released by a NLRP3 and caspase-8 dependent mechanism that was independent of pyroptosis or K^+ efflux[3]. Our study set out to investigate if this mechanism operates downstream of TLRs 1/2, 2/6, 4 and 7/8 in primary human peripheral blood monocytes.

Methods
CD14+ monocytes were isolated from peripheral blood by density centrifugation followed by iso-osmotic Percoll gradient centrifugation. Cells were then stimulated with a range of TLR ligands for 6 or 24 hours in the presence or absence of a NLRP3 inhibitor (MCC950), a potassium ionophore (nigericin), caspase-1 or -8 inhibitors, a GSDMD inhibitor (NSA) or excess extracellular potassium chloride. IL-1β secretion was measured by ELISA, pyroptosis by measurement of release of lactate dehydrogenase from monocytes and GSDMD cleavage was assessed by Western blot.
Results
TLR-induced IL-1β release was NLRP3, caspase-1 and caspase-8 dependent. It was also mediated by GSDMD but did not require K+ efflux or induce pyroptosis. Additional activation of K+ efflux with nigericin amplified GSDMD dependent IL-1β secretion by all TLRs apart from TLR7/8.
Conclusion
TLRs 1/2, 2/6, 4 and 7/8 all use the alternative NLRP3 inflammasome pathway to release IL-1β from primary human monocytes. TLR7/8 stimulation alone can induce maximal IL-1β release in the absence of pyroptosis.

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Disclosure of Interest: None Declared

LT195

USING THE COLLABORATIVE CROSS MOUSE MODEL TO DETERMINE INNATE IMMUNE CORRELATES OF RESISTANCE TO WEST NILE VIRUS

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Abstract Content: Inbred mice are inadequate in capturing the diversity of human responses to infections. The Collaborative Cross (CC) mouse model is a unique resource for modeling disease response in a genetically diverse population similar to human genetic diversity. To better understand how genetic diversity contributes to innate immune responses to West Nile infection, we examined the innate immune and cytokine gene expression following West Nile virus (WNV) infection in 114 CC mouse lines, which encompass 90% of genetic variation in laboratory mice while mirroring human genetic diversity. We measured weight loss, clinical score, viral load, and innate immune responses, including IL-12 and IFN-beta, in each CC mouse line over an acute infection time course as the host response engaged innate immunity and transitioned to adaptive immunity. We defined phenotypic categories of dynamic susceptibility to WNV and innate immune control of infection among CC strains. Our studies reveal that during WNV infection the dynamics of innate immune activation and engagement of the innate/adaptive immune interface are linked with gastrointestinal (GI) disease, and the partitioning of infection among peripheral tissue, and the central and enteric nervous systems. These outcomes reflect WNV infection dynamics in humans. Thus, the CC model is a unique resource allowing researchers to study and define host genetic features of innate immune regulation and WNV disease phenotypes.
Disclosure of Interest: None Declared

LT196

TYROSINE PHOSPHORYLATION BY EGFR IS REQUIRED FOR STING TO TRANSLOCATE TO THE ENDOSOME, ACTIVATE IRF3, INDUCE INTERFERON AND INHIBIT HSV1 REPLICATION.

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Abstract Content: Tyrosine phosphorylation by EGFR is required for STING to translocate to the endosome, activate IRF3, induce interferon and inhibit HSV1 replication.
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Stimulator of interferon genes (STING) mediates protective cellular response to microbial infection and tissue damage; however, its aberrant activation leads to autoinflammatory diseases. Upon ligand-stimulation, STING, an endoplasmic reticulum (ER) protein, translocates to the endosomes to induce interferons; but an alternate route takes it directly to the autophagosomes. Here, we report that EGFR-mediated tyrosine phosphorylation of STING was required for its ability to activate IRF3, which drives the induction of IFN. Upon cGAMP stimulation, EGFR interacted with STING in the ER membrane and directly phosphorylated its Y245 residue. Tyr phosphorylation of STING was not required for binding TBK1; STING-bound TBK1 was activated by autophosphorylation and it phosphorylated Ser366 of STING, a prerequisite for IRF3 binding. However, the Y245F mutant of STING rapidly transited to the autophagosome and did not translocate to the endosome, where, as we demonstrated, IRF3 interacted with STING. In the absence of this critical post-translational modification of STING Y245, IRF3 activation, interferon production and antiviral action were compromised in cell cultures and mice; in contrast, its autophagic activity was enhanced. Our observations illuminate a new connection between the tyrosine kinase activity of EGFR and innate immune functions of STING; they also suggest experimental and therapeutic approaches for selective regulation of STING functions.

Disclosure of Interest: None Declared

LT197

MICROGLIA UNIQUELY REGULATE THEIR PHENOTYPE AND FUNCTION IN IL-6 AND IFN-ALPHA-MEDIATED NEUROLOGICAL DISEASE

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Abstract Content: Chronic production of interleukin (IL)-6 or interferon (IFN)-alpha in the CNS is the cause of several neurological diseases including neuromyelitis optica and Aicardi-Goutières syndrome respectively. Transgenic mice that produce IL-6 (GFAP-IL6) or IFN-alpha (GFAP-IFN) in the CNS recapitulate many aspects of the respective human diseases. Microglial activation is a prominent feature in the CNS of GFAP-IL6 and GFAP-IFN mice and is implicated in the pathogenesis of these cytokine-mediated neurological diseases. To define the role of microglia in disease, we performed a detailed phenotypic comparison of microglia in GFAP-IL6 versus GFAP-IFN mice.

GFAP-IL6 microglia were highly proliferative, pY-STAT3 positive, and were smaller and had stunted processes, while GFAP-IFN microglia were pY-STAT1 positive and significantly larger with hyper-ramified processes. Purified microglia *ex vivo* had altered transcriptomic and surface marker profiles in response to IL-6 and IFN-alpha. GFAP-IL6 microglia had transcriptomic and surface marker changes associated with proliferation and phagocytosis, while GFAP-IFN microglia had changes associated with viral immunity and antigen presentation.

In order to clarify the contribution of microglia to the development of cytokine-mediated neurological disease, microglia were ablated from the CNS of GFAP-IL6 and GFAP-IFN mice using the CSF1R blocking drug, PLX5622. The capacity of surviving microglia to repopulate the empty CNS niche was differentially altered in response to the cytokine environment driven by IL-6 and IFN-alpha. GFAP-IL6 microglia repopulated the CNS more rapidly and robustly, while GFAP-IFN microglia were unable to repopulate the CNS. Microglia-depleted GFAP-IL6 and GFAP-IFN but not WT mice had exaggerated disease, with pronounced tissue destruction and severe calcification of the cerebellum. Surprisingly, although cerebral calcification is a hallmark of IFN-mediated disease, this exaggerated disease pathology was not associated with increased IFN or IFN-regulated gene expression.

We conclude that the microglia response to each cytokine environment is wide-ranging and gives rise to divergent phenotypes. The acquisition of unique phenotypic features may impart microglia with distinct functions in the neurological diseases mediated by IL-6 and IFN-alpha. Additionally, microglia depletion in both GFAP-IL6 and GFAP-IFN mice, rather than being protective, is deleterious.
Disclosure of Interest: None Declared

LT198

PROTECTIVE TRANSCRIPTOMIC SIGNATURE AND BIOMARKERS OF THE RHCMV/SIV VACCINE RESPONSE

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Abstract Content: The 68-1 RhCMV vaccine, based on Rhesus Macaque (RM) modified cytomegalovirus (CMV), can direct a protective immune response against SIV infection in RMs and serves as a preclinical model for CMV-based vaccine vectors in HIV vaccination. We longitudinally evaluated the blood transcriptional signature of the 68-1 vaccine after oral or subcutaneous vaccination of 15 RMs who underwent a prime and week 18 boost regimen before challenged with the virus multiple time starting approximately 90 weeks after prime vaccination. Using differential gene analysis and linear modeling we identified a unique transcriptional gene response linked with vaccine protection against multi-challenge SIV infection that includes genes within inflammasome, interleukin signaling, Toll-like receptor signaling, T cell receptor signaling functional pathways. We found that induction of the interleukin (IL)-15 response also tracked with vaccine protection. Our studies reveal gene correlates of 68-1 vaccine protection and identify whole blood gene biomarkers linked with vaccine efficacy.
Disclosure of Interest: None Declared

LT199

TWO DISTINCT INTERFACES MEDIATE THE ASSOCIATION BETWEEN CASPASE-1 AND GASDERMIN D

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Abstract Content: The recognition and cleavage of gasdermin D (GSDMD) by inflammatory caspases-1/4/5/11 is an essential step in initiating pyroptosis following inflammasome activation. Previous work has identified cleavage site signatures in substrates such as GSDMD and inflammatory cytokines, but it is unclear if these are the sole determinants for caspase engagement. Here we report the

crystal structure of a complex between human caspase-1 (CASP1) and the full-length murine GSDMD. Our work reveals that the GSDMD N-terminal domain does not bind CASP1, whereas the cleavage site-containing linker in GSDMD adopts a long loop structure that engages the CASP1 active site. This active site interface resembles previously reported structures of CASP1 in complex with a pan-caspase inhibitor z-VAD-FMK or a GSDMD-derived peptide inhibitor. In addition to GSDMD engagement of the caspase-1 active site, an anti-parallel b sheet at the caspase-1 L2 and L2' loops binds a hydrophobic pocket within the GSDMD C-terminal domain distal to its N-terminal domain. This "exosite" interface is in agreement with the recent publication by Wang and colleagues reporting the structures of caspases-1/4/11 in complex with the GSDMD C-terminal domains. The exosites in inflammatory caspases that bind a hydrophobic pocket formed by a5-a6 and a7'-a8 in GSDMD C-terminal domains endow a novel function for the GSDMD C-terminal domain as a caspase-recruitment module, in addition to its role in autoinhibition. Such dual functionality of the C-terminal domain may allow stringent substrate selectivity in the resting state while facilitating cleavage and pyroptosis upon inflammasome activation. The residues forming the hydrophobic pocket are conserved between human and murine GSDMD, but not in GSDME, suggesting that the exosite interface may underlie the specific recognition of GSDMD but not GSDME by inflammatory caspases. Our study thus reveals dual interface engagement of GSDMD by caspase-1, which may be applicable to other physiological substrates of caspases.
Disclosure of Interest: None Declared

LT200

ZBTB16 CONTROLS SUMOYLATION OF ASC AND INFLAMMASOME ACTIVITY

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Abstract Content: Appropriate control of Inflammasome activity is critical to limit damaging immune responses through rapidly escalates the intensity of inflammation by activating interleukin (IL)-1β, IL-18 and cell death by pyroptosis. Here we identify a novel control mechanism that modulates the central Caspase-1 and NLR (Nod-like receptor) adaptor ASC (apoptosis-associated speck-like protein containing a CARD). We identify SUMO (small ubiquitin-like modifier) modification of ASC represses the nuclear to cytoplasmic translocation and its subsequent oligomerization, ASC speck formation. We also discover that nuclear ZBTB16 (Zinc finger and BTB domain-containing protein 16) interacts with ASC to suppress the conjugation of SUMO-1, thereby promoting inflammasome activity. ZBTB16 (also known as PLZF , nuclear body protein promyelocytic leukemia zinc-finger protein) which colocalize with specialized Promyelocytic leukemia protein nuclear bodies (PML bodies) may provide interactions between SUMO and SUMO interaction motifs (SIMs) for contribution ASC speck formation. We further showed that K21,22,24,26 on ASC is essential for SUMOylation and interact with PLZF, also required for ASC speck formation. Accordingly, macrophage-specific deletion of ZBTB16 attenuates inflammation induced by Mono Sodium Urate Crystal-induce peritonitis through NLRP3 activation. Remarkably, ablating ZBTB16 also relieves inflammatory pathogenesis in a murine model of Muckle-Wells syndrome caused by hyperactive mutant NLRP3. Our findings reveal a novel role for SUMO-mediated regulation of inflammatory disease caused by ASC that is modulated through ZBTB16.
Disclosure of Interest: None Declared

LT201

MG53/TRIM72 DAMPENS TYPE I INTERFERON PRODUCTION AND PROTECTS FROM LETHAL INFLUENZA VIRUS INFECTION WHEN THERAPEUTICALLY ADMINISTERED

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Abstract Content: Tripartite motif (TRIM) family proteins play integral roles in the innate immune response to virus infections. Mitsugumin 53 (MG53), also known as TRIM72, is believed to be a muscle-specific TRIM protein that is essential for muscle cell membrane repair and maintenance. We now show that human macrophages express MG53 and that its knockdown leads to increased production of type I interferon (IFN) upon virus infection. MG53 knockout mice infected with influenza virus show comparable viral titers to WT mice, but experience increased morbidity accompanied by accumulation of inflammatory cells and elevation of IFNβ in the lung. We found that MG53 knockdown results in activation of NF-KB signaling, which is linked to an increase in intracellular calcium oscillation mediated by ryanodine receptor (RyR). Further, we observed that MG53 inhibits IFNβ induction in an RyR-dependent manner. Since MG53 is also secreted by muscle cells as a myokine to provide regenerative benefits to multiple tissues, we sought to determine the effects of enhancing systemic levels of MG53 during influenza virus infection. Intravenous administration of clinical grade recombinant human (rh) MG53 during lethal influenza virus infection protected mice from severe disease, with a 92% survival rate, and with mice remaining ambulant and active throughout infection. rhMG53 treatment of infected mice preserved lung integrity, corresponding with a decrease in lung inflammation, including decreases in IFNβ and inflammasome activation, without directly affecting viral titers. Collectively, our studies reveal a new role for MG53 in limiting tissue-damaging inflammation during virus infection, and identify rhMG53 as a promising host-directed biologic therapy for infectious lung injury.
Disclosure of Interest: A. Kenney: None Declared, M. Sermersheim: None Declared, Z. Li: None Declared, Z. Bian: None Declared, P.-H. Lin: None Declared, X. Zhou:

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LT202

STAT1 AND TYPE I IFN SIGNALING PATHWAYS REGULATE CONVENTIONAL DENDRITIC CELL DEVELOPMENT FROM COMMON LYMPHOID PROGENITORS DURING INFLAMMATION

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Abstract Content: Dendritic cells (DCs), including conventional DCs (cDCs) and plasmacytoid DCs (pDCs), are key regulators of immune response. However, the developmental origin of both subsets of DCs and the signals orchestrating DC generation remain incompletely understood. Here, we demonstrated that TLR-mediated inflammation drastically altered the developmental process of progenitors of both lymphoid and myeloid lineages by enhancing cDC production in vitro and in vivo. The ratio of cDC1 to cDC2 also increased upon TLR stimulation. More importantly, ex vivo DC development from CLPs of mice previously treated with R848, a TLR7 agonist, also favored cDC generation even though R848 is omitted in the culture conditions, suggesting that TLR signaling reprograms DC development in the progenitors. Fate mapping using IL-7R-driven reporter mice demonstrated that CLPs contributed to increased cDC population, cDC1 in particular, during inflammation. The TLR7 effect on cDC development was partially rescued in MyD88KO CLPs when cocultured with WT CLPs, suggesting that both primary and secondary signaling events downstream of TLR7 are involved. One of the secondary signaling events was dictated by IFN-I induced by the stimulation, which partially controlled TLR-dependent enhancement of cDC generation. While STAT1 is a canonical signal mediator of IFN-I, it was also directly activated by R848 through phosphorylation at S727. Either STAT1KO or S727A mutation in STAT1 impeded the effect. In sum, these findings reveal that developmental process of DCs from their progenitors is very dynamic in steady state and inflammation. Moreover, we define a novel function of STAT1 and IFN-I signaling pathway in TLR-mediated reprogramming of DC development from CLPs.
Disclosure of Interest: None Declared

LT203

RELEVANCE OF THE CELL-INTRINSIC ANTIVIRAL SIGNALING FOR THE INDUCTION OF CELL DEATH UPON DNA DAMAGE

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Abstract Content: Cell-intrinsic antiviral immunity is pivotal to combat virus infections. Sensing of conserved pathogen-associated molecular patterns (PAMPS) by cellular receptors such as retinoic acid-inducible gene I (RIG-I) results in the production and release of interferons and massive expression of interferon-stimulated genes (ISGs), conferring an antiviral state. Notably, this ISGs-driven state not only comprises antiviral effectors but also displays cytostatic and anti-tumorigenic properties, inducing apoptosis, and suppressing proliferation and migration. Surprisingly, recent work showed that this antiviral pathway is not only relevant in viral infection, but also for proper tumor-cell death following treatment with DNA damage inducing chemotherapeutics [1, 2].
Our study aims to identify and characterize the components and the mechanism of RIG-I mediated cell-death upon DNA damage. We generated A549 CRISPR/Cas9 knockout cell lines targeting different components of innate immunity and determined rates of cell death upon treatment with cytostatics or γ-irradiation by life cell imaging. We confirmed that doxorubicin treatment induces cell death dependent on a functioning RIG-I/MAVS axis, while involvement of the classical ISG response remains unclear. Moreover, we have identified proteins that contribute to cell death but are not related to the canonical RIG-I pathway. We are currently investigating the mechanistic role of these proteins in DNA damage-induced cell death.
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Disclosure of Interest: None Declared

LT204

COINFECTION WITH HELIGMOSOMOIDES POLYGYRUS INCREASES ACUTE MURINE GAMMAHERPESVIRUS-68 INFECTION IN THE PERITONEUM

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Abstract Content: In certain areas of the world, the human gammaherpesviruses, Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), create a substantial health burden through lymphoproliferative disorders. Gammaherpesviruses cause chronic infections in B cells, macrophages, and dendritic cells. While single pathogen infections in laboratory settings are common, coinfections are not, despite the fact that most infections people experience are coinfections. For example, in the places that have endemic EBV or KSHV, there is also high incidence of helminth infection. While some co-infecting pathogens induce similar immune responses, such as bacteria and virus, other combinations of pathogens induce diverse responses. Helminths induce a Th2 immune response and alternatively activate macrophages through IL-4 and IL-13 signaling. Meanwhile, viruses primarily

induce interferon, Th1 responses, and classical activation in macrophages. In order to examine how parasite coinfection affects gammaherpesvirus infection, we infected mice with a helminth, *Heligmosomoides polygyrus* (HP), and then infected with murine gammaherpesvirus-68 (MHV68) a week later. Serum from coinfected mice had increased Th2-associated cytokines, including IL-4 and IL-5, during the first week of MHV68 infection. Further, we observed increased viral titer in the coinfected mice at day 2 of MHV68 infection that was dependent on STAT6. The immune cell populations of the peritoneum were altered in single and co-infection conditions. Mice infected only with MHV68 had increased numbers of monocyte-derived macrophages, while coinfected mice had increased numbers of resident peritoneal macrophages. In addition to an increased viral replication of MHV68, coinfected mice had increased numbers of subsets of infected macrophages at day 2, day 4 and day 7 of MHV68 infection. We aim to determine the contribution of expanded macrophage populations in co-infected mice and the role of STAT6 signaling on MHV68 infection in peritoneal macrophage populations.

Disclosure of Interest: None Declared

LT204b

DYNAMIC ACCESSIBILITY UNDERLIES TH9 LINEAGE PLASTICITY AND CONFERS INNATE-LIKE FUNCTION IN VIVO TO PROMOTE ALLERGIC DISEASE

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Abstract Content: Interleukin-9 (IL-9), an inflammatory cytokine derived primarily from T helper 9 (Th9) cells, is a critical mediator of allergy, autoimmunity, and antitumor immunity. Yet Th9 cells are tissue-resident, and IL-9 expression can be transient. The mechanisms underlying Th9 lineage plasticity and the *in vivo* significance of transient IL-9 production are not well characterized. We found that human and murine Th9 cells produced IL-9 upon exposure to paracrine IL-2 and IL-4, but not other cytokines. Mechanistically, IL-2 and IL-4 induced active transcription of stable *IL9* transcript via JAK-STAT signaling. Cytokine-induced IL-9 production was independent of T-cell receptor (TCR) stimulation, indicating that it did not require antigen recognition. This mechanism was unique to IL-9, as interferon (IFN)-γ, Il-2, IL-4, IL-5, IL-13, IL-17A, and IL-10 were not induced under these conditions. IL-2 and IL-4 were sufficient to induce IL-9 production from recently activated *in vitro* differentiated or expanded Th9 cells and from *ex vivo* tonsillar Th9 cells, but not from circulating memory Th9 cells. Examining the dynamic accessibility of the extended *Il9* locus revealed three critical STAT-dependent *cis*-regulatory regions that were accessible in recently activated Th9 cells, maintained accessibility for several days, and lost accessibility thereafter. Dynamic accessibility was not seen for other extended Type 2 cytokine loci. Taken together, these results indicate that dynamic accessibility underlies STAT-dependent IL-9 production in recently activated Th9 cells. In murine asthma models, inhaled IL-2 and IL-4 were sufficient to support a population of transferred lung-resident Th9 cells, and ablating TCR signaling did not prevent Th9-mediated allergic immunopathology in sensitized mice, demonstrating an innate, antigen-independent function for tissue Th9 cells *in vivo*. Taken together, these data suggest that Th9 cells respond to STAT-dependent cytokines in a unique innate manner, independent of TCR, to promote allergic disease.

Disclosure of Interest: None Declared

LT204c

T HELPER 9 CELLS ARE ASSOCIATED WITH AN INCREASED RISK OF PSORIATIC CARDIOVASCULAR DISEASE AND DIRECTLY PROMOTE HUMAN ARTERIAL ENDOTHELIAL CELL DYSFUNCTION

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Abstract Content: $CD4^+$ T helper (Th) cells are undeniably central mediators of psoriasis, where they produce cytokines like IL-17A and IL-23, which recruit inflammatory cells to the skin and directly promote keratinocyte proliferation. Yet psoriasis is a systemic disease with various extracutaneous manifestations including arthritis, inflammatory bowel disease, and atherosclerotic cardiovascular disease. Although these extracutaneous manifestations are a major source of morbidity and mortality in patients with psoriasis, the immunopathologic mechanisms driving extracutaneous psoriatic pathology are incompletely characterized. To determine which T helper-derived cytokines might promote extracutaneous psoriatic disease, we immunophenotyped $CD4^+$ T cells from psoriasis patients and looked for associations with cutaneous and extracutaneous pathology. We tested 10 cytokines: IL-17A, IL-4, IL-5, IL-13, IL-9, IL-21, IL-10, IL-2, interferon (IFN) -γ, and tumor necrosis factor (TNF) -α. Unexpectedly, we found a correlation between circulating $IL\text{-}9^+$ T helper (Th9) cells and non-calcified coronary atherosclerotic burden (unadjusted $p = 0.039$, adjusted $p = 0.002$) that was not seen for other cytokines. Analysis of public single cell RNA-sequencing data revealed increased numbers of $CD4^+$ T cells expressing the Th9 master transcription factor (PU.1) in human atherosclerotic plaque compared with peripheral blood (FDR<0.01). This indicated that Th9 cells were plaque-resident and suggested that they might directly induce aortic endothelial cell dysfunction, which is critical to the pathogenesis of atherosclerotic cardiovascular disease. Accordingly, human aortic endothelial cells (HAoECs) expressed both subunits of the heterodimeric IL-9 receptor. *In vitro* treatment of cultured HAoECs with IL-9 caused STAT3 activation, induced STAT3 target genes, and promoted endothelial dysfunction. Taken together, these results suggest that Th9 cells promote psoriatic

cardiovascular disease by directly inducing human arterial endothelial cell dysfunction.
Disclosure of Interest: None Declared

LT204d
RED BLOOD CELLS ALLOIMMUNIZATION AND CYTOKINES IN SCD PATIENTS IN AFRICA
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Abstract Content: INTRODUCTION
Numerous factors are involved in the process of alloimmunization. Not all patients respond in the same way to alloimmunization. Some are immunological responders and others not. Inflammation has been identified as a factor and would increase the risk of immunization. In sickle cell disease the most prevalent genetic disease worldwide, inflammation is a permanently phenomenon. This work attempts to identify the pattern of few inflammatory and non inflammatory cytokines and their relationship with alloimmunization.
PATIENTS AND METHODS
50 subjects (4 to 18 years) were prospectively enrolled in the study after an informed consent. The patients were assigned in 2 groups, patients in steady state and patients admitted for crisis. Pattern of 4 cytokines were analysed (IL10, IL4, IL17 and IFNγ) and serums were measured by using LEGENDplexTM Human Inflammation Panel assays.
RESULTS
We recruit 50 patients with a diagnosis of sickle cell disease (SCD). Of these, only 31 have benefited from the research of irregular antibodies (62%) comprising 14 males (45,16%) and 17 females (54, 84%). The overall alloimmunization prevalence was 16, 12%. We noted an increased level of IL10 in nonalloimmunized patients with SCD when compared with alloimmunized patients. IL4 was higher in alloimmunized patients compared to non alloimmunized.
Concerning IL17, we noted a small increase in patients without alloantibodies and lower levels of IFNγ
CONCLUSION
The impact of biomarkers in the occurrence of alloimmunization is a constant preoccupation of researchers. Despite the small number of patients enrolled, our study shows the possible role of IL-10 as protecting from alloimmunization but this information must be explored in a larger cohort.
Disclosure of Interest: None Declared

Lightning Talk Session 3: Mucosal immunity

LT205
ORAL EPITHELIAL IL-22/STAT3 SIGNALING LICENSES IL-17-MEDIATED IMMUNITY TO ORAL MUCOSAL CANDIDIASIS
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Abstract Content: Oropharyngeal candidiasis (OPC, oral thrush) is an opportunistic infection caused by the commensal fungus *Candida albicans*. IL-17 and IL-22 are cytokines produced by Type 17 lymphocytes. Both cytokines mediate antifungal immunity yet activate quite distinct downstream signaling pathways. While much is now understood about how IL-17 promotes immunity in OPC, the activities of IL-22 are far less well delineated. We show that, despite having similar requirements for induction from Type 17 cells, IL-22 and IL-17 function non-redundantly during OPC. We find that the IL-22 and IL-17 receptors are required in anatomically distinct locations within the oral mucosa; loss of IL-22RA1 or STAT3 in the oral basal epithelial layer (BEL) causes susceptibility to OPC, whereas IL-17RA is needed in the suprabasal epithelial layer (SEL). Transcriptional profiling of the tongue linked IL-22/STAT3 to oral epithelial cell proliferation and survival, but also, unexpectedly, to driving an IL-17-specific gene signature. We show that IL-22 mediates regenerative signals on the BEL that replenish the IL-17RA-expressing SEL, thereby restoring the ability of the oral epithelium to respond to IL-17 and thus to mediate antifungal events. Consequently, IL-22 signaling in BEL 'licenses' IL-17 signaling in the oral mucosa, revealing spatially distinct yet cooperative activities of IL-22 and IL-17 in oral candidiasis.
Disclosure of Interest: None Declared

LT206
SHIGELLA DISRUPTS TYPE I AND III INTERFERON SIGNALLING IN EPITHELIAL CELLS
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Abstract Content: Type I and type III interferons (IFNs) are immune cytokines produced in response to the detection of microbes by epithelial cells (ECs) and innate immune cells. Type I IFNs, predominantly composed of IFNα and IFNβ, are secreted by almost every cell and signal through the ubiquitously expressed IFN-α receptor 1 (IFNAR1)/IFNAR2 complex receptor. In contrast, type III IFNs, comprise IFN λ1-4 and signal through the IFN-λ receptor 1 (IFNLR1)/IL-10R2 complex receptor and are restricted to epithelia at

barrier sites, thereby playing an important role in mucosal immunity. Upon binding with their respective receptor, they induce the expression of interferon-stimulated genes (ISGs) through janus kinase (Jak) – signal transducer and activator of transcription (STAT) signalling.
Although they have important roles for the clearance of numerous viruses, their roles in the defence against bacterial pathogens remain poorly understood. Our lab highlighted that type I and III interferons are secreted upon bacterial infection. The different roles of type I and III interferons as well as their mechanisms of action in the context of bacterial infections are the focus of our lab.

My project specifically investigates the **functions of type I and III IFNs in immunity against *Shigella sonnei*** (*S. sonnei*). *S. sonnei* is a gram-negative bacterium responsible for *Shigellosis,* a disease particularly life-threatening for children under five, characterized by bloody diarrhea, fever and abdominal pain. Upon ingestion, *Shigella* reaches the colon where it activates a type-3-secretion system (T3SS), through which it secretes virulence factors or effectors required for its entry, replication and dissemination into/within ECs. T3SS-effectors also interfere with innate sensing pathways and can modulate pro-inflammatory cytokine expression. However, little is known their impact on signalling downstream of IFN receptors. Using a reporter ISRE-Luc transfection system, our lab recently identified that **some *Shigella* effectors are potent inhibitors of IFN signalling**. These effectors were able to block IFNβ or IFNλ-mediated STAT1 phosphorylation and subsequent ISG expression. Furthermore, I investigated the impact of IFNβ on *S. sonnei* invasion of ECs by measuring intracellular bacterial numbers in IFNβ-treated ECs infected with *S. sonnei* for 1,3 or 5 hours. The results showed a decrease of intracellular bacteria number at each time point, suggesting that **IFNβ impairs *S. sonnei* entry into ECs**. The impact of type III IFN on *S. sonnei* infection of ECs is currently under assessment in the lab to complete these findings.

Overall, our studies greatly contribute to the understanding of interferon regulation and functions which benefits our knowledge of host-microbial interactions at mucosal surfaces.
Disclosure of Interest: None Declared

LT207

IFN-▫▫ INDUCED BY VIRAL RECOGNITION IN THE LUNG PREDISPOSES TO BACTERIAL SUPERINFECTIONS BY INHIBITING EPITHELIAL REPAIR.

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Abstract Content: RNA viruses such as influenza virus, respiratory syncytial virus and the new pandemic coronavirus SARS-CoV-2 induce severe clinical disease in the lower respiratory tract. Clinical and experimental evidence indicate that most severe and lethal cases do not depend on the viral burden and are, instead, characterized by an aberrant immune response which induce tissue damage and predisposes to lethal microbial superinfections. In this study, we analyzed how innate immune activation in response to viral RNA contributes to the pathogenesis of RNA-viruses. We demonstrate that type III interferons are produced by dendritic cells in the lung via the activation of the TLR3-TRIF pathway in response to synthetic double stranded RNA, and cause barrier damage, compromising host tolerance and resistance. In particular, type III interferons act directly on epithelial cells and inhibit tissue repair by impairing lung epithelial cell proliferation, causing susceptibility to lethal bacterial superinfections. Overall, our data describe a previously unappreciated pathophysiological role of this group of interferons which mandates a careful evaluation of their possible use in the clinical practice against endemic as well as emerging viral infections.
Disclosure of Interest: None Declared

LT208

LOC339803 LNCRNA REGULATES INTESTINAL PROINFLAMMATORY CYTOKINE RESPONSE BY AN ALLELE-SPECIFIC RNA METHYLATION-DEPENDENT MECHANISM

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Abstract Content: *LOC339803* is a lncRNA with unknown function that is located on the intestinal inflammatory disease-associated region 2p15. One of the disease-associated SNPs is within a m6A methylated region, so we hypothesized that SNP-related methylation changes could affect lncRNA function. To test this hypothesis, we used an intestinal cell line heterozygous for the associated SNP together with RNA extracted from human small intestinal samples of controls and individuals with an intestinal inflammatory condition (i.e. celiac disease).

To asses whether the associated SNP genotype was influencing m6A methylation of the lncRNA we performed an allele-specific meRIP and observed that the risk conferring G allele was preferentially methylated. Stability analysis showed that the preferentially methylated G allele is less stable, and when the m6A levels were increased by overexpressing METTL3 methylase, its levels were further decreased, pointing to an involvement of the methylation of this lncRNA in its function. Assessment of the subcellular location of the lncRNA showed that *LOC339803* is primary nuclear in intestinal cells suggesting a role in transcriptional regulation. Overexpression of *LOC339803* induced allele-dependent downregulation of the adjacent *COMMD1* gene and augmented downstream NFkB levels, while CRISPR-Cas9 mediated deletion of the lncRNA enhanced *COMMD1* and decreased NFkB expression. Additionally, overexpression of *LOC339803* activated the expression of the cytokines *IL1B* and *IL6*, a hallmark of intestinal inflammation, which was counteracted by overexpressing *COMMD1*. RNA immunoprecipitation experiments followed by mass-spectrometry, using in vitro transcribed biotinylated lncRNA, showed that HNRNPC

protein, which has been shown to bind m6A-switches, is able to interact with *LOC339803* in intestinal cells. Interestingly, proteins that bind COMMD1 promoter were also present within the immunoprecipitated complexes. Moreover, expression analysis in human intestinal biopsies revealed that this lncRNA is increased in celiac disease patients showing higher levels in those patients homozygous for the risk allele.
The results so far suggest that lncRNA *LOC339803* has an allele-specific effect in NFkB-mediated intestinal inflammation by the induction of IL1B and IL6. The allele specificity seems to be related to m6A methylation levels and to the ability of the lncRNA to regulate the expression of the nearby *COMMD1* gene probably via its interaction with the HNRNPC protein.
Disclosure of Interest: None Declared

LT209

REGULATORY T CELLS CONTROL THE DYNAMIC AND SITE-SPECIFIC POLARIZATION OF CD4 T CELLS FOLLOWING SALMONELLA INFECTION

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Abstract Content: FoxP3$^+$ regulatory T cells (Tregs) control inflammation and maintain mucosal homeostasis, but their potential to control CD4 T helper (Th) bias is poorly understood. Th1, Th2 and Th17 cells can be identified by expression of cytokines and the master transcription factors (TFs) T-bet, GATA3 and RORγT, respectively. Tregs also express these TFs, but it is unclear what role Tregs expressing Th-type TFs play during infection. To address this knowledge gap, we developed a model of non-lethal colitis using an attenuated strain of *Salmonella enterica* serotype Typhimurium. Early after infection we observed increased numbers of colonic IL17A$^+$ Th17 cells, followed by a long-lasting Th1 bias with increased IFNγ production. Unexpectedly, the early Th17 response paralleled an increase in T-bet$^+$ Tregs, and the later Th1 bias occurred in tandem with increased RORγT$^+$ Tregs. This reciprocal dynamic may indicate sub-populations of Tregs selectively suppress Th cells, shaping the overall immune response. To determine if Tregs were required for the dynamic Th bias, depletion experiments were carried out. Treg depletion 1-2 days post-infection shifted the early Th17 response to a Th1 bias; and depletion 6-7 days post-infection abrogated the Th1 bias. Thus, Tregs are necessary for the early Th17 response, and for a maximal Th1 response later. These data show that Tregs shape the colonic CD4 T cell response in a nuanced and fine-tuned way. This highlights the potential for subpopulations of Tregs to be targeted in novel immunotherapies to restrain pathogenic T cell responses.
Disclosure of Interest: None Declared

LT210

INHIBITION OF SALMONELLA ENTERICA INVASION AND INTRACELLULAR REPLICATION 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. Once consumed it invades host cells, including epithelial cells, M cells and dendritic cells within the gastrointestinal tract. Recognition of the pathogen by the host immune system induces a pro-inflammatory state that usually results in clearance of the pathogen by neutrophils and other immune cells. Interferons (IFNs) are archetypal antiviral cytokines that act in an autocrine and paracrine manner to activate JAK/STAT signalling and induce the expression overlapping sets of hundreds of interferon-stimulated genes (ISGs). IFNs have also been shown to be expressed during bacterial infections, although whether they help the host or the pathogen is highly variable between bacterium, biological context, and IFN type. As they are induced in concert, the individual roles of many ISGs are not well understood and there is a dearth of knowledge regarding their effects in bacterial infections. In this study, epithelial cell lines were incubated with IFNs prior to infection with *S.* Typhimurium. Data indicates that IFNb can inhibit the invasion of *S.* Typhimurium into epithelial cells and may also inhibit intracellular replication. The project aims to conduct a comprehensive screen of ISGs to better understand how they restrict bacterial invasion into, and replication within, host epithelial cells.
Disclosure of Interest: None Declared

LT211

MODC'S-TARGETING TNF FUSION PROTEINS ENHANCE CYCLIC DI-GMP MUCOSAL VACCINE ADJUVANTICITY IN MIDDLE-AGED AND AGED MICE

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Abstract Content: Since the discovery of alum in the late 1920's, vaccine adjuvants have been the "immunological bullets" for nearly a century. Cyclic dinucleotides (CDNs) are promising mucosal vaccine adjuvants and have been reported to induce a balanced, potent humoral and cellular immune responses. However, CDNs' efficacy in an aged environment is unclear. We examined the mucosal vaccine efficacy of cyclic di-GMP (CDG), the founding member of CDNs, in 1-year-old (middle-aged), and 2-year-old (aged) C57BL/6J mice. We found that 1-year-old and 2-year-old C57BL/6J mice are defective in CDG-induced memory Th1 and Th17 responses and high-affinity serum IgG, mucosal IgA production. Next, we generated two novel TNF fusion proteins; soluble TNF and transmembrane TNF to target TNF receptors in monocyte-derived DCs (moDCs) to enhance CDG vaccine efficacy in 1-year- old and 2-year-old mice. The moDCs -targeting TNF fusion proteins restored CDG-induced memory Th1, Th17, and high-affinity IgG, IgA

responses in the 1-year-old and 2-year-old mice. Together, the data suggested that aging negatively impacts CDG vaccine adjuvanticity. However, moDC- targeting TNF fusion proteins enhanced CDG adjuvanticity in the aging mice. It can be said that, age related immune impairment is not irreparable and can be recovered with precise targeting.
Disclosure of Interest: None Declared

LT212

INTRAVAGINAL ZIKA VIRUS INFECTION INITIATES TISSUE-SPECIFIC INNATE IMMUNE PROGRAMS IN THE FEMALE REPRODUCTIVE TRACT

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Abstract Content: Introduction: Innate immunity plays a critical role in protection of the female reproductive tract (FRT) to mediate tissue-specific defense against invading pathogens while maintaining tolerance toward commensal bacteria, sperm and fetal products. We have shown that innate immune defenses against ZIKV are triggered through RIG-I. Importantly, ZIKV suppresses IFN signaling to facilitate infection, but how these processes direct innate immune actions, gene expression, and viral dissemination from the FRT are not known. We found that ZIKV targets and persistently replicates in the vaginal mucosa after subcutaneous challenge in nonhuman primates, thus defining the FRT and as a relevant site of viral tropism and persistence.
Methods: To define the innate immune correlates that respond to ZIKV and impart antiviral defense or susceptibility to infection, we established a small animal model of intravaginal ZIKV challenge with mouse-adapted ZIKV/Dakar in C57xBL6/J mice featuring knock-in of the human STAT2 gene (S-KI). This model is fully immune competent and is susceptible to ZIKV infection and viral antagonism, displaying virus/host dynamics similar to NHP and human infection.
Results: We found that intravaginal infection in S-KI mice resulted in virus dissemination to the upper reproductive tract and draining lymph nodes as assessed by qRT-PCR as early as one day post-infection (dpi). Virus was subsequently detected in the spleen and gastrointestinal tract at 4-6 dpi, and spinal cord at 8-10 dpi. We next conducted bulk RNA sequencing on tissues of the lower and upper reproductive tract, draining lymph nodes, and spleen over the infection time course. Innate immune induction in the lower female reproductive tract - as measured by differential gene expression - followed a biphasic pattern, with a rapid response via the RIG-I pathway followed by a subsequent induction of genes downstream of types I and III IFN. Complimentary co-expression analysis of the entire LRT gene set identified a suite of keratinization and cornification genes upregulated in infected animals as early as 1 dpi that may suggest an acceleration in programmed epithelial cell death following ZIKV infection.
Discussion: Homeostatic cell renewal in stratified squamous epithelial tissues requires suppression of inflammation, and the keratinization modules we identified were associated with an upregulation of genes that regulate inflammatory signaling, apoptosis, and necroptosis. Ongoing single-cell RNAseq analysis aims to discern the role of RIG-I-directed responses in epithelial cells of the vaginal mucosa and to determine the potential role of tissue-specific programmed cell death in controlling ZIKV infection. At this time, we hypothesize that accelerated keratinization serves as a host-directed effort to shed infected cells rapidly, and that ZIKV persistence in the FRT and dissemination to other sites may benefit from the resultant anti-inflammatory state.
Disclosure of Interest: None Declared

LT213

A SMALL-MOLECULE RIG-I AGONIST ENHANCES VACCINE PROTECTION AGAINST PANDEMIC AND HIGHLY PATHOGENIC AVIAN INFLUENZA A VIRUS INFECTION

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Abstract Content: Despite the constant threat of pandemic influenza virus emergence, vaccines against influenza remain suboptimal in eliciting an effective adaptive immune response. Novel strategies to develop new adjuvants may aid in increasing the functionality of currently existing influenza virus vaccines. In order to address this need, we leveraged knowledge of host innate immune signaling pathways to develop a novel small-molecule RIG-I agonist, KIN1148, to adjuvant vaccination against pandemic H1N1 (pH1N1) or highly pathogenic avian influenza (H5N1). C57Bl/6 mice were vaccinated intramuscularly (i.m.) with inactivated split vaccine against pH1N1 or H5N1 and adjuvanted with formulated KIN1148 or vehicle. Thirty days following vaccination mice were challenged with homologous virus for survival studies or the lungs were harvested at day 5 p.i. for analyses of immune response. Alternatively, mice were administered two homologous vaccinations on days 0 and 14 with immune response assessment of serum, dLN, and/or spleen performed on day 19. Our results demonstrate that KIN1148 binds to and activates RIG-I leading to the activation of IRF3 and innate immune genes. *Ex vivo* treatment of dendritic cells with KIN1148 leads to cellular activation and maturation. Administration of KIN1148 with pH1N1 or H5N1 i.m. vaccination significantly increased IAV-specific antibody responses compared to vaccination alone, and led to an increase in HI titers against other H5 HAs. Intriguingly, KIN1148 also induced IAV-specific CD4 and CD8 T cell responses following vaccination, a unique and cross-protective cellular immune response not typically observed downstream of i.m. split vaccination. This broad humoral and cellular immune response induced following a single vaccination with KIN1148 led to enhanced protection during high dose pH1N1 or H5N1 challenge and reduced virus titers and pathology in the lungs. In sum, KIN1148 administered with pH1N1 or H5N1 vaccination leads to the induction of a broad and enhanced anti-IAV immune

responses compared to vaccination alone. Overall, these results suggest targeting of the RIG-I pathway typically activated during infection to confer immunity with a small molecule compound can enhance vaccine protection, highlighting the potential of KIN1148 to enhance vaccine-induced protection against RNA virus infection.
Disclosure of Interest: None Declared

LT214

IFN-LAMBDA INDUCTION BY MURINE ASTROVIRUS REFLECTS GOBLET CELL TROPISM AND IS RECAPITULATED BY ENTEROID CULTIVATION

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Abstract Content: Despite being a global cause of viral gastroenteritis in children, astroviruses are understudied due to the lack of a well-defined small animal model. Murine astroviruses (muAstVs) chronically infect immunodeficient mice, but an *in vitro* cultivation system and understanding of their *in vivo* pathogenesis has been lacking. Here, we describe a platform to cultivate muAstV using air-liquid interface (ALI) cultures derived from mouse enteroids. The ALI system supports apical infection of and viral release by epithelial cells. MuAstV also stimulates IFN-λ production in ALI, recapitulating our *in vivo* findings. We report that chronic muAstV infection in immunodeficient mice is predominantly in the small intestine, is microbiota-independent, and correlates with higher IFN-λ expression. We demonstrate that goblet cells and presumptive enterocytes are the *in vivo* targets for chronic muAstV infection. During chronic infection, muAstV stimulates IFN-λ production in goblet cells, in turn inducing widespread ISG expression in the intestinal epithelium. Collectively, these findings provide insights into the cellular tropism for and innate immune responses to muAstV, and demonstrate an enteroid-based culture system to readily propagate muAstV *in vitro*.
Disclosure of Interest: None Declared

LT215

IDENTIFICATION OF A TRANSMISSIBLE EARLY LIFE GAMMA DELTA INTRAEPITHELIAL LYMPHOCYTE HYPERPROLIFERATIVE PHENOTYPE THAT IS ASSOCIATED WITH CHANGES IN THE INTESTINAL MICROBIOME

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Abstract Content: Intestinal intraepithelial lymphocytes expressing the γδ T cell receptor (γδ IEL) function as a first line of defense against injury or microbial invasion. The majority of the γδ IELs express the Vγ7 T cell receptor (TCR); however, the epithelial compartment also contains Vγ7$^-$ cells typically found in the periphery. Given that the intestinal microbiota can induce tonic type I interferon (IFN) and interferon a/b receptor (IFNAR) signaling is a known regulator of lamina propria lymphocyte populations at steady-state, we hypothesized that microbiota-induced IFNAR signaling also influences γδ IEL homeostasis. Morphometric analysis of jejunum from 8-week-old GFP γδ T cell reporter mice (TcrdEGFP, WT) and TcrdEGFP; IFNAR KO mice showed that the number of GFP$^+$ γδ IELs was increased 2-fold in IFNAR KO mice compared to WT. Administration of 5-Ethynyl-2´-deoxyuridine (EdU) revealed that γδ IELs isolated from IFNAR KO mice exhibit a 50% increase in proliferation compared to WT. Surprisingly, the relative proportion of γδ IELs was skewed toward Vγ7$^-$ subsets in IFNAR KO mice. EdU incorporation within each Vγ subset revealed that IFNAR KO mice exhibit a 66% and 22% increase in proliferation in Vγ7$^-$ and Vγ7$^+$ IEL populations, respectively, relative to WT. Bulk TCR sequencing and clonotype analysis of γδ IELs isolated from WT and IFNAR KO mice showed polyclonal TCRγδ repertoires, indicating that the expanded γδ IEL population was not due to clonal expansion. We observed a 35-fold increase in γδ IELs in IFNAR KO mice one week after birth, indicating that this phenotype begins early in life. Interestingly, IFNAR KO mice rederived into a murine norovirus (MNV)-free facility had normal γδ IEL compartment similar to WT mice, indicating that loss of type I IFN signaling alone is not sufficient to induce the hyperproliferative phenotype, but that an environmental factor may also be involved. To test this, MNV$^-$ IFNAR KO breeding pairs were housed with dirty bedding from IFNAR KO mice from our barrier facility. The mice born from these pairs had increased numbers of γδ IELs, indicating that the γδ IEL hyperproliferative phenotype is transmissible and may be independent of IFNAR signaling. To control for maternal effects on the microbiome, we crossed separately-housed WT and IFNAR KO mice from our barrier facility to generate F2 littermates. Adult F2 WT mice had a 2-fold increase in the number of γδ IELs, similar to parental SH IFNAR KO mice. Further, F2 WT γδ IELs exhibited a 71% increase in proliferation compared to SH WT. Fecal 16S rRNA sequencing analysis showed a greater diversity in SH IFNAR KO and F2 littermates compared to SH WT or MNV$^-$ controls (Shannon Index, $p<0.01$). While the precise contribution of IFNAR signaling and the intestinal microbiome remains to be determined, we have identified a novel, vertically-transmitted γδ IEL hyperproliferative phenotype that develops during early life prior to weaning.
Disclosure of Interest: None Declared

LT216

THE CYCLIC DINUCLEOTIDES-SENSING INDEPENDENT FUNCTION OF HAQ STING IN BODY METABOLISM

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Abstract Content: The human R71H-G230A-R293Q (HAQ) STING allele is common in non-Africans. The population frequencies for HAQ ranges from ~23% in

Caucasians to ~63% in East Asians. However, HAQ is extremely rare in Africans (<1%). In contrast, ~40% of Africans have the AQ (G230A-R293Q) STING, whose population frequency in non-Africans is <1%. HAQ STING evolved from the AQ STING. The modern anatomically humans migrated from Africans about ~40,000 years ago. The rapid replacement of AQ-STING by the HAQ-STING allele in non-Africans suggests a strong natural selection for HAQ in early human migration out of Africa. STING senses cyclic dinucleotides (CDNs) including bacterial cyclic di-GMP, cyclic di-AMP and mammalian 2'3'-cyclic GMP-AMP. We previously showed that the HAQ STING is defective in responses to cyclic dinucleotides (CDNs) sensing *in vivo* and *in vitro* (Jin et al., 2011; Patel et al., 2017; Sebastian et al., 2020). To address the question of why HAQ replaced AQ, we generated an AQ knock-in mouse. We found that AQ mice responded to CDNs similarly to the WT mice. Interestingly, we noticed that mature adult AQ mice had less body weight than the HAQ or WT mice on a normal diet. STING$^{-/-}$ mice had similarly lean phenotypes as the AQ mice. Thus, the evolution of AQ to HAQ STING, while resulted in the loss of CDNs sensing ability, restored STING function in body metabolism, which appeared to be essential for early humans' survival. We will present data further characterizing this previously unrecognized, CDNs-sensing independent function of STING in calibrating body metabolism. We propose that the de facto role of STING in humans is to control body metabolism.

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Disclosure of Interest: None Declared

LT217

GUT EPITHELIAL IL-27 CONFERS INTESTINAL IMMUNITY THROUGH THE INDUCTION OF INTRAEPITHELIAL LYMPHOCYTES

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Abstract Content: IL-27 is known to control a diverse range of immune responses in many different disease settings. Multiple cell types are capable of expressing IL-27 with innate immune cells considered to be the dominant cellular sources. Here, we identify intestinal epithelial cells (IECs) as one of the major IL-27-producing cell types in the gut-associated tissue. Whereas IL-27 expression in conventional dendritic cells (DCs) or myeloid cells is respectively required for the differentiation of a specialized regulatory T (Treg) cell subset and IL-10 production, gut epithelial IL-27 specifically promotes a distinct CD4CD8αα intraepithelial lymphocyte (IEL) population that acquires their functional differentiation upon arrival at the intestinal epithelium. Either loss of IL-27 in IECs or disruption of its receptor in T cells could lead to a selective defect in CD4CD8αα IELs over time. Consequently, mice with IEC-specific IL-27 ablation exhibited uncontrolled pathogen burden during parasitic infection and this could be rescued by transfer of exogenous CD4CD8αα IELs. Collectively, our data reveals that in addition to its known regulatory properties in preventing immune hyperactivity, gut epithelial IL-27 confers barrier immunity through inducing a specific IEL subset and further suggests that IL-27 derived from different cellular sources plays distinct roles in maintaining intestinal homeostasis.

Disclosure of Interest: None Declared

LT218

DEVELOPMENTAL PATHWAYS REGULATE CYTOKINE-DRIVEN EFFECTOR AND FEEDBACK RESPONSES IN THE INTESTINAL EPITHELIUM

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Abstract Content: The intestinal tract is a common site for infection, and it relies on an appropriate immune response to defend against pathogens. The intestinal epithelium has an important role in effector responses, which is coordinated by immune-type specific cytokines. It is incompletely understood how cytokines drive epithelial responses. Here, using organoid-cytokine co-cultures, we provide a comprehensive analysis of how key cytokines affect the intestinal epithelium, and relate this to *in vivo* infection models. We use image analysis based on a convolutional neural network to automatically categorize organoids into "spheroid" and "budding". We show that these categories correlate to the maturity of organoids and that cytokines affect the relative percentage of these categories. Furthermore, we use transcriptomic analysis to reveal that cytokines use developmental pathways to define intestinal epithelial effector responses. For example, we find that IL-22 and IL-13 dichotomously induce goblet cells, in which only IL-13 driven goblet cells are associated with NOTCH signaling. We further show that IL-13 induces BMP signaling to act as a negative feedback loop in IL-13 induced tuft cell hyperplasia, an important aspect of type 2 immunity. Together, we show that targeting developmental pathways may be a useful tool to tailor epithelial effector responses that are necessary for immunity to infection.

Disclosure of Interest: None Declared

LT219

A NOVEL INTERFERON-BETA SIGNALING PATHWAY CONTROLS THE TOLEROGENIC PROGRAM IN PULMONARY TNFR2+ CDC2 SUBSET IN VIVO

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Abstract Content: Introduction: Dendritic cells (DCs) play a vital role in maintaining lung immune tolerance by generating regulatory T cells (Tregs). Lung tolerance must be tightly controlled to avoid the development of chronic inflammatory lung diseases. We recently identified the tolerogenic TNF**R2**$^{+}$ c**D**C**2** subset (**R2D2**) in the lung that induces regulatory T cells and maintains lung immune tolerance (Mansouri et al., 2020)(PMID: 31959883). R2D2 require type I interferon signaling to induce Tregs. Type I IFNs, including IFNb and IFNa, signal through the shared IFANR1/IFNAR2 receptors, however their signaling outcomes can have different outcomes. Here, we sought to delineate the tolerogenic IFNb signaling in R2D2.
Methods: To identify the molecular mechanisms responsible for the tolerogenic IFNb signaling *in vivo*, various DC-specific conditional knock-out mice were intranasally treated with IFNb, IFNa, aIFNAR1 and aIFNAR2. Characterization of lung DCs was examined by flow cytometry 24 hours after treatment. Induction of Tregs was examined by flow cytometry on day 14.
Results: We have delineated a non-canonical signaling pathway in which IFNb activates the anti-inflammatory properties of the tolerogenic R2D2. Interestingly, inhaled IFNb and IFNa had opposing effects on R2D2. IFNb promoted the tolerogenic program in R2D2 and enhanced Treg generation. IFNa repressed the tolerogenic program and inhibited Treg induction in the lung. Furthermore, IFNb signaling acts through IFNAR1, not IFNAR2, to generate Tregs in the lung. We will present data further characterizing the non-canonical IFNb-IFNAR1 signaling pathway in the tolerogenic R2D2 cells.
Conclusions: We delineated an IFNb-specific signaling pathway in the lung tolerogenic DC subset that is essential for maintaining lung tolerance. These findings elucidate a fundamental mechanism controlling lung tolerance and identifies a strategy to restore lung tolerance in inflammatory lung diseases.
Disclosure of Interest: None Declared

LT221

THE PERSISTENCE OF NONTYPEABLE HAEMOPHILUS INFLUENZAE FUELS TYPE 17 IMMUNITY IN THE LUNG

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Abstract Content: Introduction
Cystic fibrosis (CF) and chronic obstructive respiratory disease (COPD) are characterized by a pulmonary inflammatory status coordinated by a network of cytokines, often associated with bacterial colonization. Nontypeable *Haemophilus influenzae* (NTHi) is a bacterium commonly isolated from the airways of patients that suffer from CF or COPD. However, to what extent NTHi persistence contributes to the lung inflammatory burden during chronic airway disease is controversial. Here, we aimed at determining the pro-inflammatory role of NTHi persistence in a cohort of CF patients and in a newly generated mouse model of NTHi persistence.
Methods
Nasopharyngeal aspirates from 19 CF patients with variable genotypes were collected from the Regional CF Center at Milan's Ospedale Maggiore Policlinico during routine care visits, and levels of CF pro-inflammatory cytokines were evaluated by ELISA.
Long-term chronic infection was established with NTHi-embedded agar beads by adapting a murine model previously described for other pathogens (Lorè NI et al, Sci. Rep. 2016). Cytokines levels and lung infiltrating cells were evaluated by ELISA, flow cytometry and histological analysis during the development of chronic infection (2 and 14 days).
Results
In our study cohort, we found that CF patients chronically colonized by NTHi had significantly higher levels of IL-8 and CXCL1 than those who were not colonized. To better define the impact of NTHi persistence in fuelling the airway inflammatory response, we developed a novel mouse model using both laboratory and CF clinical strains. NTHi persistence associated with a sustained inflammation of the lung, characterized by recruitment of neutrophils and by the release of related pro-inflammatory cytokines (KC, Mip-2, G-CSF, IL-6) at 2 and 14 days post-infection. Moreover, an increased burden of T cell mediated response (CD4^{+} and γδ T cells) was observed in the lungs at 14 days post-infection. We also found that both CD4^{+}IL-17^{+} cells and levels of IL-17A cytokine (IL-17A and IL-17F) were enriched in mice at advanced stage of NTHi chronic infection, in association with higher levels of matrix metalloproteinase 9 as a marker of tissue remodelling.
In addition, we were able to demonstrate by immunohistochemistry that that CD3, B220 and CXCL-13 expressing cells co-localized in bronchus-associated lymphoid tissues(BALT)-like structure in infected lungs at day 14 post-infection.
Conclusion
Our results demonstrate that NTHi persistence exerts a pro-inflammatory activity, mediated by type 17 immunity, and could therefore contribute to the exaggerated burden of lung inflammation in patients with chronic respiratory diseases.
Disclosure of Interest: None Declared

LT222

INTERFERON-LAMBDA RECEPTOR 1 EXPRESSION IS DECREASED IN THE SMALL INTESTINE OF PEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS

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Abstract Content: Introduction: Type III interferons (IFN-lambdas, IFN-λs) are important antiviral cytokines that modulate immune responses by acting through a unique IFN-λR1/IL-10R2 heterodimeric receptor that is highly expressed on epithelial cells especially at mucosal surfaces. Mouse colitis models clearly demonstrate *Ifnlr1* deficiency leads to exacerbated inflammation, while Ifn-λ treatment promotes gut barrier integrity and ameliorates colitis in wildtype mice. With little 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 pediatric IBD patients.

Methods: IFN-λR1 expression was assessed by immunohistochemistry of terminal ileum formalin-fixed paraffin-embedded biopsies from healthy (n=8), Crohn's disease (n= 7), and ulcerative colitis (n=10) pediatric patients, along with healthy patient liver biopsies (antibody positive control). Fresh patient biopsies (terminal ileum) were cultured *ex vivo* in media with or without IFN-λ3 overnight and gene expression was quantified by RT-qPCR.

Results: A novel monoclonal antibody was identified that accurately stains IFN-λR1 in human mucosal tissue samples for the first time. We found distinct IFN-λR1 staining in intestinal epithelial cells (average 74% positive), but also in specific immune cells, which was not previously described in mouse models. This was corroborated by examining single cell RNA sequencing datasets. There was a significantly lower percentage of epithelial and immune cells positive for IFN-λR1 in pediatric Crohn's disease and ulcerative colitis patients compared to pediatric healthy controls ($P<0.01$). IFN stimulated gene (ISG) transcript levels also differed between healthy and IBD patients after IFN-λ3 stimulation of fresh biopsies.

Conclusion: Our novel finding of decreased expression of IFN-λR1 in pediatric IBD compared to healthy patients indicates patients may be less able to induce critical IFN-λ-mediated antiviral responses and protective anti-inflammatory pathways. This work supports the possibility for new therapeutic approaches to promote IFN-λ signaling to improve regulation of inflammatory pathways in pediatric IBD patients.

Disclosure of Interest: None Declared

LT223

NEWLY ISOLATED PORCINE EPIDEMIC DIARRHEA VIRUS RESISTANCE TO INTERFERON AND NEUTRALIZING ANTIBODY

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Abstract Content: Porcine epidemic diarrhea virus (PEDV) infects pigs and causes enteric disease characterized by watery diarrhea and dehydration. Despite the extensive vaccination, PEDV strains with variations in the spike gene have been reported in several countries, including Korea, China, and the United States. Variant PEDV belonging to genogroup G2 has higher pathogenicity and morbidity than conventional PEDV belonging to genogroup G1. To understand the pathogenesis of the variant PEDV, we examined the susceptibility of the variant PEDV to interferon and PEDV specific neutralizing antibodies, which are known to important for the control of PEDV infection. We found that variant PEDV (WG strain) showed higher resistance to interferon than conventional PEDV (KPEDV-9 strain). Similarly, KPEDV-9 strain grew well in interferon-deficient Vero cells but did not grow in interferon-releasing porcine alveolar macrophages, whereas WG strain showed similar infectivity in both cells. We also found that WG strain is not blocked by the neutralizing antibodies against KPEDV-9, suggesting the differences in the antigenicity of two strains. To elucidate the possible mechanism of resistance to interferon and neutralizing antibody in variant PEDV, sequencing analysis was performed. We found huge variations in spike and ORF3 proteins. To test the impacts of these mutations on the acquisition of interferon and neutralizing antibody resistance, we are currently generating pseudotyped PEDV and recombinant PEDVs with mutations. Our results indicate that the recently introduced variant PEDV exhibits higher resistance to the interferon than the existing PEDV and avoids the neutralizing antibody against the existing PEDV. These results also provide understanding of the occurrence of variant PEDV and its pathogenesis.

Disclosure of Interest: None Declared

LT224

INFLUENZA-INDUCED MONOCYTE-DERIVED ALVEOLAR MACROPHAGES CONFER PROLONGED ANTIBACTERIAL PROTECTION VIA INTERLEUKIN-6

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Abstract Content: Despite the prevalence and clinical importance of influenza, its long-term effect on lung immunity is unclear. Here we describe that following viral clearance and clinical recovery, at 1 month after infection with influenza, mice are better protected from*Streptococcus pneumoniae* infection due to a population of monocyte-derived alveolar macrophages (AMs) that produce increased interleukin-6 (IL-6). We show that IL-6 is dispensable for antibacterial protection in naïve mice, but

crucial for enhanced protection in influenza-experienced mice. Influenza-induced monocyte-derived AMs have a surface phenotype similar to resident AMs but display a unique functional, transcriptional and epigenetic profile that is distinct from resident AMs. In contrast, influenza-experienced resident AMs remain largely similar to naive AMs. Over longer time periods, recruited AMs become transcriptionally more similar to resident AMs. Thus, influenza changes the composition of the AM population to provide prolonged antibacterial protection. Monocyte-derived AMs persist over time but lose their protective profile. Our results help to understand how transient respiratory infections, a common occurrence in human life and a feature of the current COVID-19 pandemic, can constantly alter lung immunity by contributing monocyte-derived, recruited cells to the AM population.
Disclosure of Interest: None Declared

LT225

T HELPER 2 CELL RESPONSES ARE DEPENDENT ON TISSUE-RESIDENT BASOPHILS AND THE NOTCH SIGNALING PATHWAY IN BASOPHILS DURING HELMINTH-INDUCED TYPE 2 INFLAMMATION

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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. Basophils are innate immune cells that accumulate in the intestine during infection with the helminth *Trichuris muris*, and are potent producers of inflammatory mediators. However, the molecular mechanisms that control basophil function and gene expression during helminth-induced type 2 inflammation have been unclear. We previously showed that during *T. muris* infection, basophils upregulated the Notch signaling pathway, which regulates gene expression programs during development and inflammation. Transcriptional profiling of Notch-deficient basophils revealed that Notch directs basophil responsiveness to inflammatory cues and effector gene expression. *In vivo*, basophils displayed decreased interaction with $CD4^+$ cells in the cecum. Consequently, mice lacking basophil-intrinsic Notch signaling had reduced $Gata3^+$ T helper 2 cells in the intestinal site and decreased type 2 inflammation following *T. muris* infection, resulting in impaired worm clearance. We are now investigating the role of productive basophil-T cell interactions in driving fulminant type 2 immunity in the intestine. These studies highlight that inhibition of Notch signaling in basophils inhibits infection-induced gene expression changes in $CD4^+$ T cells, while *in vitro* coculture experiments demonstrate that basophils drive Th2 responses in $CD4^+$ T cells. These findings broaden our understanding of type 2 immunity, providing important insights for development of effective therapeutics aimed at this arm of host defense.
Disclosure of Interest: None Declared

LT226

IL-17 DEPENDENT FIBROBLASTIC RETICULAR CELLS SET THE THRESHOLD FOR PROTECTIVE ANTIBODY PRODUCTION IN THE GUT

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Abstract Content: Attaching/Effacing (A/E) bacterial infection of the intestine is a significant cause of mortality and morbidity worldwide. Bacteria specific antibody production are critical for clearing the murine A/E bacteria, *Citrobacter rodentium*. Interleukin 17 (IL-17) promotes B-cell activation and T cell-dependent antibody responses in autoimmune models, and is known to contribute to *C. rodentium* clearance. Here we report that IL-17 acts via lymph node fibroblastic reticular cells (FRC) to drive *C. rodentium* specific antibody production. Mice with FRC-restricted IL-17RA deletion ($CCL19^{Cre}IL17RA^{fl/fl}$), had reduced germinal center (GC) formation in gut-draining LN, and reduced *C. rodentium*-specific antibody in both serum and feces, along with higher *C. rodentium* burden and increased colon inflammation. Besides, loss of IL-17RA specifically in FRC causes defective protective immunity against N, and reduced *C. rodentium*-specific antibody in both serum and feces, along with higher *C. rodentium* re-infection. On the other hand, previous IL-17 dependent activation of gut-draining LN FRC by inducing mild colitis with dextran sodium sulfate enhanced anti-*Citrobacter* antibody production, leading to quicker *C. rodentium* clearance and less colon inflammation. In conclusion, these data demonstrate an important mechanism for IL-17 in mucosal immunity, acting indirectly through LN stromal cell activation to promote protective antibody responses.
Disclosure of Interest: None Declared

LT227

MODULATING IFNG PRODUCTION AS A NEW EFFECTIVE WAY IN COMBATING RECURRENT UTI

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Abstract Content:
Urinary tract infection (UTI) is one of the most common bacteria infections, mostly initiated by uropathogenic *E. coli* (UPEC) invading bladder. UTI is mainly affecting women, young children and aging population, and it was estimated that about 50% of women will get at least one UTI during lifetime. Besides its high incidence rate, high recurrence rate is another major problem of UTI. Various clinical research showed that 27% to 44% of UTI patients will have UTI recurrence. Actually, a previous history of UTI is widely accepted as a strong risk factor for another UTI in clinics. This is striking, considering that bacteria infections in other body sites such as lung and gut have relatively low recurrence rate, as adaptive immunity activated during the initial infection should establish memory preventing infections of the same pathogen. Due to incomplete understanding of the adaptive immunity of bladder, no effective treatment is available in preventing recurrent UTI.

To get a better understanding of bladder adaptive immunity and to develop an effective way preventing recurrent UTI, we studied CD4 T cells, a pivotal regulator of adaptive immunity, in bladder. Previous studies on bladder CD4 T cells failed to determine their function in bladder due to lack of proper tools. With newly developed tools, such as cytokine reporter mice, several knock-out mice and bladder transplantation technique, we are able to study the dynamics of cytokine secretion from bladder CD4 T cells and determine their function. We found that both IFNg secreting Th1 cells and IL4 secreting Th2 cells are activated during UTI, and their activation is potentially modulated by bladder microbiota. However, the percentage of IL4 secreting Th2 cells is significantly higher than the percentage of IFNg secreting Th1 cells. Those bladder Th2 cells are induced to repair bladder epithelium damaged during UTI, but they are inhibiting bacterial clearance. The Th2 biased adaptive immunity was further reinforced during the second and third infections. Consequently, the bladder obtains an abnormal structure due to overwhelmed IL4 mediated epithelium repair and an inhibited bacterial clearance capability due to reduced IFNg secretion. We further identified that these cytokine secreting activities were modulated by a novel bladder specific OX40L+CD301b+ dendritic cell. Knowing CD4 T cells didn't contribute to bladder clearance during recurrent UTI because of inhibited IFNg secretion, we explored whether we can enhance the IFNg production from CD4 T cells by immunization to combat recurrent UTI. By using UPEC antigens and different adjuvants prone to induce Th1 cells such as CpG and IL-12, we successfully increased IFNgproduction in bladder and enhanced bacterial clearance during recurrent UTIs. Mechanism study on CpG induced IFNg mediated bacterial clearance found out that it is not dependent on antibody production. Considering that no effective UTI vaccine is available now and most of the attempts are focusing on inducing high antibody production, inducing local IFNg secretion by CD4 T cells is a new effective method for combating UPEC in patients with one or recurrent UTIs.

Disclosure of Interest: None Declared

Lightning Talk Session 3: SARS-CoV-2 or COVID-19

LT228

ΔACE2: A NOVEL PRIMATE-SPECIFIC INTERFERON-STIMULATED GENE AND ITS POTENTIAL ROLE IN COVID-19

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Abstract Content: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes COVID-19, utilizes angiotensin-converting enzyme 2 (ACE2) for entry into target cells. *ACE2* has been proposed as an interferon-stimulated gene (ISG). Thus, IFN-driven variability in *ACE2* expression levels or functionality could be important for susceptibility to COVID-19 and its outcomes. Here, we report the discovery of a novel, N-terminally truncated primate-specific isoform of ACE2, designated as deltaACE2 (dACE2), which we demonstrate is an ISG but not ACE2. In The Cancer Genome Atlas (TCGA), *dACE2* expression was enriched in squamous epithelial tumors of the respiratory, upper gastrointestinal, and urogenital tracts. *In vitro*, dACE2, which lacks 356 aa of the peptidase domain, neither interacted with the SARS-CoV-2 spike protein nor affected binding between the spike protein and ACE2. In contrast with ACE2, the carboxypeptidase activity of dACE2 was undetectable. The existence of two functionally distinct ACE2 isoforms reconciles several functional properties previously attributed to ACE2, with dACE2 being an ISG, and ACE2 being the SARS-CoV-2 entry receptor and carboxypeptidase. We propose that the ISG-type induction of dACE2 is unlikely to affect the cellular entry of SARS-CoV-2 and would not promote infection. Our results warrant further studies on other functions of dACE2 and their potential relevance for COVID-19.

Disclosure of Interest: None Declared

LT232

SARS-COV-2 RECEPTOR ACE2 IS AN INTERFERON-STIMULATED GENE: IMPLICATIONS FOR CYTOKINE REGULATION OF DIVERGENT HOST OUTCOMES DURING COVID-19

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Abstract Content: There is pressing urgency to understand the pathogenesis of the severe acute respiratory syndrome coronavirus clade 2 (SARS-CoV-2) which causes the disease COVID-19. SARS-CoV-2 spike (S)-protein binds ACE2, and in concert with host proteases, principally TMPRSS2, promotes cellular entry. The cell subsets targeted by SARS-CoV-2 in host tissues and how local cytokine activity influences divergent host outcomes, remain unknown. Importantly, the COVID-19 pandemic has disproportionately affected Black, Indigenous, and Latinx communities which are significantly underrepresented in biomedical research. Here, we leverage pre-COVID-19 human, non-human primate, and mouse single-cell RNA-sequencing (scRNA-seq) datasets across health and disease to uncover the putative targets of SARS-CoV-2 amongst tissue-resident cell subsets and guide our ongoing collection and analysis of COVID-19 samples. We identify *ACE2* and *TMPRSS2* co-expressing cells within lung type II pneumocytes, ileal absorptive enterocytes, and nasal goblet secretory cells. Strikingly, we discover that *ACE2* is a human interferon-stimulated gene (ISG) *in vitro* using airway epithelial cells, and extend our findings to *in vivo* viral infections. Our data suggest that SARS-CoV-2 could exploit species-specific interferon-driven upregulation of *ACE2*, a tissue-protective mediator during lung injury, to enhance infection.

In ongoing work, our group is using scRNA-seq to characterize the direct cellular targets of viral infection, and bystander responding cells, from the nasal mucosa of a predominantly Black cohort (enrollment n=15 non-infected controls and n=60 SARS-CoV-2+) of COVID-19 patients. We have successfully started to profile this cohort, illustrating technical feasibility, and will report on all of our collected data through November 1st, as well as making all cell x gene matrices publicly available on a rolling basis before and beyond Cytokines 2020. We hypothesize that the intrinsic response of *ACE2+TMPRSS2+* goblet secretory cells to infection, as well as their interactions with innate and adaptive immune cells in the nasal mucosa, play a crucial role in limiting COVID-19 disease severity. Identifying whether and how upper airway host-virus interactions specify divergent outcomes is essential to understand SARS-CoV-2 pathogenesis and identify treatments and vaccine strategies uniquely suited to individuals suffering from COVID-19.

Disclosure of Interest: None Declared

LT233

THE KINASE DYRK1A CONTROLS TRAF3 TURNOVER AND IS POTENTIALLY MODULATED BY SARS-COV-2

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Abstract Content: Progression of SARS-CoV-2 infected patients to life threatening disease may result from a virus-mediated, dysregulated immune response associated with excessive production of inflammatory cytokines, cytokine release syndrome. Coronaviruses are known to suppress the innate, type I interferon (IFN-I, IFNαβ) response by targeting TRAF3, a critical switch point for the induction of IFN-I by TNF receptors, innate sensors (TLRs, RLRs) and inflammatory cytokines (IL1β, IL18). Viral inactivation of TRAF3 can limit IFN-I secretion, leading to dysregulated cytokine production and a hyper-inflammatory state. Using a multiomic screen we discovered a novel set of antiviral proteins within the LTβR-TRAF3 pathway that are potentially involved in controlling LTβR-TRAF3->IFN-I response (Virgen-Slane et al., 2020 J. Immunol). At least two conserved SARS-CoV-2 proteins (Nsp3/PLpro and Nsp9) target the novel components in the TRAF3 interactome. Nsp3/PLpro deubiquitinates TRAF3 suppressing activation of NIK and IFN-I. Nsp9 targets DCAF7 (DDB1 and CUL4 E3 Ub ligase associated factor 7) that forms a complex with DYRK1A (Dual-specificity tyrosine phosphorylation regulated kinase 1A). DYRK1A has been shown to be involved in the development of B and T cells, however specific pathway contribution and the functional role remain elusive. We show that DYRK1A specifically binds to TRAF3 protein and regulates the stability of TRAF3. In the absence of Dyrk1A, Traf3 protein is abnormally degraded and induces the survival of germinal center B cells. Further, Dyrk1A-deficient B cells have higher non-canonical NF-κB signaling. Together, we show a crucial regulatory role for DYRK1A in TRAF3 and TNFR-mediated signaling pathways. These pathways may play important roles during the infection with SARS-CoV-2.

Disclosure of Interest: None Declared

LT234

COVID19 AND ENDOCRINOLOGY

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Abstract Content: The unexpected appearance of the viral infection COVID-19, with tremendous global consequences, creates thoughts and concerns to doctors and researchers, regarding the possible impacts on other systems of the organism.Various researches, show that an interesting correlation seems to exist between the COVID-19 infection and particular endocrine and metabolic pathways. Those pathways are related to the existence of the ACE2 receptor, Additionally, a correlation seems to exist between COVID-19 and production of proinflammatory factors or cytokines. We know that cytokines and especially **Interleukin 6 (pleiotropic),** are produced in various endocrine systems and glands throughout the organism, as well as in other organs.

A further examination of those endocrine and metabolic pathways might be important in understanding, dealing and finding the disease severity markers, as well as in studying the genetic differences between the two sexes or between different populations.

the- 2 (ACE2 / the equivalent of the known ACE, which converts Ang I to Ang II) .Normally, ACE2 cleaves Angiotensin II (Ang II) to Angiotensin (1-7) and consequently converts Ang I to Ang (1-9). ACE2 receptors play a role in the pathogenesis of hypertension and diabetes mellitus, and they are expressed in the epithelial cells of many organs.

Angiotensin II stimulates the release of the antidiuretic hormone (ADH), the adrenocorticotropic hormone (ACTH), the prolactin, the luteinizing hormone (LH), the oxytocin, the

aldosterone and others. However, it is important that drugs, such as diuretics, mineralocorticoids, glucocorticoids, estrogens, oral contraceptives, adrenocorticotropic hormone, sodium, potassium, as well as the body posture during blood collection, influence Angiotensin II levels. In COVID-19, the levels of Angiotensin II appear to increase.
If there is an involvement, the question is what is the importance of the neuroendocrine system and the endocrine glands, (e.g. thyroid, adrenal, hypothalamus, pituitary, fatty tissue of the pancreas), and hormones, (such as prolactin, estrogens, testosterone, pituitary hormones, thyroxin, insulin, various prohormones, etc.), and the genital systems of the two sexes, (ovaries, testes), as well as the involvement in the pathophysiology of various diseases (e.g. metabolic syndrome, type 2 diabetes mellitus, insulin resistance, thyroid disorders, Addison, hypertension, obesity, etc.) lThe Hypothalamic – Pituitary – Adrenal axis is a particularly intricate complex, with their interaction as feedback. This axis and the interaction of the three, compose the neuroendocrine system, defining and regulating the responses of many biological procedures of the organism, including stress mechanism, metabolism, defensive system, affect, emotions, sexuality and energy storage.
The Hypothalamic – Pituitary – Adrenal (HPA) axis, the Hypothalamic – Pituitary – Gonadal (HPG) axis, the Hypothalamic – Pituitary – Thyroid (HPT) axis and the Hypothalamic – Neurohypophyseal system, form the four neuroendocrine systems, through which the hypothalamus and the pituitary, guide and perform the neuroendocrine functions.
Hormones Potentially, these neuroendocrine axes are involved, through the cytokines and other proinflammatory factors, and play an important role in the pathophysiology and the development of a hyperreaction of the immune system and the evolution of the disease, triggered by coronavirus infection, and this should be further investigated.
Disclosure of Interest: None Declared

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INTERFERON GAMMA MAY BE A CRITICAL TARGET FOR CHRONIC STRESS-ASSOCIATED SARS-COV-2 DISPARITIES

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Abstract Content: COVID-19, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a global pandemic that has exacerbated existing cardiometabolic disease disparities in the US and across the globe. SARS-CoV-2 mortality has been seen to disproportionately affect individuals who live in lower resourced neighborhoods, are of lower socioeconomic status (SES), and are of minority background; these factors are associated with increased chronic stress-related neural activity measurable by amygdala activity in 18FDG-PET/CT scans. Yet, chronic stress-related neural activity and its underlying mechanism has not been explored in the context of SARS-CoV-2 disparities. In this study, 60 African American individuals without SARS-CoV-2 were recruited to the NIH Clinical Center (n=60 , 93% female, median age=60.8, median BMI=33.0). Amygdala activity (AmygA) was measured by standard uptake value of 18FDG-PET/CT and plasma cytokines (IFNγ, TNFα) were measured using an ELISA-based multiplex technique. Coronavirus mortality risk scores (CMRS), a score indicative of potential infection-related death if the participants were to contract SARS-COV-2, were calculated based on U.S. available data. Linear regression modeling was performed to identify associations between each individual's amygdala activity, cytokine serum level, and CMRS while adjusting for body mass index (BMI). Increasing AmygA was associated with increasing TNFα and IFNγ (ß=0.43, p=<0.01 and ß=0.31, p=0.02, respectively). In addition, IFNγ associated with CMRS (ß=0.39, p=<0.01) but TNFα only trended towards significance (ß=0.24, p=0.06). After adjusting for both neighborhood- and individual-level SES, the association with CMRS holds true for IFNγ (ß=0.32, p=0.04), but is eliminated in TNFα (ß=0.22, p=0.16), suggesting that the association between TNFα and CMRS is dependent on SES. Structural equation modeling identified cytokine mediators between AmygA and CMRS. IFNγ accounted for 52.6% of the AmygA-CMRS relationship, suggesting that IFNγ may be an important mediator between chronic stress-related neural activity and SARS-CoV-2 mortality risk. We therefore present that chronic stress-related neural activity may relate to SARS-CoV-2 mortality risk by way of increased IFNγ levels in circulation in an African American, community-based cohort. Interestingly, IFNγ overproduction by immune cells has recently been connected with SARS-CoV-2-related death. The mechanism linking circulatory IFNγ levels and SARS-CoV-2 mortality remains to be elucidated, but our data show that IFNγ may potentially be used as an early biomarker to identify individuals at greater risk for SARS-CoV-2 mortality, especially in the most vulnerable populations. Identifying these individuals is crucial to allow for earlier therapeutic intervention to decrease SARS-COV-2 mortality in disadvantaged populations.
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