O01
TREATMENT WITH HETERODIMERIC IL-15 SHAPES THE CYTOKINE AND CHEMOKINE MILIEU OF THE TUMOR, PROMOTING TUMOR INFILTRATION BY CYTOTOXIC LYMPHOCYTES: A GENERAL METHOD FOR LYMPHOCYTE ENTRY IN TUMORS
C. Bergamaschi1, B. Nagy1, S. M. Jensen2, K. Dimas1, D. Stellas1, B. A. Fox2, B. K. Felber1, G. N. Pavlakis1
1National Cancer Institute at Frederick, Frederick, 2Robert W Franz Cancer Research Center, Earle A Chiles Research Institute, Providence Cancer Center, Providence Portland Medical Center, Portland, United States

Introduction: The presence of tumor-infiltrating effector T cells is considered an important predictive biomarker for clinical benefit in response to immunotherapies. IL-15 is a cytokine important for the proliferation, activation and mobilization of lymphocytes, including natural killer (NK) and CD8+ T cells. Bioactive IL-15 in vivo comprises a complex of the IL-15 chain with the IL-15 receptor alpha chain that are together termed heterodimeric IL-15 (hetIL-15). Several preclinical models have supported the anti-tumor activity of IL-15, and based on these results, hetIL-15 has advanced to clinical trials. The objective of the study was to explore whether hetIL-15 is a universal factor promoting lymphocyte entry into the tumor, thus converting “cold” (lymphocyte-poor) tumors into “hot”.

Methods: We have produced hetIL-15 and tested its anti-tumor activity in several murine cancer models. Analysis of lymphocytes in lymphoid organs and in tumors was performed by flow cytometry and multi-color immunohistochemistry. Chemokine and cytokine levels were assessed using electrochemiluminescence (MSD) and ELISA assays.

Results: hetIL-15 treatment in mice was effective in delaying tumor growth in the MC38 colon carcinoma, TC-1 cervical carcinoma and B16 melanoma models. The combination of hetIL-15 and adoptive cell transfer of melanoma specific Pmel-1 cells showed anti-tumor efficacy in B16-bearing mice in the absence of lymphodepletion. The E0771 and 4T1 orthotopic breast cancer models showed delay in tumor progression and significantly reduced lung metastases upon hetIL-15 treatment. Flow cytometry and multi-color immunohistochemistry assays showed increased trafficking and persistence of NK and CD8+ T cells, including tumor specific T cells, into the tumors and an increased CD8+/Treg ratio, upon hetIL-15 administration. Tumor-resident CD8+ T and NK cells showed features of activated effector cells, with enhanced proliferation (Ki67+) and high cytotoxic potential (Granzyme B+). Upon ex-vivo stimulation, an increased frequency of both CD8+ and CD4+ T cells producing IFN-gamma was observed in the tumors of mice treated with hetIL-15. Increased levels of IFN-gamma were also found in plasma and tumor lysates. The increased tumor infiltration by cytotoxic lymphocytes promoted by hetIL-15 was accompanied by increased levels of the IFN-gamma dependent chemokines CXCL9 and CXCL10 in the tumor microenvironment and in plasma. Splenic and circulating NK and CD8+ T cells were also characterized by an elevated expression of the CXCL9/10 chemokine receptor CXCR3. IFN-gamma knockout mice showed an accelerated tumor growth in comparison to wild type mice, and administration of hetIL-15 failed to upregulate plasma CXCL9 and CXCL10 in the absence of IFN-gamma.

Conclusion: Our results show that hetIL-15 administration may be a general method to enhance T cell entry in tumors, increasing the success rate of immunotherapy interventions. Preclinical cancer studies support the use of hetIL-15 in tumor immunotherapy approaches to promote the development of anti-tumor responses by favoring effector over regulatory cells and by promoting T cells localization into tumors through the modification of the tumor cytokine/chemokine milieu.

Disclosure of Interest: C. Bergamaschi: None Declared, B. Nagy: None Declared, S. Jensen: None Declared, K. Dimas: None Declared, D. Stellas: None Declared, B. Fox: None Declared, B. Felber: None Declared, G. Pavlakis Grant / Research support from: Admune/Novartis

O02
REGULATION OF NOVEL PATTERN RECOGNITION RECEPTOR SIGNALING AND IFN INDUCTION BY UNANCHORED K48-LINKED POLYUBIQUITIN CHAINS
A. Hage1, P. Bharaj1, R. Rajbaum1
1Microbiology and Immunology, University Of Texas Medical Branch, Galveston, United States

Introduction: The type-I interferon (IFN-I) pathway is required for mediating the host antiviral response during infection. This pathway is highly regulated in order to avoid excessive inflammation and damage to the host. We previously reported that unanchored K48-linked poly-ubiquitin (poly-Ub) chains, which can associate with target proteins through non-covalent interactions, can promote IFN-I responses via activation of the IKKε kinase (1). In an effort to identify other host factors that have potential functions regulated by non-covalent interactions with unanchored poly-Ub in a more physiological context, we developed a novel approach to isolate these Ub chains from the lungs of influenza virus (IAV)-infected mice. Using this approach, we identified DHX16, an RNA helicase previously reported to be involved in pre-mRNA splicing (2). The participation of DHX16 in host innate immunity and regulation of its activity have not been described. Here we report a novel role of the RNA helicase DHX16 as host pattern recognition receptor (PRR) and regulation of its activity via unanchored poly-Ub chains.

Methods: To identify host factors that interact with unanchored poly-Ub we used a pull-down assay using as a bait the Ub-binding domain of the Isopeptidase T, which is known to specifically interact with unanchored poly-Ub chains. This approach combined with mass spectrometry analysis identified the RNA helicase DHX16 as an unanchored Ub-interacting protein. To elucidate the role of DHX16 in innate immunity we utilized a series of
knockdown and overexpression assays followed by next generation sequencing, qPCR and immunoblot analyses. Finally, to elucidate the mechanism by which DHX16 activity is regulated by viral RNA and unanchored Ub, we performed in vitro ATPase assays in the presence or absence of purified unanchored poly-Ub chains and viral RNA.

Results: Our data show that during IAV infection, the levels of unanchored K48-linked poly-Ub that associate with DHX16 are significantly increased. We further demonstrate that these Ub chains are synthetized by the E3-Ub ligase TRIM6. DHX16 knockdown analyses showed that expression of a subset of interferon-stimulated genes (ISGs) is significantly downregulated in DHX16 knockdown cells. Consistent with this, overexpression of DHX16 induced TBK1 and IRF3 phosphorylation, which can be further enhanced upon IAV infection. In vitro ATPase assays showed that the ATPase activity of DHX16 is enhanced upon addition of purified unanchored K48-linked poly-Ub, and this occurred only in the presence of viral RNA, suggesting that DHX16 recognizes viral RNA and that unanchored poly-Ub regulates its activity.

Conclusion: Together our data suggest that DHX16 recognizes viral RNA and that unanchored poly-Ub synthetized by TRIM6 stimulates its ATPase activity for induction of downstream IFN-I responses. We propose that DHX16 acts as a novel PRR involved in the IFN-I pathway and its activity is regulated by TRIM6 and unanchored K48-linked poly-Ub chains.

Disclosure of Interest: A. Hage: None Declared, P. Bharaj: None Declared, R. Rajsbaum Grant / Research support from: R21AI126012-01A1, R21AI132479-01, R01AI134907-01

O03
DELINEATION OF THE INFLAMMATORY PATHWAY BEHIND PROTEASOME-ASSOCIATED AUTOINFLAMMATORY SYNDROME
S. Davidson1,*, F. Moghaddas1, P. Baker1, D. Calleja2, B. T. Kille3, R. T. Goldbach-Mansky4, S. L. Masters1
1Inflammation, Walter and Eliza Hall Institute for Medical Research, 2Inflammation, Walter and Eliza Hall Institute, Parkville, 3Monash Biomedicine Discovery Institute, Monash University, Melbourne, Australia, 4Translational Autoinflammatory Disease Studies (TADS) Laboratory of Clinical Immunology and Microbiology, LCIM, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, United States

Introduction: Interferonopathies are autoinflammatory diseases driven by constitutive type I interferon (IFNαβ) signalling. Proteasome-associated autoinflammatory syndrome (PRAAS) is a newly described interferonopathy characterised by genetic mutations that result in a decrease or loss of proteasome function. Proteins which are misfolded, damaged, or redundant, are tagged with ubiquitin and consequently degraded by the proteasome. Accordingly, aggregates of ubiquinated proteins are observed in PRAAS patient cells. Interestingly, this loss of proteostasis associates with an interferon stimulated gene (ISG) signature1, yet how IFNαβ is induced in response to proteotoxic stress is currently unknown.

Methods: In order to identify a possible cellular sensor for protein aggregation we employed CRISPR/Cas9 gene editing. Proteasome subunits genes associated with disease of were deleted in a monocytic human cell line to generate a cellular model for PRAAS. Candidate pattern recognition receptor (PRR) genes were then deleted from the established PRAAS model cells. IFNαβ and ISG mRNA expression, accumulation of ubiquinated proteins and activation of key signalling molecules, were characterised by qPCR, ELISA, flow cytometry and western blotting. Peripheral blood mononuclear cells (PBMCs) from PRAAS patients and control samples were similarly investigated.

Results: Cells deficient for specific proteasome subunits (PRAAS model cell lines) recapitulate the accumulation of ubiquinated protein and constitutive IFNαβ signalling. Interestingly, genetic deletion or chemical inhibition of the PRR: protein kinase R (PKR) in PRAAS model cell lines abrogated IFNαβ and ISG expression. Intriguingly, preliminary data indicates that PKR activation is not mediated by its canonical ligand: double stranded RNA but, by accumulation of interleukin 24 (IL-24) in the cytosol. IL-24 is degraded from the cytosol via the proteasome and previous studies have demonstrated direct interaction between PKR and IL-24 (ref. 2). In line with PKR deficiency, genetic deletion of IL-24 also decreased IFNαβ and ISG expression in PRAAS model cells. Ex vivo analysis of PBMCs from PRAAS patients revealed increased presence of phosphorylated PKR and accumulation of IL-24 in these cells. Of note, chemical inhibition of PKR decreased IFNαβ and ISG gene expression and abolished activation of key IFNαβ signalling molecules in PBMCs from PRAAS patients but not in PBMCs from other interferonopathies. Thus, this PKR-mediated inflammation is specific to PRAAS.

Conclusion: Study of PRAAS has revealed a novel role for PKR as a sensor for the accumulation of ubiquinated protein. These findings have significant implications not only for PRAAS, but for other human diseases associated with proteotoxic stress and inflammation such as neurodegenerative disorders.

Disclosure of Interest: None Declared

O04
DI- AND TRIMERIC BIOLOGICAL SWITCHES MADE OF NANOBODY-CYTOKINE RECEPTOR FUSION PROTEINS SIMULATE NATURAL SIGNAL TRANSDUCTION
E. Engelowski1,*, P. Lang2, J. Scheller1
1Institute of Biochemistry and Molecular Biology II, 2Institute of Molecular Medicine II, Medical Faculty, Heinrich-Heine-University, 40225 Duesseldorf, Germany, Duesseldorf, Germany

Introduction: Synthetic biology deconstructs and reassembles biological bits and pieces to construct
biological devices for applications such as biological sensors, releasers, and switches. Cytokine-induced signal transduction is executed by natural biological switches among many other functions control immune related processes. In principle, cytokine receptors are in an off-state in the absence of cytokines and in an on-state in the presence of cytokines. Here, we developed Synthetic Cytokine Receptors (SyCyRs) which can be activated by synthetic, modular ligands and phenocopy signal transduction of natural cytokine receptors.

**Methods:** The synthetic cytokine receptors have a strictly modular design consisting of a nanobody, followed by a short linker-peptide sequence, the transmembrane and intracellular domain of a naturally occurring cytokine receptor of choice. Multimeric fusion proteins are modular synthetic ligands of fluorescent GFP and mCherry. The ligands specifically control homo- and heterodi- and trimeric receptor complex assembly. As biological read-out system, we used IL-23- and IL-6/IL-11- and TNF-signaling. Consequently, the extracellular sensors were fused to intracellular IL-23-, gp130 and TNF receptor chains.

**Results:** The switchable Synthetic Cytokine Receptor system resembled IL-23- and IL-6/IL-11- and TNF-signaling and revealed that homodimeric IL-23R were biologically active. Importantly, the synthetic receptors appear to be active in vivo, since we demonstrated the activation of Gver-gp130 by 3xGFP in the liver of mice after hydrodynamic injection. Moreover, we demonstrated that the Janus kinase activity and the receptor STAT3 phosphorylation binding site can be separated on two different receptor chains, a phenomenon which is referred to as trans-phosphorylation.

**Conclusion:** The synthetic cytokine receptor system allows tailor-made activation and analysis of cytokine signaling by recruitment of defined numbers and compositions of receptor chains. This system simulates signal transduction without relevant background activation and will allow a widespread area of potential applications for studying cell-type specific receptor activation by synthetic ligand application in transgenic mice and might also be applicable to novel tumor-immune therapy approaches.

**Disclosure of Interest:** None Declared

---

**O05**

A METABOLITE-TRIGGERED TUFT CELL-ILC2 CIRCUIT DRIVES SMALL INTESTINAL REMODELING

C. Schneider1, C. E. O’Leary1, J. von Molke1,2, H.-E. Liang1, Q. Y. Ang1, P. J. Turnbaugh1, S. Radhakrishnan3, M. Pellizzon3, A. Ma1, R. M. Locksley4,1

1University of California, San Francisco, San Francisco, 2Current position: University of Washington, Seattle, 3Research Diets, Inc., New Brunswick, 4Howard Hughes Medical Institute, UCSF, San Francisco, United States

**Introduction:** The small intestinal tuft cell-ILC2 circuit mediates epithelial responses to intestinal helminths and protists by tuft cell chemosensory-like sensing and IL-25-mediated activation of lamina propria ILC2s. Our laboratory and others recently demonstrated that activation of this circuit after helminth infection stimulated lamina propria ILC2s to secrete IL-13, which directly biased cell-fate decisions in epithelial stem cell progenitors, resulting in increased goblet and tuft cell frequencies that accompany the ‘weep-and-sweep’ response to worms. Functions of this circuit in intestinal tissue homeostasis and the ligands and receptors that trigger circuit activation are unknown.

**Methods:** We undertook a comprehensive study of factors that impact the small intestinal tuft cell – ILC2 circuit in mice. Specific deletion of A20 (Tnfaip3) in ILC2s was used to demonstrate a function as a cell-intrinsic brake on the response of small intestinal ILC2s to IL-25. We used this model, together with cytokine treatment and pathosymbiotic colonization, to study the consequences of chronic circuit activity in the small intestine. We studied these models in the context of deficiency in IL-25, IL-4Rα and tuft cells (Pou2f3/-), and we analyzed the impact of dietary fibers and pathosymbiotic-associated metabolites on the activity of the circuit using cytokine reporter alleles and knock-out mice.

**Results:** A20-deficiency in ILC2s spontaneously triggers the circuit, and, unexpectedly, promotes adaptive small intestinal lengthening and remodeling. Circuit activation occurs upon weaning, and is enabled by dietary polysaccharides that render mice permissive for colonization with the protist *Trichomonas*, resulting in luminal accumulation of acetate and succinate, metabolites of the protist hydrogenosome. Tuft cells express GPR91, the sucinate receptor, and dietary succinate, but not acetate, activates ILC2s via a tuft-, TRPM5-, and IL-25-dependent pathway. Also induced by parasitic helminths, circuit activation and small intestinal remodeling impairs infestation by new helminths, consistent with the phenomenon of concomitant immunity.

**Conclusion:** Our studies reveal a tripartite interplay between the small intestinal tuft cell – ILC2 circuit, diet, and microbiota, and identify the pathosymbiotic-associated metabolite succinate as a potent circuit activator. We describe a metabolic sensing circuit that may have evolved to facilitate mutualistic responses to luminal pathosymbionts.

**Disclosure of Interest:** None Declared

---

**O06**

TYPE I INTERFERON SIGNALING ATTENUATES REGULATORY T CELL FUNCTION IN VIRAL INFECTION AND IN THE TUMOR MICROENVIRONMENT

A. Gangapala1,2, C. Martens2, E. Dahstrom2, A. Metidji3, A. S. Gokhale1, D. D. Glass1, M.-L. Ocasio1, R. Baur2, K. Kanakabandi3, E. M. Shevach1

1Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, 2Genomics Unit, Research Technologies Branch, National Institute of Allergy and Infectious Diseases, Bethesda, 3The Francis Crick Institute, London, United Kingdom

**Introduction:** The small intestinal tuft cell-ILC2 circuit mediates epithelial responses to intestinal helminths and protists by tuft cell chemosensory-like sensing and IL-25-mediated activation of lamina propria ILC2s. Our laboratory and others recently demonstrated that activation of this circuit after helminth infection stimulated lamina propria ILC2s to secrete IL-13, which directly biased cell-fate decisions in epithelial stem cell progenitors, resulting in increased goblet and tuft cell frequencies that accompany the ‘weep-and-sweep’ response to worms. Functions of this circuit in intestinal tissue homeostasis and the ligands and receptors that trigger circuit activation are unknown.

**Methods:** We undertook a comprehensive study of factors that impact the small intestinal tuft cell – ILC2 circuit in mice. Specific deletion of A20 (Tnfaip3) in ILC2s was used to demonstrate a function as a cell-intrinsic brake on the response of small intestinal ILC2s to IL-25. We used this model, together with cytokine treatment and pathosymbiotic colonization, to study the consequences of chronic circuit activity in the small intestine. We studied these models in the context of deficiency in IL-25, IL-4Rα and tuft cells (Pou2f3/-), and we analyzed the impact of dietary fibers and pathosymbiotic-associated metabolites on the activity of the circuit using cytokine reporter alleles and knock-out mice.

**Results:** A20-deficiency in ILC2s spontaneously triggers the circuit, and, unexpectedly, promotes adaptive small intestinal lengthening and remodeling. Circuit activation occurs upon weaning, and is enabled by dietary polysaccharides that render mice permissive for colonization with the protist *Trichomonas*, resulting in luminal accumulation of acetate and succinate, metabolites of the protist hydrogenosome. Tuft cells express GPR91, the succinate receptor, and dietary succinate, but not acetate, activates ILC2s via a tuft-, TRPM5-, and IL-25-dependent pathway. Also induced by parasitic helminths, circuit activation and small intestinal remodeling impairs infestation by new helminths, consistent with the phenomenon of concomitant immunity.

**Conclusion:** Our studies reveal a tripartite interplay between the small intestinal tuft cell – ILC2 circuit, diet, and microbiota, and identify the pathosymbiotic-associated metabolite succinate as a potent circuit activator. We describe a metabolic sensing circuit that may have evolved to facilitate mutualistic responses to luminal pathosymbionts.

**Disclosure of Interest:** None Declared
Introduction: Regulatory T cells (Tregs) play a cardinal role in the immune system by suppressing detrimental autoimmune responses, but their role in acute, chronic infectious diseases and tumor microenvironment remains unclear. We recently demonstrated that IFN-α/β receptor (IFNAR) signaling promotes Treg function in autoimmunity. Here we dissected the functional role of IFNAR-signaling in Tregs using Treg-specific IFNAR deficient (IFNAR\(^{−/−}\)x Foxp3\(^{YFP-Cre}\)) mice in acute, chronic viral infection, and transplantable tumor models.

Methods: Here we used LCMV Armstrong, chronic Clone-13 viral infection and MC38 colon adenocarcinoma and B16F10 melanoma tumor models.

Results: In both viral infection and tumor models, IFNAR\(^{−/−}\)x Foxp3\(^{YFP-Cre}\) mice Tregs expressed enhanced Treg associated activation antigens. LCMV-specific CD8\(^{+}\) T cells and tumor-infiltrating lymphocytes from IFNAR\(^{−/−}\)x Foxp3\(^{YFP-Cre}\) mice produced less antiviral and antitumor IFN-γ and TNF-α. In the chronic viral model, the numbers of antiviral effector and memory CD8\(^{+}\) T cells were decreased in IFNAR\(^{−/−}\)x Foxp3\(^{YFP-Cre}\) mice and the effector CD4\(^{+}\) and CD8\(^{+}\) T cells exhibited a phenotype compatible with enhanced exhaustion. IFNAR\(^{−/−}\)x Foxp3\(^{YFP-Cre}\) mice cleared Armstrong infection normally but had higher viral titers in sera, kidneys, and lungs during chronic infection, and higher tumor burden than the WT controls. The enhanced activated phenotype was evident through transcriptome analysis of IFNAR\(^{−/−}\)x Foxp3\(^{YFP-Cre}\) mice Tregs during infection demonstrated differential expression of a unique gene signature characterized by elevated levels of genes involved in suppression and decreased levels of genes mediating apoptosis.

Conclusion: Thus above results suggest that IFN signaling in Tregs is beneficial to host resulting in a more effective antiviral response and augmented antitumor immunity.

This work is supported by the intramural research program of the NIAID, NIH.

Disclosure of Interest: None Declared

O08 GSMD IS CRITICAL FOR AUTOINFLAMMATORY PATHOLOGY IN A MOUSE MODEL OF FAMILIAL MEDITERRANEAN FEVER

A. Kanneganti\(^{1,2,3,4}\), M. Lamkanfi\(^{1,2,4}\)

1Center for Inflammation Research, VIB, 2Department of Internal Medicine, Ghent University, Ghent, Belgium, 3Center for Computational and Integrative Biology, Massachusetts General Hospital, Harvard Medical School, Boston, United States, 4Janssen Immunosciences, World Without Disease Accelerator, Janssen Pharmaceutica, Pharmaceutical Companies of Johnson and Johnson, Beerse, Belgium

Introduction: Pyroptosis is an inflammasome-induced lytic cell death mode, the physiological role of which in chronic inflammatory diseases is unknown. Familial Mediterranean Fever (FMF) is the most common monogenic autoinflammatory disease worldwide, affecting an estimated 150,000 patients. The disease is caused by missense mutations in Mefv that activate the Pyrin inflammasome, but the pathophysiologic mechanisms driving autoinflammation in FMF are incompletely understood.

Methods: Bone marrow-derived macrophages (BMDMs) of Mefv\(^{V726A/V726A}\) mice were infected with C. difficile to analyze inflammasome-induced maturation of caspase-1 and the pyroptosis executioner GSDMD. To address the in vivo role of pyroptosis in secretion of IL-1β in a more physiologically relevant context of autoinflammation, we measured the levels of IL-1β in serum of Mefv\(^{V726A/V726A}\) mice that were either sufficient or deficient in GSDMD expression. We additionally examined the impact of (cGAMP) to result in cell intrinsic activation of STING. However, whether epithelial cGAMP can modulate the trans-activation of immune cells is poorly defined.

Methods: To address this question we rely on co-culture models and genetically modified cell lines deficient in cGAS, STING or connexins 43-45.

Results: In this work, we demonstrate that cGAMP can be transferred from epithelial cells to primary monocytes, resulting in the stimulation of monocytes in trans. This activation in the monocytic compartment is independent of cGAS, but reliant on STING and the formation of functional gap junctions with epithelial cells. As such, we show that cGAMP producing epithelial cells lacking connexins (43/45) cannot trans-activate co-cultured monocytes. Critically, we demonstrate that this transfer of cGAMP from damaged cells to immune cells can be observed in the context of UVB-treated keratinocytes.

Conclusion: Collectively, our results suggest a previously unappreciated mechanism used by patrolling immune cells to detect damaged cells and facilitate their clearance. This repositions gap junctions as critical regulators of immune responses to cellular damage, while suggesting a novel therapeutic point of action in select diseases where DNA damage is at the root of aberrant inflammation.

Disclosure of Interest: None Declared

O07 GAP-JUNCTION DEPENDENT TRANS-ACTIVATION OF HUMAN MONOCYTES BY CGAMP.

G. Pepin\(^{1,2}\), D. De Nardo\(^{2}\), M. Gantier\(^{1}\)

1Department of Molecular and Translational Science, Monash University, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, 2Inflammation Division, Department of Medical Biology, The University of Melbourne, The Walter & Eliza Hall Institute of Medical Research, Parkville, Australia

Introduction: Accumulating evidence suggests that cGAS sensing of cytosolic DNA is directly involved in the detection of genomic integrity and DNA damage, to promote senescence of damaged cells. Such activation of cGAS by nuclear DNA released into the cytosol instigates the synthesis of the second messenger cyclic GMP-AMP (cGAMP) to result in cell intrinsic activation of STING. However, whether epithelial cGAMP can modulate the trans-activation of immune cells is poorly defined.
GSDMD deficiency on serum levels of a panel of inflammatory cytokines and chemokines. To further assess the role of GSDMD in IL-1β-mediated autoinflammation using histopathological means, we examined signs of systemic neutrophilia, leukocyte infiltration and splenomegaly.

**Results:** C. difficile infection of FMF knock-in macrophages that express a chimeric FMF-associated MefV726A Pyrin elicited pyroptosis and gasdermin D (GSDMD)-mediated IL-1β secretion. Importantly, in vivo GSDMD deletion abolished spontaneous autoinflammatory disease. GSDMD-deficient FMF knock-in mice were fully protected from the runted growth, anemia, systemic inflammatory cytokine production, neutrophilia and tissue damage that characterize this autoinflammatory disease model.

**Conclusion:** This work thus clearly delineated the dominant contribution of GSDMD-mediated pyroptosis to IL-1β dependent pathology in this mouse model of FMF-associated autoinflammation and suggests that pyroptosis may similarly exert a crucial role in the etiology of other chronic IL-1β-mediated autoinflammatory and autoimmune diseases. Therefore, pyroptosis is established as a critical in vivo mechanism of autoinflammation, opening up the possibility of pyroptosis inhibition as an anti-inflammatory strategy in inflammasome-dependent autoinflammatory and autoimmune pathology.

**Disclosure of Interest:** None Declared

---

**O9**

THE AIM2 INNATE IMMUNE DNA SENSOR FACILITATES STAT3-DRIVEN GASTRIC TUMORIGENESIS.

V. Deswaerte1*, B. J. Jenkins1

1Centre for Innate Immunity and Infectious Diseases, HUDSON INSTITUTE OF MEDICAL RESEARCH, Clayton, Australia

**Introduction:** The potent pro-inflammatory and oncogenic transcription factor signal transducer and activator of transcription (STAT)3 is hyperactivated in approximately half of all stomach cancer cases. However, the identity of innate immune gene networks that are deregulated by hyperactive STAT3 which trigger inflammation-associated carcinogenesis in the stomach are poorly understood. Among all known pattern recognition receptors (PRRs) which form inflammasome complexes, only the gene promoter region of absent in melanoma 2 (AIM2), which encodes a cytosolic innate immune DNA sensor, contains two putative consensus STAT3 binding motifs, making it an attractive new target in promoting STAT3-driven inflammation-associated carcinogenesis.

**Methods:** AIM2 and STAT3 expression was assessed by quantitative real-time PCR in both human gastric cancer patient biopsies and mouse gastric tumours. The effect of AIM2 on tumour formation was assessed using the gp130F/F spontaneous GC model crossed onto backgrounds deficient in genes encoding AIM2. The inflammatory status in mice gastric tumours was assessed by numerous techniques including immunohistochemistry and western blot.

**Results:** Here, we reveal that hyperactivation of STAT3 promotes oncogenic processes in the gp130F/F gastric cancer mouse model via the AIM2 innate immune DNA sensor. Gastric (tumour) Aim2 expression levels were significantly upregulated in these mice in a STAT3-dependent and disease-associated manner, and genetic ablation of AIM2 suppressed tumorigenesis, but not inflammation. Mechanistically, we identified that Aim2 is a novel transcriptional target of STAT3. Moreover, increased Aim2 expression coincided with STAT3 hyperactivation in human gastric cancer biopsies, and correlated with poor patient survival.

**Conclusion:** Collectively, these data define the existence of a novel functional link between STAT3 hyperactivation and upregulated AIM2 production in the pathogenesis of gastric cancer.

**Disclosure of Interest:** None Declared

---

**O10**

MODULATION OF INTERLEUKIN-22 ALLEVIATE RESPIRATORY VIRAL INFECTION BY PROMOTING EPITHELIAL INTRINSIC TYPE I INTERFERON RESPONSE


1Mater Research Institute - The University Of Queensland, Brisbane, 2South Australia Health and Medical Research Institute, Adelaide, 3Immunopathology Group, Mater Research Institute - The University of Queensland, 4Diamantina Institute & Faculty of Medicine, The University of Queensland, 5Immunology and Infectious Diseases, QIMR Berghofer Medical Research Institute, 6Centre for Children’s Health Research (CCHR) and Institute of Health and Biomedical Innovation (IHB), Queensland University of Technology, Brisbane, 7Faculty of Medicine, Dentistry and Health Sciences, the University of Melbourne, Melbourne, 8Immunopathology Group, The University of Queensland, Brisbane, Australia

**Introduction:** We have discovered that immunity regulates protein synthesis in mucosal epithelial cells via the modulation of endoplasmic reticulum (ER) stress. We show that IL-22, an immune mediator, can alleviate ER stress and enhance protein production in secretory cells. Respiratory viruses utilize the host ER to produce viral proteins via bypassing host ER stress response. We hypothesise that locally elevated IL-22 during respiratory viral infection could increase viral replication by promoting viral protein synthesis.

**Methods:** To confirm the cellular stress modulatory role of IL-22, we treated the differentiated primary human bronchial epithelial cells (HBECs) with ER stressors (tunicamycin, IL-23, IL-24, IL-17A, and IFN-γ) in combination with IL-22. We also infected Hela cells with human Respiratory Syncytial Virus or Human
Rhinovirus and co-treated with IL-22 to determine IL-22's effect in modulating viral load. We also utilised pneumovirus of mouse (PVM) infection model in neonates to study whether IL-22 exacerbates PVM infection via reducing ER stress to facilitate viral replication.

**Results:** In *in vitro* HBEC culture, IL-22 suppressed both cytokine (IL-23, IL-24, IL-17A, IFN-g) and N-glycosylation inhibitor tunicamycin induced ER stress, and promoted appropriate mucin production in HBECs. Supporting our hypothesis, IL-22 treatment increased viral load in HeLa cells infected with human Respiratory Syncytial Virus and Human Rhinovirus *in vitro*. In PVM infection, rIL-22 administration prior viral infection significantly increased viral load and lead to severe lung pathology and ~80% mortality before 6 day post-infection (dpi). Meanwhile, neutralisation of IL-22 at the time of infection reduced viral load, lung injury and airway mucin hyperplasia at 10 dpi. This was accompanied by a marked increase in the Type I IFNs. As the IL-22 receptor is not expressed on any immune cells and mainly expressed on the epithelial cells, we believe that IL-22 intrinsically regulates Type I IFN responses in epithelial cells via the regulation of cellular stress. Our data suggests that blocking endogenous ER stress suppressor IL-22 at early stage of viral infection could limit viral replication and immunopathology via promoting ER stress and boosting autonomous Type I IFN responses in respiratory epithelial cells.

**Conclusion:** Our data suggests that IL-22 can be therapeutically manipulated at appropriate times to help limit respiratory viral infection and mucosal tissue damage.

**Disclosure of Interest:** None Declared

---

### O11

**ADAM17/IL-6 TRANS-SIGNALLING AXIS: A PROMISING THERAPEUTIC TARGET FOR LUNG ADENOCARCINOMA**

M. I. Saad1, S. Alhayyani1, L. McLeod1, L. Yu1, C. Garbers2, S. Ruwanpura1, I. Sagi2, S. Rose-John2, B. Jenkins1

1Hudson Institute Of Medical Research - Monash University, Clayton, Australia, 2Institute of Biochemistry, Christian-Albrechts-University, Kiel, Germany, 3Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel

**Introduction:** Oncogenic KRAS mutations are a major driver of lung cancer, the most fatal among all cancer types. Considering the direct therapeutic targeting of KRAS has been problematic, the identification of “druggable” cooperating factors of KRAS provides an attractive alternative approach to develop new therapeutic strategies that indirectly block the oncogenic actions of KRAS. Since the protease a disintegrin and metalloproteinase 17 (ADAM17) is responsible for the ectodomain shedding of bioactive protumourigenic interleukins, growth factors and/or their receptors implicated in lung cancer, including TNFα, soluble IL-6 receptor (sIL-6R), and EGF receptor ligands, we investigated the role of ADAM17 in the pathogenesis of lung cancer.

**Methods:** Genetic (*KrasG12D*) and carcinogen (NNK)-induced mouse models of lung adenocarcinoma(LAC) were coupled with *Adam17*−/− mice, which are homozygous for a hypomorphic *Adam17* allele resulting in a dramatic reduction in *Adam17* protein expression. Oncogenic *KrasG12D* was activated using intranasal inhalation of Adenovirus Cre recombinase, while NNK (100mg/kg) was injected intraperitoneally into *Adam17*−/− on the tumour-sensitive A/J background. CRISPR-Cas9-mediated knockdown of *Adam17* in human KRAS-mutant LAC cell lines was employed to evaluate the effects of ADAM17 deletion in *vivo* xenograft. The *in vivo* anti-tumour activity of the newly engineered recombinant ADAM17 prodomain (A17pro), which is a highly-specific inhibitor of ADAM17 cell surface activity, was evaluated in *KrasG12D-*driven LAC and KRAS-mutant LAC patient-derived xenograft (PDX) models.

**Results:** Genetic deficiency of ADAM17 significantly reduced tumour burden in both *KrasG12D* and NNK-induced LAC models, which was accompanied by reduced cellular proliferation and inflammation. CRISPR-Cas9-driven ADAM17 deficiency in human KRAS-mutant LAC cell lines similarly reduced cell proliferation and tumour growth in *vivo*. Furthermore, A17pro treatment significantly reduced the tumour burden in *KrasG12D* mice, as well as tumour growth of a KRAS-mutant LAC PDX. Among ADAM17 substrates, only sIL-6R - which drives IL-6 trans-signalling that promotes *Kras*-induced LAC - was preferentially reduced in LAC mouse models upon the targeted blockade of ADAM17 expression and/or activity, which was associated with a concomitant reduction in downstream ERK1/2 MAPK activation. In the lungs of both mouse models and LAC patients, when compared with their non-LAC counterparts, threonine (Thr235) phosphorylation of ADAM17 was enhanced via a p38 MAPK-induced mechanism.

**Conclusion:** Collectively, our data suggest that ADAM17 plays a pivotal role in LAC development. Moreover, targeting ADAM17 activity using the A17pro prodomain inhibitor represents an attractive strategy in tackling LAC, and paving the way for the development of therapies for *KRAS*-mutant LAC.

**Disclosure of Interest:** None Declared

---

### O12

**DECOUPLING IL-6 BIOLOGICAL EFFECTS USING PROTEIN ENGINEERING**

J. Martinez-Fabregas1, S. Wilmes1, I. Moraga Gonzalez1

1Cell Signaling and Immunology, University Of Dundee, Dundee, United Kingdom

**Introduction:** IL-6 is a highly pleiotropic cytokine that acts as a central regulator of the immune response by eliciting both pro-inflammatory and anti-inflammatory responses. IL-6 induces Th-17 cell differentiation and inhibition of T...
regulatory (reg) and Th-1 cell differentiation. In addition, IL-6 signaling is often found deregulated in human diseases, making this cytokine highly relevant for human health. IL-6 exerts its activities by engaging two surface receptors, IL-6Rα and gp130. IL-6 binds first to IL-6Rα with high affinity and then in a second step gp130 is recruited to the complex with low affinity, leading to the activation of STAT1 and STAT3 transcription factors. How activation of STAT1 and STAT3 by IL-6 produces such a large functional diversity remains an open question.

Methods: Here, we have used a multidisciplinary approach encompassing the engineering of surrogate IL-6 ligands with different receptor complex half-life, their biophysical characterization, the study of their surface dynamics and endosomal traffic, as well as characterization of their signaling patterns and functional properties to identify cellular and molecular determinants contributing to IL-6 functional diversity.

Results: We have isolated IL-6 variants that activate 100%, 50% or 30% of the signaling amplitude induced by IL-6 wild type (wt). These differences did not result from different kinetics of signal activation nor could they be rescued by higher doses of ligand. Additionally, not all signaling molecules were equally affected, with STAT1 activation being more sensitive to binding affinity changes than STAT3. We identified endosomes as a critical contributor to the biased signal activation by IL-6 variants. Inhibition of the endosomal trafficking of IL-6 receptor complexes by the clathrin inhibitor, PITSTOP, prevented IL-6 variants from activating STAT1, but did not affect STAT3 activation. Short-lived complexes engaged by low affinity IL-6 variants did not traffic to the endosomes, suggesting that the bulk of STAT1 activation takes place in endosomes. Next we studied the ability of IL-6 variants to fine-tune differentiation of human CD4 T cells in order to understand how different signaling profiles engaged by IL-6 variants shape their biological properties. We found that while all mutants could induce Th-17 differentiation to a similar extent as IL-6 wt, their ability to inhibit Treg and Th-1 differentiation differed significantly from the wt, with some of the IL-6 variants inducing good Th-1 and good Treg inhibition, others inducing good Th-1 and poor Treg inhibition, and a third group promoting poor inhibition of both T cell subsets.

Conclusion: Our data show that modulation of the IL-6-gp130 complex half-life with engineered IL-6 surrogate ligands fine tunes the amplitude and nature of the signaling responses induced by this complex, which ultimately result in IL-6 variants with more defined immune-modulatory properties. In principle, this approach could be used to design more specific and less toxic cytokine variants that could rescue the translation of cytokines into the clinic.

Disclosure of Interest: None Declared

O14
CRITICAL ROLE OF IFNS IN GASTRO-INTESTINAL INJURY REPAIR
C. McElrath1,2, J.-D. Lin3, V. Espinosa4, J. Peng1,2, R. Sridhar1,2, H.-C. Tseng1,5, S. Smirnov2, H. Risman5, M. Sandoval3, M. Galan5, A. Rivera4,6,7, J. Durbin4,5,7, S. Kotenko5,6,7
1School of Graduate Studies, 2Microbiology, Biochemistry, and Molecular Genetics, Rutgers University, Newark, 3Pathology, New York University, New York, 4Center for Immunity and Inflammation, 5Pathology and Laboratory Medicine, 6Pediatrics, 7Cancer Institute of New Jersey at University Hospital, Rutgers University, Newark, United States

Introduction: The etiology of ulcerative colitis (UC) is poorly understood, but involves perturbation of the complex interactions between the mucosal immune
system and the commensal bacteria of the gut, with cytokines acting as important cross-regulators. Type I and type III interferons (IFNs) are recognized for their non-redundant importance in limiting viral infection in a variety of tissues, including the gastro-intestinal tract. However their involvement in the maintenance of mucosal barrier homeostasis and injury repair is less well characterized.

**Methods:** To study the contributions of type I and type III IFNs to the formation, progression, and resolution of UC, we used mice deficient in type I IFN, type III IFN, and type I/III IFN signaling and a murine model of acute UC, which follows exposure of mice to dextran sulfate sodium (DSS)-containing water. 

**Results:** Unexpectedly, mice lacking both type I and type III IFN receptors did not survive even low levels of DSS-exposure. This enhanced susceptibility to DSS-inflicted colonic injury in type I/III IFN receptor-deficient mice was associated with diffuse destruction of the colonic epithelium, progressive weight loss and increased disease activity index. Impaired proliferative capacity of intestinal epithelial cells in double IFN receptor-deficient mice, resulting in incompetent colonic tissue repair, was demonstrated to be responsible for DSS-induced mortality. Analysis of experiments conducted with bone marrow chimera mice revealed that IFN signaling in either the epithelial or hematopoietic compartment supports epithelial regeneration following DSS-induced damage, and is sufficient for protection. Moreover, epidermal growth factor receptor (EGFR) signaling was identified as a critical pathway that mediates the action of IFNs and triggers proliferation of intestinal epithelial cells leading to colonic tissue restoration.

**Conclusion:** This study reveals a new unexpected role for IFN signaling in supporting intestinal epithelial regeneration following acute colonic injury. Therefore, compartmentalized and coordinated action of type I and III IFNs within the gastro-intestinal tract is important not only for the efficient antiviral protection, but also contributes to maintaining intestinal tissue homeostasis and balancing tissue repair and inflammatory responses.

**Disclosure of Interest:** None Declared
Introduction: Type I interferons (IFNs) are among the soluble mediators of the anti-viral immune response. Although innate immune cells are responsible for the majority of type I IFN production in the body, recently we showed that B cells, when stimulated with lipid conjugated CpG-A type TLR9 agonists, produce Type-I IFNs. Additionally, the production of type I IFNs in response to intravenously injected CpG-lipid conjugates were significantly lower in B cell deficient mice as compared to WT mice indicating that B cells are important interferon producers in this setting. However, other pathways that can induce Type I IFN in B cells and the overall significance of B cell specific IFN production have yet to be characterized. 

Methods: To identify different stimulation patterns that lead to type I IFN production in B cells, purified mouse splenic B cells were cultured in the presence of various B cell stimulants. The autocrine effects of B cell mediated IFN production was tested using the type I IFN inducing stimuli on B cells purified from either WT or type I IFN receptor (IFNAR) KO mice. To test the developmental effects of type I IFN signalling on B cells, a bone marrow chimera was generated by reconstituting irradiated animals with congenically labelled WT and IFNAR KO bone marrow cells at 1:1 ratio. Upon reconstitution, these animals wereinfected with Lymphocytic choriomeningitis virus (LCMV) to show differences in immune response depending on IFN signalling.

Results: Here, upon testing a range of stimulation conditions, we showed that dual stimulation of TLR3 and TLR4 by Poly IC and LPS is a potent inducer of type I IFNs in B cells. Furthermore, by comparing WT and IFNAR KO mice, we identified that TLR induced upregulations of CD86 and CD317 on B cells are dependent on autocrine effects of IFNs. Having observed these, we tested the extent to which B cell responses are mediated by IFNs by generating bone marrow chimeric mice using 1:1 ratio of WT and IFNAR KO bone marrow cells. Upon successful reconstitution, 1:1 ratio was conserved in total B cells and follicular cell compartments. However, majority of B1 and marginal zone cells came from WT mice while transitional cells showed IFNAR KO predominance indicating differential effects of IFN signaling in B cell development. Once these chimeric mice were infected with LCMV, we observed a significant increase in WT/IFNAR KO ratio in class switched B cells despite relatively unchanged ratios in germinal center and plasma cell compartments.

Conclusion: These findings indicate that Type I IFNs play major roles at various stages of B cell development, activation and differentiation.

Disclosure of Interest: None Declared

O17 ALLERGIC INFLAMMATORY MEMORY OF TYPE 2 CYTOKINES IN HUMAN RESPIRATORY EPITHELIAL PROGENITOR CELLS

Introduction: Epithelial dysfunction is a fundamental aspect in the evolution of chronic inflammatory diseases at barrier tissues.1,2 Allergic inflammation in the upper airway barrier can develop from persistent activation of the Type 2 immune (T2I) cytokine module, resulting in the disease spectrum known as chronic rhinosinusitis (CRS): ranging from inflamed mucosa to the severe tissue reorganization seen in nasal polyps3. Identifying the principal cell types and states which maintain and propagate disease in humans is vital to treating these conditions. Here, we hypothesized that T2I cytokines act directly on epithelial stem cells to influence their state and differentiation trajectory towards mature functional epithelial cell subsets.

Methods: Profiling twelve primary human CRS samples surgically isolated from ethmoid sinus that span the range of clinical severity with the Seq-Well platform4 for massively-parallel single-cell RNA-sequencing (scRNA-seq), we report the first single-cell transcriptomes for human respiratory epithelial cell subsets, immune cells, and stromal cells (18,036 cells) from a T2I inflammatory disease, and map key cytokines (e.g. TSLP) with subset-level resolution. Through comparison with scrapings derived from healthy turbinate, inflamed turbinate, and polyp sinus tissues (additional 18,704 cells), we define core, healthy, inflamed, and polyp secretory cell signatures.

Results: We find striking differences between the epithelial compartments of the non-polyp and polyp cellular ecosystems. More specifically, across 10,383 epithelial cells, we identify a global reduction in epithelial diversity in polyps characterized by basal cell hyperplasia, a concomitant decrease in glandular and ciliated cells, and phenotypic shifts in secretory cell antimicrobial function. Furthermore, we detect that the immune effector cytokines IL-4/IL-13 act directly on these basal progenitors and propose that they drive an aberrant tissue stem cell differentiation trajectory in polyps. We functionally validate this idea by demonstrating that basal cells ex vivo retain an intrinsic memory of IL-4/IL-13 exposure characterized by a persistent activation of the Wnt/CTNNB1 pathway. Finally, we test the potential for clinical administration of in vivo IL-4Ra blockade to modify basal and secretory cell states through in vivo serial sampling at single-cell resolution in humans.

Conclusion: Our data provide the first-in-human evidence for immune effector cytokines acting to rewire tissue stem cells and indicate that T2I barrier dysfunction stems from intrinsically altered epigenetic “memories” in basal cells. Collectively, we nominate a cellular mechanism for the persistence and chronicity of severe human respiratory disease.

Disclosure of Interest: None Declared
O18
TISSUE SIGNALS IMPRINT ILC2 TRANSCRIPTIONAL IDENTITY AND PREEMPTIVE FUNCTION
S. Van Dyken1,2,*, R. Ricardo-Gonzalez2, C. Schneider2, J. Lee2, J. Nasbbaum2, A. Molofsky2, R. Locksley2
1Pathology & Immunology, Washington University School of Medicine in St. Louis, St. Louis, 2University of California, San Francisco, San Francisco, United States

Introduction: ILC2s are defined by their ability to produce type 2 cytokines, in particular IL-5 and IL-13, by integrating inputs from multiple ligands, including cytokines, neuropeptides and eicosanoids. The epithelial cytokine IL-33, IL-25 and TSLP are powerful ILC2 activating ligands, and mice deficient in these signaling pathways display substantial compromise in the capacity of mature ILC2s to generate type 2 cytokines in response to helminths and allergens. Recent analyses of murine and human ILCs have indicated transcriptomic heterogeneity of ILC2s in tissues; however, direct comparisons of ILC2s among normal resting tissues are limited and complicated by sorting strategies that rely on surface markers that are variably expressed by ILCs. Furthermore, functional characterization of the signals that establish the homeostatic cytokine activation of ILC2s across and within disparate tissues, particularly in skin, are lacking.

Methods: We combine analysis of mouse cytokine reporter alleles with next-gen RNA sequencing approaches to comprehensively assess the constitutively function-marked populations of ILC2s present in resting bone marrow and peripheral tissues. We further analyze germ-free mice and mice triple-deficient in IL-33, IL-25 and TSLP signaling, to uncover key determinants of homeostatic effector cytokine function that are intact in the absence of commensal microbiota, as assessed by the ILC2-defining cytokines IL-5 and IL-13.

Results: Remarkably, although IL-5+ ILC2s require IL-33, IL-25 and TSLP signaling for optimal homeostatic type 2 cytokine competency in a tissue-dependent manner, the transcriptomic signatures imprinted by each tissue in which ILC2s reside dominates their identity. Receptiveness to particular tissue-derived activating signals segregates by tissue, and tissue ILC2 subsets are normal in germ-free mice. Single-cell profiling confirms a tissue-organizing transcriptome and identifies ILC2 subsets expressing distinct activating receptors, including the major subset of skin ILC2s, which are activated preferentially by IL-18, independent of other activating cytokines.

Conclusion: Our data suggest that endogenous, tissue-derived signals drive the maturation of ILC2 subsets by controlling expression of distinct patterns of activating receptors, thus anticipating tissue-specific patterns to perturbations occurring later in life.

Disclosure of Interest: None Declared

O19
STAT3 SUPPRESSES PRIMARY TUMOUR GROWTH, BUT INCREASES METASTASIS IN MYC-DRIVEN SMALL CELL LUNG CANCER
A. C. Guanizo1,†, J. Chen1, W. S. N. Jayasekara1, D. N. Watkins2, D. J. Gough1
1Centre for Cancer Research, Hudson Institute Of Medical Research, Clayton, 2Garvan Institute, Darlinghurst, Australia

Introduction: Signal transducer and activator of transcription 3 (STAT3) is a transcription factor critical for cellular growth, differentiation, immune function, metabolism and survival. Aberrant STAT3 activation has been observed in approximately 50% of human cancers, and has been linked to tumour progression and therapeutic resistance. One key target gene of STAT3 is MYC which is a potent transcription factor with oncogenic potential as observed in at least 40% of tumours. The STAT3-dependent induction of MYC expression in tumours is a key event in STAT3-driven tumourigenesis. It would therefore, be expected that STAT3 is redundant in tumours with MYC amplification in which MYC expression is not responsive to STAT3.

Methods: To determine the requirement for STAT3 in MYC-driven tumours, we generated a Myc-driven mouse model of small cell lung cancer (SCLC). In this model, Myc is expressed from the Rosa26 locus following delivery of adenoviral Cre into the airways via inhalation. These mice were crossed with Stat3 conditional mice resulting in simultaneous overexpression of Myc and loss of Stat3. MYC amplification is observed in up to 30% of SCLC patients, however this is always in combination with loss-of-function mutations in pRb1 and Tp53. Therefore, we also generated more clinically relevant mouse models in which Myc is overexpressed and Tp53 and pRb1 are deleted (RPM mice) with or without Stat3 loss. We performed immunohistochemical analysis on the primary and metastatic tumours that formed in these mouse models.

Results: Our data show that Stat3 loss corresponds to a decrease in primary tumour burden in both the Myc-amplified and the RPM mice. However, we observed a significant increase in the liver metastasis in RPM mice following Stat3 deletion. This is accompanied by a significant increase in lipid deposition in the liver. Analysis of primary tumour cell lines derived from our mouse models shows that the deletion of Stat3 leads to increased expression of enzymes involved in lipogenesis. Finally, the deletion of Stat3 from metastatic human SCLC cell lines leads to accelerated growth and earlier tumour onset in xenografted animals.

Conclusion: Together, our data show that STAT3 is crucial for the onset of primary tumours in MYC-driven SCLC. However, STAT3 concomitantly increases lipogenesis and liver metastasis.

Disclosure of Interest: None Declared
O20
SUCCESSFUL ANTI-PD-1 CANCER IMMUNOTHERAPY REQUIRES T CELL-DENDRITIC CELL CROSSTALK INVOLVING IFN-αα AND IL-12
C. S. Garris1,*, S. P. Arlauckas1, R. Weissleder1, M. J. Pittet1
1Center for Systems Biology, Massachusetts General Hospital - Harvard Medical School, Boston, United States

Introduction: Anti-PD-1 immune checkpoint blockers can induce sustained clinical responses in cancer but how they function in vivo remains incompletely understood. To date, anti-PD-1 mAbs have proved particularly efficacious in the clinic among immune checkpoint blockade therapies. However, we still have a limited understanding of how immune checkpoint blockers engage complex tumor microenvironments and which mechanisms define treatment success during the time when tumor rejection occurs. To address these knowledge gaps, we sought to track key readouts of immunotherapy function in vivo at single cell resolution and during tumor rejection, and decipher how immune-mediated tumor control is achieved.

Methods: Using mouse models of cancer, we combined intravital real-time imaging of key TH1 cytokines IFN-αα and IL-12 with single cell-RNAseq analysis to uncover anti-PD-1 pharmacodynamics directly within tumors. Fluorochrome labeling of checkpoint immunotherapy further enabled tracking of immunotherapy targeted cells in vivo in relation to cytokine producing cells in tumors.

Results: Treatment of mice with anti-PD-1 resulted in intratumoral increases of both IFN-αα and IL-12, and these cytokines were produced by discrete cell types. Morphology, flow cytometry, and sequencing analysis identified IL-12 producing cells as intratumoral dendritic cells. These DCs do not bind anti-PD-1 but produce IL-12 upon sensing interferon (IFN)-αα that is released from neighboring T cells. In turn, IL-12 stimulates anti tumor T cell immunity. Furthermore, we find that targeting the non-canonical NFκB pathway, identified as elevated in a tumor infiltrating DC population, can amplify IL-12-producing DCs and sensitize tumors to anti-PD-1 treatment.

Conclusion: These findings suggest that full-fledged activation of anti tumor T cells by anti-PD-1 is not direct, but rather involves T cell:DC crosstalk and is licensed by IFN-αα and IL-12.

Disclosure of Interest: None Declared

O21
INTERLEUKIN-11 SIGNALING INDUCES A UNIQUE PATHOGENIC CD4+ T HELPER-17 CELL POPULATION
K. Y. Fung1,2,*, A. Preaudet1, P. Nguyen1,2, C. Burstrom1, K. Edwards3, N. Wilson3, T. Putoczki1,2
1Inflammation Division, Walter and Eliza Hall Institute of Medical Research, 2Department of Medical Biology, University of Melbourne, 3CSL Ltd., Melbourne, Australia

Introduction: It has been well documented that in vitro polarization of naïve CD4+ T-cells towards the Th17 lineage requires a combination of T-cell antigen receptor stimulation and IL-6 together with transforming growth factor β (TGFβ) in mice, or IL-1b, IL-23 and to a lesser extent IL-6 in humans. We explored the role of other IL-6 family cytokines in Th17 differentiation.

Methods: To fully compare the IL-11 mediated Th17 gene signature to that of IL-6, we performed cell-seq analysis of differentiated naïve mouse CD4+ T cells. In order to assess the potential of IL-11 to modulate Th17-associated pathologies in vivo, we compared female C57Bl/6 WT, il6KO and il11KO mice in experimental autoimmune encephalomyelitis (EAE), a classic model of multiple sclerosis (MS) and Th17-associated pathology. Since neutralizing antibodies against IL-23p19 are in clinical trials for MS, Il23aKO mice were included for comparison of therapeutic relevance. In order to definitively demonstrate the pathogenic role of IL-11 responsive CD4+ T-cells in EAE we generated a novel IL-11R floxed allele through CRISPR-Cas9 gene editing, which was crossed to a constitutive Cd4Cre allele to generate compound mutant Cd4Cre; Il-11Rfloxp mice on a C57Bl/6 background. To demonstrate the therapeutic utility of IL-11 signalling inhibition, we utilized neutralizing antibodies targeting the mouse IL-11r or human IL-11r, which potently inhibit the activation of STAT3, an essential transcription factor for Th17 polarization.

Results: We have identified that both mouse and human CD4+ cells can be polarized towards Th17 cells in the presence of IL-11, suggesting conservation of the requirement of this signaling molecule between species. Importantly, we show that IL-11 drives a unique Th17 population compared to its sibling IL-6, with both cytokines contributing to the pathogenesis of EAE. Importantly, we show that inhibition of IL-11 signaling specifically in CD4+ T-cells alleviates disease in a mouse model of MS, a phenotype that can be mimicked by the therapeutic administration of clinically relevant anti-IL-11R antibodies.

Conclusion: These studies highlight overlapping, yet diverging functions, for IL-6 and IL-11 signaling in CD4 T-cell biology and identify alternative therapeutic opportunities for patients suffering from Th17-associated pathologies.

Disclosure of Interest: None Declared

O22
THE STROMAL TISSUE COMPARTMENT AS AN ORCHESTRATOR OF IL-6-DRIVEN ANTI-MICROBIAL HOST DEFENCE
J. Uceda1, D. Millrine1, A. Cardus1, R. Andrews2, C. Rice1, V. Tyrrell1, C. Fielding1, B. Szomolya2, B. Coles1, C. Colmont1, N. Topley1, P. Taylor1, N. Williams3, G. W. Jones1, S. A. Jones1
1Division of Infection and Immunity, School of Medicine, 2Systems Immunity Research Institute, 3Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff, United Kingdom

Introduction: Interleukin (IL)-6 promotes anti-microbial host defence against bacteria, viruses, parasites and fungi. In acute resolving infection IL-6 restricts bacterial dissemination and drives bacterial clearance by regulating...
innate immune responses. However repeated bouts of infection promotes tissue damage through an IL-6-mediated expansion of pro-fibrotic effector CD4 T-cells. Thus, IL-6 compromises tissue repair by shifting acute resolving inflammation into a chronic pro-fibrotic state. Using murine models of sterile peritonitis and live bacterial infection, we have examined how IL-6 signaling in the stromal tissue compartment reconciles the development of these two distinct inflammatory outcomes.

**Methods:** Acute peritoneal inflammation was established in WT, Il6–/– and Il6ra–/– mice – stimulated using a cell-free supernatant of *Staphylococcus epidermidis* (SES), or a fluorescently labelled *S. epidermidis* isolate. Experiments tracked bacterial clearance and dissemination, and changes in leukocyte infiltration and activation, body temperature, and regulation of the acute phase response. At optimal timepoints for IL-6-driven STAT1 and STAT3 signalling, mesothelial lining tissue was isolated for RNA-seq and ChIP-seq. Analysis was performed during acute resolving infection, and following transfer of ex vivo polarised interferon (IFN)-γ secreting CD4 T-cells.

**Results:** Molecular pathway analysis of RNA-seq data, and ChIP-seq analysis of STAT1 and STAT3 showed that IL-6 activation of the stromal tissue compartment promotes responses relevant to the maintenance of anti-microbial immunity. These include effects on iron sequestration, complement regulation, and leukocyte trafficking and activation. While IL-6 was unable to directly activate neutrophil function, infiltrating neutrophils from Il6–/– mice showed reduced phagocytic capacity to *S. epidermidis* infection. In this respect, we will present data showing that reconstitution of IL-6 activity in Il6–/– and Il6ra–/– mice, or supplementation with IFN-γ, rescues the defective anti-microbial host response. We will further show that adoptive transfer of pro-fibrotic CD4 T-cells together with SES triggers substantive alterations in the capacity of the stromal peritoneal tissues to respond to acute inflammation, and enhances the transcriptional output displayed by challenged Il6–/– mice. Importantly, the IFN-γ-mediated activation of STAT1 in the peritoneum restored the anti-microbial activities observed in the peritoneal membrane of Il6–/– mice. This enhanced contribution of STAT1 also impacted genes linked with the onset of peritoneal fibrosis. Thus, pro-fibrotic IFN-γ secreting CD4 T-cells may promote antimicrobial host defence and bacterial containment within the infected tissue.

**Conclusion:** These data provide a novel holistic perspective into the dynamic relationship between IL-6 and IFN-γ signalling, and highlight the importance of STAT1 and STAT3 in linking anti-microbial host defence with inflammation-induced tissue damage.

**Disclosure of Interest:** None Declared

**O23**

**IFN-LAMBDA FACILITATES PROTECTIVE T CELL IMMUNITY BY MODULATION OF MIGRATORY DENDRITIC CELL FUNCTION DURING INFLUENZA VIRUS INFECTION**

E. A. Hemann1, R. Green1, R. A. Langlois2, R. Savan1, M. Gale1

1Immunology, University of Washington, Seattle, 2Microbiology and Immunology, University of Minnesota, Minneapolis, United States

**Introduction:** Influenza A virus (IAV) is a public health threat that has the potential to cause pandemic outbreak in addition to annual, seasonal epidemics. While an IAV vaccine is available, it is rapidly subverted through viral genetic drift, necessitating strategies to limit IAV infection and drive robust, protective immune responses. Type III interferon (IFN), IFN-lambda (IFNA), is the most highly produced IFN in the lung during IAV infection and optimally restricts IAV *in vivo* compared to type I IFN. IFNA influences pulmonary CD4 T cell function, however the contribution of IFNA to the critically protective CD8 T cell response against IAV has not been defined. Here we evaluated the role of IFNA signaling in the development of CD8 T cell responses against IAV infection in a murine model.

**Methods:** To determine the contribution of IFNA to the development of protective immune responses against IAV, WT, IFNA receptor knock out (*Ifnlr<sup>-/-</sup>), *Ifnlr<sup>-/-</sup> floxed* (*Ifnlr<sup>f/f</sup>*) or *Ifnlr<sup>-/-</sup> mice crossed to CD11c<sup>Cre</sup> or Lysm<sup>Cre</sup> all on a C57Bl/6 background were infected intranasally with IAV and morbidity/mortality was monitored. At various timepoints following infection, lungs and lung-draining lymph nodes (dLN) were harvested and virus titers, cellular migration, and immune responses were assessed. In addition, dendritic cells (DCs) were sorted from dLN of WT and *Ifnlr<sup>-/-</sup> mice following infection and subjected to mRNA sequencing and analysis.

**Results:** Our studies revealed an essential role for IFNA signaling to support cytokine-producing IAV-specific effector CD8 T cells. We found that the CD8 T cell response against IAV was significantly reduced in IFNA receptor knock-out (*Ifnlr<sup>-/-</sup>*) mice compared to WT. Importantly, *Ifnlr<sup>-/-</sup> mice exhibited reduced immune protection upon heterologous IAV re-challenge that is dependent upon T cell memory responses. This immune defect was not T cell intrinsic but instead mapped to altered function and migration of DCs in *Ifnlr<sup>-/-</sup>* mice. These observations link IFNA signaling in DCs with generation of a protective CD8 T cell response. Additionally, conditional knockout of the *Ifnlr* in DCs confirmed their role in generation of optimal CD8 T cell responses against IAV infection. Transcriptomic analyses revealed the dysregulation of a specific set of IFNA-dependent genes in migratory DCs that impart regulation of antigen uptake, processing and/or presentation.

**Conclusion:** Our findings reveal a unique role for IFNA in the governance of DC function and subsequent development of protective CD8 T cell responses against IAV infection. These results may be broadly applicable to development of therapeutics against respiratory and other viral infections.

**Disclosure of Interest:** None Declared
O24
INACTIVATION OF TYPE I INTERFERON RESPONSES IN THE TUMOR MICROENVIRONMENT TRIGGERS THE STROMAGENIC SWITCH AND DESMOPLASIA
C. Cho1,*, R. Mukherjee1, J. Gui1, K. V. Katlinski1, Y. V. Katlinskaya1, S. Y. Fuchs1
1Biomedical Sciences, University Of Pennsylvania, Philadelphia, United States

Introduction: Desmoplasia—the excessive and abnormal deposition of extracellular matrix (ECM) proteins around malignant cells in solid tumors—is known to support tumor growth and progression and is long associated with resistance to therapy and poor prognosis. Desmoplastic ECM is assembled by cancer-associated fibroblasts (CAF), which are derived from resident fibroblasts and recruited mesenchymal cells that are pathologically activated into CAFs through a process called the stromagenic switch. The molecular mechanisms underlying this switch remain poorly understood. Our current studies reveal a novel mechanism wherein downregulation of the type I interferon receptor (IFNAR1) in the non-immune cells of tumor microenvironment (TME) induces the stromagenic switch to generate a desmoplastic microenvironment supportive of tumor growth.

Methods: Transplantable mouse models of colorectal carcinoma were utilized to study the biological significance of IFNAR1 inactivation in the stromagenic switch, desmoplasia and primary tumor growth. Bone marrow transplantation and co-injection studies were used to examine the role of stromal IFNAR1 in CAF activation and the generation of a tumor-supportive desmoplastic microenvironment. The expression of fibroblast activating protein (FAP)—a CAF marker—was confirmed by immunofluorescent analysis and the desmoplastic reaction was studied by immunohistochemistry for hyaluronic acid (HA), fibronectin, and versican.

Results: Our previous studies demonstrated that IFNAR1 undergoes ubiquitination-dependent degradation in the TME leading to the inactivation of type I interferon signaling. Using a syngeneic mouse model of colorectal carcinoma, we found that robust tumor growth is associated with the accumulation of CAFs expressing FAP, and high levels of desmoplasia manifested by the abundance of HA, versican and fibronectin. Remarkably, tumors grew poorly in mice that express the ubiquitination-deficient stable mutant form of IFNAR1 (IFNAR1S526A) and these tumors exhibited reduced FAP expression and a diminished desmoplastic reaction. Bone marrow transplantation experiments demonstrated that the stromagenic switch, desmoplasia and maximal tumor growth require the inactivation of IFNAR1 in non-immune tissues. Furthermore, co-injection of wild-type fibroblasts partially restored the ability of colorectal carcinoma cells to grow in the IFNAR1S526A knock-in mice. Altogether the data demonstrate that IFNAR1 downregulation in stromal cells promotes the generation of a desmoplastic microenvironment that stimulates tumor growth.

Conclusion: Our studies show that loss of IFNAR1 expression in stromal cells is a critical step in developing a tumor supportive microenvironment. Efforts to stabilize IFNAR1 in the TME could serve to prevent the stromagenic switch and ensuing desmoplasia and ultimately curtail tumor growth.

Disclosure of Interest: None Declared

O25
TYPE I INTERFERON SENSING UNLOCKS DORMANT ADIPOCYTE INFAMMATORY POTENTIAL
1Immunobiology, Cincinnati Children's Hospital, Cincinnati, 2UCLA, Los Angeles, 3Cincinnati Children's Hospital, Cincinnati, 4Children's Hospital Colorado, Aurora, United States

Introduction: White adipose tissue (WAT) inflammation, in part via myeloid cell contribution, is a central pathogenic promoter of obesity-associated sequelae. Despite some conserved inflammatory capabilities between myeloid cells and adipocytes, mechanisms regulating adipocyte inflammation remain poorly understood. We hypothesized that activation of the type I IFN/IFNAR axis in adipocytes uncovers immune-like inflammatory signatures and exacerbates adipocyte-intrinsic inflammatory vigor.

Methods: Primary Adipocyte Cultures: Stromal vascular fractions from mice or humans were differentiated into primary adipocytes, primed with IFNβ, subsequently challenged with LPS and analyzed for gene/protein expression and cellular bioenergetics. Gene expression of adipocytes and macrophages were compared by RNA-sequencing.

Obesity studies: WT or IFNAR1−− mice or bone marrow chimera mice (WT and IFNAR1−−) were fed a high fat or chow diet for 22 weeks with body weight/food intake monitored weekly. Adipose tissue inflammation and severity of obesity-associated sequelae were quantified at harvest.

Human cohorts: Bariatric surgery participants were stratified into metabolically healthy or metabolically challenged groups according to well-established clinical parameters.

Results: We show that activation of the type I interferon (IFN)/IFNa receptor (IFNAR) axis robustly amplified adipocyte inflammatory vigor and uncovered dormant gene expression patterns resembling inflammatory myeloid cells. IFNb-sensing altered adipocyte cellular energetics, while counter regulation of cellular energetics impeded IFNb-driven amplification of intra-adipocyte inflammation. Obesity-associated induction of type I IFN axis and activation of IFNAR signaling on non-hematopoietic cells strongly contributed to obesity pathogenesis in mice. Notably, IFNb effects were conserved in human adipocytes and detection of the type I IFN/IFNAR axis-associated signatures positively
correlated with obesity-driven metabolic derangements in humans.

**Conclusion:** Our findings reveal the capacity for the type I IFN/IFNAR axis to uncover unifying features in the regulation of both myeloid cell and adipocyte inflammatory vigor. Further, these data hint at a potential underappreciated role of intra-adipocyte inflammation in the pathogenesis of obesity as well as other type I IFN-associated diseases.

**Disclosure of Interest:** None Declared

**O26**

**THE LONG NON-CODING RNA LUCAT1 TRANSCRIPTIONALLY REGULATES TYPE-I INTERFERON RESPONSES**

S. Agarwal\(^1\), S. Ghosh\(^1\), P. Vangala\(^2\), E. Ricci\(^3\), D. Caffrey\(^4\), M. Garber\(^2\), K. Fitzgerald\(^4\)

\(^1\)Department of Medicine, \(^2\)University of Massachusetts, Worcester, United States, \(^3\)Centre international de recherche en infectiologie (CIRI), Ecole normale supérieure de Lyon, Lyon, France

**Introduction:** The transcription of Type 1 interferons (IFN-I) and ISGs is a hallmark of innate antiviral defenses critical to eliminate viral infections. Failure to shut down this response can result in unrestricted and deleterious immune activation and autoimmune diseases such as Lupus. Using RNA sequencing approaches we have obtained a deeper understanding of this transcriptional response. Numerous protein coding genes and non-coding RNAs including both miRNA and long non-coding RNAs are regulated during the IFN response. Long non-coding RNAs (lncRNAs) are important regulators of gene expression and recent evidence suggests some of these fine tune the inflammatory response in immune cells.

**Methods:** In this study, we are set out to profile the expression of long non-coding transcripts that may contribute to the regulation of innate immune responses. We performed high throughput RNA-sequencing (RNA-seq) in a time course of primary human dendritic cells infected with both herpes simplex virus (HSV1, a DNA virus) and influenza A virus (IAV, an RNA virus). We identified several lncRNA that were dynamically regulated in virus infected cells.

**Results:** One of these lncRNA, LUCAT1 was one of the most potently induced lncRNAs in cells infected with both IAV and HSV1. LUCAT1 expression was also increased in THP-1 cells in response to HSV1, IAV or in cells stimulated with various innate immune ligands. To gain insights into the potential function of LUCAT1 in inflammatory responses, we used both loss and gain of function approaches. We generated LUCAT1 deficient THP-1 cell lines using CRISPR-cas9 and shRNA techniques. Gene expression analysis of LUCAT1 deficient cell lines showed increased Type IFN-I response and cytokines including CXCL10 in response to TLR ligands and virus infection when compared to respective controls cells. Furthermore, using western blot analysis, we found prolonged phosphorylation of STAT1 in addition to increased levels of total STAT1 suggesting hyper activation of signaling events in LUCAT1 deficient cells. Conversely, LUCAT1 overexpression cell lines, generated using CRISPR-cas9 SAM activation showed decreased Type I Interferon responses in response to TLR ligand and virus infections, suggesting a role for LUCAT1 transcript in modulating immune responses. Fractionation of stimulated THP-1 cells, revealed that LUCAT1 was enriched in the nuclear compartment. Fluorescent in-situ hybridization studies and RNA immunoprecipitation confirmed chromatin localization of LUCAT1. Increased expression of Type-I IFN and ISGs along with chromatin localization suggested transcriptional regulation of immune gene expression by LUCAT1. Chromatin-immunoprecipitation using active hallmarks of transcription showed increased enrichment of RNA polymerase II and H3K4 tri-methylation at the promoter regions of IFN-b and ISGs like Viperin.

**Conclusion:** Collectively, these findings reveal the inducible expression of IncRNA LUCAT1, which in turn restrains the type I IFN response. Ongoing efforts are focused on defining the molecular and biochemical strategies and protein binding partners that enable LUCAT1 to restrain interferon responses.

**Disclosure of Interest:** None Declared

**O27**

**A LONG NONCODING RNA ATTENUATES DENDRITIC CELL CYTOKINE PRODUCTION AND DECREASES AUTOIMMUNE DISEASE SUSCEPTIBILITY**

T. Brodnicki\(^1\), M. Ashton\(^1\), C. Elzo\(^1\), E. Chu\(^1\), S. Ford\(^2\), N. Payne\(^3\), M. Alsaby\(^4\), H. Thomas\(^5\), S. Manning\(^1\), T. Kay\(^1\), T. Papenfuss\(^5\), E. Gurzov\(^5\), R. Kitching\(^6\), C. Bernard\(^6\), H. Acha-Orbea\(^6\), G. Morahan\(^6\), K. Shortman\(^6\), M. O’Keeffe\(^6\)

\(^1\)Immunology & Diabetes Unit, St Vincent’s Institute, Fitzroy, \(^2\)Department of Medicine, \(^3\)Australian Regenerative Medicine Institute, Monash University, Clayton, \(^4\)The Walter & Eliza Hall Institute, Parkville, Australia, \(^5\)Université Libre de Bruxelles, Brussels, Belgium, \(^6\)Department of Biochemistry, University of Lausanne, Lausanne, Switzerland, \(^7\)Harry Perkins Institute, University of Western Australia, Nedlands, \(^8\)Department of Biochemistry & Molecular Biology, Monash University, Clayton, Australia

**Introduction:** A complex network of mechanisms allows appropriate immune responses while preventing destruction of an organism’s own tissues. Recent studies indicate that long noncoding RNAs (lncRNAs) are a component of this network, but their impact upon the development of autoimmune disease is poorly understood. LncRNAs are broadly defined as RNAs that are >200 nucleotides in length and perform molecular functions without encoding proteins.

**Methods:** In this study, a combination of genetic and transcriptome profiling, immunological and biochemical assays, knockout mice and models of autoimmune disease were used to investigate the role of lincRNAs in dendritic cell function and autoimmunity.
Results: We have identified more than twenty putative murine IncRNAs that are differentially expressed in dendritic cells upon toll-like receptor (TLR) activation. Sequence variation for one of these IncRNAs is associated with autoimmune diabetes in the nonobese diabetic mouse strain. This IncRNA is detected in the nucleus and cytoplasm of dendritic cells after induction by TLR activation, and binds SHIP1, an inositol phosphatase that regulates dendritic cell function. To further investigate its function, we established C57Bl/6 mice that lack expression of this IncRNA. Upon TLR activation, dendritic cells from these mice have enhanced cytokine production associated with decreased SHIP1. Moreover, genetic disruption of this IncRNA increased susceptibility in different models of autoimmune disease.

Conclusion: Our findings highlight a role for IncRNAs in promoting immune tolerance by stabilizing regulatory proteins and attenuating specific innate immune responses that may otherwise precipitate pathological tissue destruction.

Disclosure of Interest: None Declared

O28
AIM2-DEFICIENT DENDRITIC CELL VACCINE FACILITATES ADOPTIVE T-CELL THERAPY AND ANTI-PD-1 IMMUNOTHERAPY VIA THE ACTIVATION OF STING-TYPE I IFN SIGNALING
K. Fukuda1, R. L. Riding1, J. E. Harris1
1 Dermatology, University of Massachusetts Medical School, Worcester, United States

Introduction: The production of type I IFN through the recognition of tumor-derived, cytosolic DNA by STING signaling in tumor infiltrating dendritic cells (DCs) has been known to induce a spontaneous T-cell response against melanoma and to correlate with the prognosis of immunotherapies. However, cytosolic DNA is also a ligand of AIM2, an innate immune sensor that generates mature forms of IL-1β and IL-18 and induces pyroptosis, whose function in melanoma is still unknown.

Methods: To determine whether AIM2 would exhibit a positive or negative regulatory role in anti-melanoma immunity, we subcutaneously challenged Aim2−/− mice with B16F10 melanoma and examined the immune cells that infiltrated in the tumor by flow cytometry. In addition, we performed biochemical analyses of bone marrow-derived DCs (BMDCs) of Aim2−/− mice (Aim2−/− BMDCs) stimulated with B16F10 melanoma-derived DNA to assess the role of AIM2 in STING-type I IFN signaling. Furthermore, to assess the applicability of AIM2-deficient DC vaccination as a treatment option to enhance the anti-melanoma response of immunotherapies, we performed adoptive T-cell therapy (ACT) using PMEL-specific CD8+ T cells (PMEls) or anti-PD-1 immunotherapy in combination with intravenous injection of PMEL peptide-pulsed Aim2−/− BMDCs (PMEL Aim2−/− BMDCs) in wild-type (WT) mice inoculated with B16F10 melanoma.

Results: We observed that Aim2−/− mice with B16F10 melanoma exhibit significantly lower tumor burden with fewer tumor-infiltrating regulatory T cells (Tregs) and higher CD8+/Treg ratio than WT mice, indicating that AIM2 exerts an immunosuppressive effect in the melanoma microenvironment. In vitro experiments confirmed that in response to tumor-derived DNA in a dose-dependent manner, the Aim2−/− BMDCs enhanced STING signaling activation and significantly increased Ifnb and Cxcl10 expression, which promotes CD8+ T-cell infiltration, and decreased Ccl22 expression, which promotes Treg migration, compared with WT BMDCs. The mice that received ACT with PMEL Aim2−/− BMDCs and anti-PD-1 immunotherapy with PMEL Aim2−/− BMDCs had significantly lower tumor burden, higher number of tumor-infiltrating PMELs, lower percentage of tumor-infiltrating Tregs in the CD4+ T-cell population, and higher CD8+/Treg ratio than those that received ACT with PMEL WT BMDCs and anti-PD-1 immunotherapy with PMEL WT BMDCs, respectively. Importantly, this enhanced the effect of AIM2 deficient DC vaccination was largely due to the recognition of tumor-derived DNA in the tumor microenvironment by STING. The addition of intratumoral injection of DNaseI or the use of PMEL Aim2−/−Sting−/− BMDCs rescued the phenotype.

Conclusion: AIM2 signaling in DCs suppresses STING-mediated production of type I IFN and elimination of AIM2 during DC vaccination could improve the anti-melanoma responses of ACT and anti-PD-1 immunotherapy via enhanced activation of STING-type I IFN signaling.

Disclosure of Interest: None Declared

O29
THE EXPANDING FUNCTIONS OF THE INFLAMMASOME COMPLEXES
V. Rathinam1,2
1Immunology, UConn Health School of Medicine, Farmington, United States

Introduction: Gasdermin D is a pore forming protein. Inflammasome-activated caspase-1 cleaves gasdermin D to unmask its pore forming activity, the predominant consequence of which is pyroptosis. Gasdermin D can also mediate the secretion of IL-1 cytokines via its pores independent of pyroptosis.

Methods: We utilized various macrophage cell types and a Francisella in vivo model to discover a new role for gasdermin D.

Results: Here, we report a new biological role for gasdermin D in limiting cytosolic DNA surveillance. Cytosolic DNA is sensed by the Aim2 inflammasome as well as cyclic GMP-AMP synthase (cGAS) to activate gasdermin D and type I interferon expression, respectively. We found that gasdermin D activated by the Aim2 inflammasome suppresses cGAS-driven type I interferon responses of macrophages to cytosolic DNA and Francisella novicida, a cytosol-invasive bacterium. Similarly, F. novicida-infection induced IFN-b response and lethality were significantly elevated in gasdermin D-
deficient mice. Gasdermin D-mediated negative regulation of IFN-b occurs in a cell death- and IL-1/IL-18-independent manner. Mechanistically, gasdermin D, via its membrane pores, disrupts intracellular ionic homeostasis, which is necessary and sufficient to inhibit cGAS-dependent type I interferon responses.

**Conclusion:** Thus, our findings uncovered a new interferon regulatory module involving gasdermin D.

**Disclosure of Interest:** None Declared

**O30**

**THE NLRC4 INFLAMMASOME REQUIRES IRF8-DEPENDENT PRODUCTION OF NAIPS**

R. Karki1,*, E. Lee1, T.-D. Kanneganti1

1Immunology, St. Jude Children’s Research Hospital, Memphis, United States

**Introduction:** Cells are equipped with various pattern-recognition receptors (PRRs) to mount an appropriate immune response. Certain cytosolic receptors, including NLRP3, NLRC4, AIM2 and Pyrin have ability to activate inflammasomes, a molecular platforms that assemble upon sensing various intracellular stimuli to process caspase-1 into its active form, allowing production of mature IL-1b and IL-18. During microbial infection, type I interferons (IFNs) are rapidly induced and multiple intersections exist between type I IFN signaling and inflammasome activation, however, the regulation is limited to NLRP3 and AIM2 inflammasome activation.

**Methods:** We used biochemical, molecular and genetic approaches to examine the role of IRF8 in the activation of the inflammasome.

**Results:** Here we found that IRF8 is required for optimal activation of the NLRC4 inflammasome in bone marrow-derived macrophages infected with Salmonella Typhimurium, Burkholderia thailandensis, or Pseudomonas aeruginosa but is dispensable for activation of the canonical and non-canonical NLRP3, AIM2, and Pyrin inflammasomes. IRF8 governs the transcription of Naips to allow detection of flagellin or T3SS proteins to mediate NLRC4 inflammasome activation. Furthermore, we found that IRF8 confers protection against bacterial infection in vivo, owing to its role in inflammasome-dependent cytokine production and pyroptosis

**Conclusion:** Our findings show that IRF8 is a transcriptional regulator of Naips that are prerequisite for NLRC4 inflammasome activation, ultimately contributing to host defense against bacterial pathogens.

**Disclosure of Interest:** None Declared

**O31**

**GERM-CELL SPECIFIC INFLAMMASOME COMPONENT NLRP14 NEGATIVELY REGULATES CYTOSOLIC NUCLEIC ACID SENSING TO PROMOTE FERTILIZATION**

S. Shapira1,2; T. Abe1, A. Lee1, R. Rabadan1

1Systems Biology, 2MicroBiology and Immunology, Columbia University, New York, United States

**Introduction:** Cytosolic sensing of nucleic acids initiates tightly regulated programs to limit infection. Oocyte fertilization represents a scenario when inappropriate responses to exogenous yet non-pathogen-derived nucleic acids would have negative consequences.

**Methods:** We hypothesized that germ cells express negative regulators of nucleic acid sensing (NAS) in steady state and applied an integrated data-mining and functional genomics approach to identify a rheostat of DNA and RNA sensing – the inflammasome component NLRP14.

**Results:** We demonstrated that NLRP14 interacted physically with the nucleic acid sensing pathway and targeted TBK1 (TANK Binding Kinase 1) for ubiquitination and degradation. We further mapped domains in NLRP14 and TBK1 that mediated the inhibitory function. Finally, we identified a human nonsense germline variant associated with male sterility that results in loss of NLRP14 function and hyper-responsiveness to nucleic acids.

**Conclusion:** This discovery points to a mechanism of nucleic acid sensing regulation that may be of particular importance in fertilization and suggests that development of sexual reproduction required an evolutionary leap that enabled the repression of innate immunity and genome protection.

**Disclosure of Interest:** None Declared

**O32**

**"THE MULTIFACETED M152 PROTEIN SELECTIVELY MODULATES STING-DEPENDENT SIGNALING TO ENHANCE MURINE CYTOMEGALOVIRUS REPLICATION"**

M. Stempel1, B. Chan1, V. Juranic Lisnic2, S. Paludan3, S. Jonić2, N. Lemmermann4, M. M. Brinkmann1,*

1Helmholtz Centre for Infection Research, Braunschweig, Germany, 2Center for Proteomics, University of Rijeka, Rijeka, Croatia, 3Biomedicine, University of Aarhus, Aarhus, Denmark, 4Institute for Virology, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

**Introduction:** Here we present the identification of a novel viral antagonist, namely the m152 protein encoded by murine cytomegalovirus, which targets the STING-mediated antiviral type I interferon (IFN) response. More importantly, through our investigation into the consequences of m152 antagonism of STING, we have uncovered new insights into the precise steps of the STING signaling pathway.

**Methods:** Immunofluorescence, live cell imaging, in vitro and in vivo infection experiments, immunoprecipitation, luciferase reporter assays, ELISA, qPCR

**Results:** We show that the type I transmembrane protein m152 binds to the two ER-luminal loops of STING via its own luminal domain. m152 accompanies STING to the Golgi upon cGAS stimulation, resulting in a delay of trafficking of STING to this compartment. Strikingly, we have observed that whilst m152 specifically diminishes the cGAS-STING-mediated activation of IRF3, it does not
O33
ACTIVATION OF MITOCHONDRIAL APOPTOTIC SIGNALING TRIGGERS NLRP3 INFLAMMASOME ACTIVATION
J. E. Vince¹, D. De Nardo¹, W. Gao², A. Vince¹, C. Hall¹, K. McArthur³, S. Vijayaraj¹, L. M. Lindqvist¹, J. M. Chambers⁴, M. A. Rizzacasa³, J. Silke¹, S. L. Masters¹, G. Lessene³, D. H. Gray¹, B. T. Kile³, F. Shao², K. E. Lawlor⁵
¹The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, ²National Institute of Biological Sciences, Beijing, China, ³Monash University, Clayton, ⁴The University of Melbourne, Melbourne, ⁵Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Australia

Introduction: Apoptotic cell death is classically regarded as immunologically silent. The mitochondrial or “intrinsic” apoptotic pathway is an evolutionarily conserved, BCL-2 family regulated, process that promotes the death and phagocytic clearance of stressed, damaged or infected cells. BAX and BAK-mediated mitochondrial membrane damage is essential for mitochondrial apoptosis, and is required for the downstream activation of apoptotic effector caspase activity. Consequently, the study of BAX/BAK regulation in cancer cells has resulted in the development of rationally designed BH3-mimetics compounds, which trigger BAX/BAK to induce cancer cell death. However, research into the consequences of activating mitochondrial apoptosis in innate immune cells, such as macrophages, is less well studied.

Methods: Wildtype and gene knockout (deficient in apoptotic or inflammasome signaling machinery) macrophages were primed with LPS and treated with a range of targeted BH3-mimetic compounds, and other cancer chemotherapeutics. At specified times, cytokine levels and cell death were measured by ELISA and flow cytometric analysis of propidium iodide uptake, respectively. Inflammasome and cell death activity was further assessed by Western blot.

Results: Using our targeted BH3-mimetic compounds and gene knockout macrophages, we discovered that BCL-XL and MCL-1 are the key pro-survival BCL-2 family members required to prevent spontaneous BAX/BAK-dependent apoptotic cell death in macrophages. Unexpectedly, we also observed that activation of BAX/BAK signaling in macrophages triggered inhibitor of Apoptosis (IAP) protein loss to promote caspase-8-mediated proteolysis of Interleukin-1β (IL-1β). In parallel, BAX/BAK signaling induced NLRP3 inflammasome activation via potassium efflux. Remarkably, using a panel of mice deficient in apoptotic and inflammasome signaling machinery, we found that the apoptotic executioner caspases, caspase-3 and -7, acted upstream of both caspase-8 and NLRP3. In contrast, pyroptotic cell death effectors, gasdermin D and gasdermin E, were not essential for cellular demise or IL-1β secretion.

Conclusion: These data reveal for the first time how specific activation of mitochondrial cell death can promote inflammation. We believe this novel pathway may underpin observations linking chemotherapeutic and cellular stress responses to inflammasome and IL-1β-driven inflammation.

Disclosure of Interest: None Declared

O34
IL-38 Restricts Anti-Tumor Immunity by Limiting IL-17 Production from Gamma-Delta T Cells Through IL1RAPL1
J. Mora¹,², Y. Han¹, B. Bruene¹, A. Weigert¹
¹Institute of Biochemistry I, Frankfurt University, Frankfurt, Germany, ²Facultad de Microbiología, University of Costa Rica, San Jose, Costa Rica

Introduction: Interleukin-38 (IL-38; IL1F10) is a poorly characterized cytokine of the IL-1 family. While accumulating evidence from our group and others suggests a role in modulating chronic inflammation downregulating production of inflammatory cytokines such as IL-6 and IL-17, its main cellular targets and receptor(s) remain obscure. We have previously described the release of IL-38 from apoptotic tumor cells, nevertheless the role of this anti-inflammatory cytokine in tumor biology has not been explored. Establishment of an immunosuppressive
milieu is an essential requirement for tumor progression, therefore we aimed to dissect the role of IL-38 as a novel immune suppressor within the tumor microenvironment.

Methods: We used the PyMT oncogene-driven mammary tumor model, un wild type and IL-38 knock out mice. Tumor cytokine expression and tumor infiltration were analyzed by flow citometry and RT-PCR. In vitro experiments were performed using gamma delta T cell from IL-1RAPL1 knock out mice and cytokine production was measured by Citometric Beads Array.

Results: We found that the growth of PyMT oncogene-driven mammary tumors was limited in IL-38 mice, accompanied by an increase in infiltration of anti-tumoral cell populations such as CD8+ T lymphocytes, γδ T cells, NK and NKT cells, as well as an increase in expression of pro-inflammatory cytokines. Based on previously described role of IL-38-mediated IL-17 regulation, we identified γδ T cells as main sources of increased IL-17 production in IL-38 KO mice. Mechanistically, IL-38 antagonized the orphan IL-1 family receptor X-linked interleukin-1 receptor accessory protein-like 1 (IL1RAPL1), which was upregulated upon γδ T cell activation to feed-forward amplify IL-17 production. Clinical data base analysis indicated an association of IL1RAPL1 with γδ T cells in mammary carcinoma patients.

Conclusion: In conclusion, support that an anti-tumor γδ T17 response is activated in the absence of the anti-inflammatory cytokine IL-38. We identified γδ T cells as a primary target for IL-38 to limit anti-tumor immunity through antagonizing IL1RAPL1.

Disclosure of Interest: None Declared

O35 SIGNIFICANT REDUCTION OF TUMOR GROWTH AND METASTASIS BY INTRATUMOR TREATMENT OF A SHRNA TARGETING IL-17RA IN A MURINE B16F10 MELANOMA MODEL

Y.-S. Chen1, C.-L. Liu2, H.-S. Chen3, M.-H. Lee1, C.-R. Shen1

1Chang Gung University, Graduate Institute of Biomedical Sciences, Taoyuan City, 2Ming Chi University of Technology, Department of Chemical Engineering, New Taipei City, 3Chang Gung University, Department and Graduate Institute of Medical Biotechnology and Laboratory Science, Taoyuan City, Taiwan, Province of China

Introduction: Interleukin 17 (IL-17) and IL-17-expressing cells appear to involve in promoting tumor development and associated with survival in cancer patients. Here we intend to develop a potential therapeutic strategy by intratumoral targeting IL-17/IL-17 receptor (IL-17RA) axis in a IL-17RA-expressing B16F10 melanoma tumor model.

Methods: IL-17RA knockdown B16F10 cells were utilized to identify the effects of the IL-17/IL-17RA signaling on tumor growth, migration, invasion as well as the tumor development. The therapeutic efficacy of adenoviral shRNA against IL-17RA were evaluated in the tumor-bearing animals, and its impact on anti-tumor immunity was also analyzed.

Results: Treatment with γmIL-17 significantly enhanced cell proliferation in B16F10 cells, and tumor growth and metastasis rates were significantly lower in IL-17-deficient mice, indicating the crucial role of IL-17. When IL-17RA was knocked down in tumor cells, the γmIL-17 elicited cell growth, migration, invasion as well as VEGF and MMP production were significantly suppressed. Most importantly, locally administering an adenovirus to deliver an shRNA against IL-17RA significantly suppressed tumor growth in tumor-bearing mice. Such treatment consequently enhanced anti-tumor immunity via marked increase of IFNγ production and reduction of Treg infiltration.

Conclusion: These results suggest that the reduction of IL-17RA on tumor cells suppressed IL-17 signaling, which promotes tumor cell proliferation and invasion. The intratumoral targeting on the IL-17RA appears to be a potential therapeutic strategy for cancers.

Disclosure of Interest: None Declared

O36 MITOCHONDRIAL DOUBLE STRANDED RNA TRIGGERS ANTIVIRAL SIGNALLING IN HUMANS

A. Dhir1,*, 1Sir William Dunn School of Pathology, University of Oxford, OXFORD, United Kingdom

Introduction: Mitochondria are descendants of endosymbiotic bacteria and retain essential prokaryotic features such as a compact circular genome. Bidirectional transcription of mtDNA is an extreme example of convergent transcription in mammalian cells owing to symmetrical synthesis of both the heavy (H) and the light (L) strands. Notably, nearly the entire L-strand transcript undergoes rapid RNA decay by the RNA degradosome. This decay process likely prevents the formation of potentially deleterious mtdsRNA. Indeed, among different cellular compartments, mitochondrial RNA (mtRNA) is known to be especially immunogenic. Cellular nucleic acid sensors must discriminate viral nucleic acids from the vast excess of often biochemically indistinguishable cellular RNA and DNA as part of the innate immune response. To achieve this, nucleic acid metabolism is pivotal in suppressing immune responses to self-nucleic acids. We sought to determine whethermitochondria are also a source of dsRNA in vivo, and in so doing uncover a pathway that suppresses the formation of immunostimulatory mtdsRNA.

Methods: We employed an anti-dsRNA monoclonal antibody as a tool for dsRNA detection by immunofluorescence and IP-seq based approach.

Results: Immunofluorescence (IF) signals were also observed in uninfected HeLa cells suggesting the existence of cellular dsRNA. To further characterize these cellular IF signals, fixed cells were pretreated with structure specific RNases. IF signals were sensitive to
dsRNA specific RNase III but not ssRNA specific RNase T1 or TURBO DNase confirming the presence of dsRNA at a single cell level. We next performed a J2 IP based dsRNA-seq to identify selected cellular dsRNA. Notably the mitochondrial genome generates nearly all detectable cellular dsRNA with 99% of the reads attributable to the mitochondrial genome. Notably, the RNA-seq profile showed widespread reads from both H and L-strand of mtDNA, implying the presence of intermolecular dsRNA. This was confirmed by IF as 95% of J2 foci colocalized with mitochondria. The mtdsRNA are highly turned over by combined action of mitochondrial proteins dsRNA helicase SUV3 and an exonuclease PNase. Loss of either enzymes in mtdsRNA accumulation resulting in IFN signalling in case of PNase depletion. This was verified in HeLa, PNase KO mice and Patients carrying deleterious PNase mutations. Loss of PNase results in mtdsRNA release into the cytosol and seems to be mediated by Bax/Bak pores.

Conclusion: Here, we describe the presence of a highly unstable native mtdsRNA species at single cell level and identify key roles for the degradosome components, mitochondrial dsRNA helicase SUV3 and exonuclease PNase in restricting mtdsRNA levels. Loss of either enzyme results in massive accumulation of mtdsRNA that escapes into the cytoplasm in a PNase dependent manner. This engages an MDA5 driven antiviral signalling pathway that triggers a type I interferon response. Consistent with these data, patients carrying hypomorphic mutations in PNPT1 display mtdsRNA accumulation coupled with upregulation of interferon stimulated genes (ISGs) and other markers of immune activation. The localization of PNase to both the mitochondrial inter-membrane space (IMS) and matrix suggests its dual role in preventing formation and release of mtdsRNA into the cytoplasm. This in turn prevents the activation of potent innate immune defence mechanisms evolved to protect eukaryotic cells against microbial and viral attack.

Disclosure of Interest: None Declared

O38
MYELOID P38 MAPK PROMOTES TUMORIGENESIS BY SUPPRESSING CYTOTOXIC T CELL RECRUITMENT
M.-K. Choo1,*, J. M. Park1
1Massachusetts General Hospital and Harvard Medical School, Charlestown, United States

Introduction: The last decade has seen explosive progress in understanding how the tumor microenvironment contributes to the development, metastasis, and therapy resistance of cancer. Cellular and molecular components of the tumor microenvironment are investigated as targets for cancer therapy. Tumor-
associated macrophages are one of the well-studied cell types that impact tumorigenesis either positively or negatively. However, the precise mechanisms for their function remain to be fully elucidated. The p38 MAP kinase is known to play a pivotal role in cellular responses to stress, mitogenic stimuli, and immune signaling. Evidence from clinical observations and cell culture experiments reveals a role for p38 in many aspects of cancer and immunity. However, little is known about the functions of p38alpha, the most abundant and ubiquitously expressed p38 isoform, in tumor-associated macrophages.

Methods: DMBA-TPA-induced skin carcinogenesis and isogenic tumor engraftment models were applied to myeloid specific-p38alpha knockout mice. Tumor phenotype and immune cell populations were analyzed by flow cytometry and immunohistochemistry. Tumor-infiltrating macrophages were isolated using F4/80 antibody-coated microbeads. Bone marrow-derived macrophages (BMDM) and PMA-induced THP-1 cells were treated with LPS, and target gene expression and downstream signaling were analyzed by qPCR and immunoblotting. Transcriptome analysis was performed to identify p38alpha target genes in BMDM. CRISPR/Cas9 genome editing was performed for gene ablation in human cells.

Results: Myeloid-specific p38alpha ablation resulted in a decrease in tumor incidence and multiplicity in a DMBA-TPA carcinogenesis model. Tumors from these mutant mice displayed markedly reduced proliferation and poorly developed blood vessels compared to those from control WT mice. Genome-wide expression analysis revealed that p38alpha ablation in macrophages resulted in enhanced CXCL9 expression, a chemokine crucial for cytotoxic T cell recruitment. Indeed, we observed greater number of tumor-infiltrating CD8+ T cells in myeloid-specific p38alpha-knockout mice. p38alpha indel mutations in THP-1 cells also led to elevated CXCL9 induction. TCGA data analysis revealed that higher intratumoral expression of CXCL9 was associated with prolonged survival of cancer patients, suggesting a beneficial effect of CXCL9 in human cancers. Next, we sought to identify the molecular mechanism linking p38alpha to the regulation of CXCL9 expression, and found that p38alpha directly phosphorylates and enables the induction of ATF3, a transcription factor that represses CXCL9 expression. Consistent with this finding, ATF3-deficient macrophages exhibited higher CXCL9 expression similar to p38a-deficient macrophages.

Conclusion: p38alpha in macrophages promotes tumorigenesis by suppressing CXCL9 expression and thereby incapacitating CD8+ T cell-mediated antitumor immunity. p38alpha regulates CXCL9 expression in macrophages via the transcription repressor function of ATF3. Macrophage-specific targeting of p38alpha signaling may promote cytotoxic T cell recruitment to the tumor microenvironment and maximize the potential of checkpoint inhibitor and CAR T cell therapies and other therapeutic modalities that rely on tumor-killing CD8+ T cells.

Disclosure of Interest: None Declared

O39
A PROGRAMMABLE SIGNALING COMPLEX CONTROLS CELL FATE DECISIONS UPON TOLL-LIKE RECEPTOR ACTIVATION
Y. Tan1,2,*, J. Kagan3
1Boston Children’s Hospital, 2Harvard Medical School, 3GI/Nutrition, Boston Children’s Hospital, Harvard Medical School, Boston, United States

Introduction: The signaling platforms of the mammalian innate immune system consist of distinct helical oligomeric protein complexes known as supramolecular organizing centers (SMOCs). Examples of SMOCs include the myddosome, the inflammasome, and the RIG-I-MAVS complex, which respectively regulate TLR-, NLR-, and RLR-mediated responses. Upon microbial detection, pattern recognition receptors induce SMOC assembly, and these structures represent the principal sites where inflammatory signals emanate to promote host defense. However, the spectrum of activities that occur within SMOCs remains unclear.

Methods: To fill these gaps in our knowledge, we combined multi-disciplinary approaches, such as biochemistry, genetics, microscopic analysis, seahorse metabolic analysis, and synthetic biology, to interrogate the biological functions of the myddosome, a key signaling protein complex in the TLR pathway.

Results: Herein, we report that the myddosome is indeed a multifunctional organizing center. In addition to promoting proinflammatory transcription factor activation, the myddosome also drives the rapid induction of aerobic glycolysis. This metabolic reprogramming activity depends on the recruitment and activation of a novel component, TBK1, to the myddosome. This mechanistic insight was leveraged by using synthetic immunology approaches to further diversify the signaling functions of the myddosome, as we engineered this SMOC to induce type-I interferon production or necroptosis upon TLR activation.

Conclusion: These discoveries therefore demonstrate that the myddosome is an organizing center that orchestrates distinct cellular responses, and further highlights SMOCs as modular platforms that can be programmed to rewire cell fate decisions in the innate immune system.

Disclosure of Interest: None Declared

O40
A NEW ANTIVIRAL ISG TDRD7 INHIBITS CELLULAR AUTOPHagy TO CONTROL VIRUS REPLICATION
S. Chattopadhyay1,*, G. Subramanian1, K. Chawla1, R. Chakravarti2
1Medical Microbiology and Immunology, 2Physiology & Pharmacology, University of Toledo College of Medicine and Life Sciences, Toledo, United States

Introduction: The interferon (IFN) system provides the first line of host defense against virus infection. Virus
infection activates the pattern recognition receptors, which via a series of signaling proteins and transcription factors, trigger the synthesis of IFNs and IFN-stimulated genes (ISGs). The ISG-encoded proteins, either alone or in combination with other ISGs, inhibit specific stages of viral life cycle. Here, we report a new antiviral ISG, Tudor domain containing 7 (TDRD7), which inhibits autophagy pathway to suppress viral replication.

**Methods:** We applied a high throughput genetic screen using human ISG shRNA library to identify antiviral ISGs against Sendai virus (SeV), a model paramyxovirus. Using lentiviral expression of the shRNA library followed by flow cytometry-based screening of the virus-infected cells, we isolated a small subset of anti-SeV ISGs. We studied the antiviral activities of the new ISGs using shRNA-mediated stable knockdown (KD), or CRISPR/CAS9-mediated knockout (KO), human and mouse cells.

**Results:** Our screen identified a new antiviral ISG TDRD7, which is transcriptionally induced by virus infection or IFN-treatment. TDRD7 KD or KO human and mouse cells display a robust increase in paramyxovirus replication. Mechanistically, TDRD7 inhibits ‘virus-induced autophagy’ to suppress paramyxovirus replication (Subramanian et al. 2018). The paramyxoviruses, including the clinically relevant human pathogens (RSV, HPIV3), activate cellular autophagy pathway to facilitate virus replication. TDRD7 inhibits AMP-dependent kinase (AMPK), the enzyme required for initiating autophagy. Genetic or chemical inhibition of AMPK blocks paramyxovirus replication. We have expanded the antiviral activity of TDRD7 against the DNA viruses, which similarly rely on host autophagy pathway. Using herpesvirus model, we show that virus infection or cytoplasmic DNA signaling triggers the transcriptional induction of TDRD7 in cells and mouse tissues. The anti-autophagic activity of TDRD7 is required to inhibit these viruses. In contrast, TDRD7 acts as a provilar factor for EMCV, a member of the picornavirus family.

**Conclusion:** We discovered a new facet of the IFN system to regulate the cellular autophagy pathway to differentially control the viruses of various families.

**Disclosure of Interest:** None Declared

O41

NEW TRICKS FOR AN OLD DOG: NON-CANONICAL STAT3 MEDIATES TLR4 MITOCHONDRIAL REPROGRAMMING AND INFLAMMATION

A. S. Mansell1,*, D. Gough2
1Pattern Recognition Receptors and Inflammation, Centre for Innate Immunity and Infectious Diseases; 2Centre for Cancer Research, Hudson Institute of Medical Research, Melbourne, Australia

**Introduction:** Activated macrophages undergo metabolic repurposing following Toll-like receptor (TLR) challenge which drives their proinflammatory phenotype. Recent studies have demonstrated that stimulation of TLRs causes TLR-induced aerobic glycolysis, a phenomenon known as the Warburg effect. Following TLR4 activation, macrophages become more glycolytic and reprogram their mitochondria to generate reactive oxygen species (ROS) from ETC complex I. Together, these events induce transcription factor hypoxia-inducible factor-1a (HIF-1a)-dependent expression of pro-IL-1b. To date however, the mechanism as to how TLR activation induces mitochondrial reprogramming are unknown.

**Methods:** We have utilised primary and immortalised macrophages to dissect the role of STAT3 Ser727 phosphorylation in TLR induced metabolic reprogramming.

**Results:** We have discovered a novel role for non-canonical STAT3 serine phosphorylation which is required for TLR4-mediated mitochondrial reprogramming. We found that TRAF6 interacts with STAT3 following TLR stimulation that results in rapid STAT3 Ser727 phosphorylation, but not pTyr705 within 10 mins of challenge. Importantly, STAT3 pSer727 translocates to the mitochondria where we observed increased mtROS production. We further found that macrophages generated from STAT3 mice with Ala substituted for Ser at position 727 (STAT3 S727A; herein termed STAT3 SA) display dysregulated extracellular acidification and aberrant mitochondrial oxygen consumption compared to WT responses indicative of disrupted glycolytic shift. Critically, this results in reduced expression of inflammatory mediators, such as IL-1b, IL-6, and reduced HIF-1a mRNA. Importantly, IL-10 mRNA and protein expression was unchanged in STAT3 SA macrophages.

**Conclusion:** Our findings identify a novel mechanism of direct cross-talk between the TLR and STAT3 signalling pathways to mediate TLR4-induced mitochondrial repurposing and the proinflammatory response. These studies reveal non-canonical STAT3 signaling as a critical mediator of immunometabolism and metabolic reprogramming. Targeting STAT3 serine phosphorylation may represent an approach to specifically modulate TLR-induced mitochondrial function as a novel approach to restricting inappropriate inflammation.

**Disclosure of Interest:** None Declared

O42

THE MOONLIGHTING PROTEIN 14-3-3 ETA CONTROLS MDA5 ACTIVATION AND THE MDA5-DEPENDENT ANTIVIRAL INNATE IMMUNITY

J.-P. Lin1, H. M. Liu1,2
1Biochemistry and Molecular Biology, National Taiwan University, Taipei, Taiwan, Province of China

**Introduction:** During RNA virus infections, cytosolic RIG-I-like receptors (RLRs), such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5), play an essential role in recognizing foreign viral RNA and subsequently activate downstream cascade signaling pathway to produce type I interferon (IFN). It has been reported that RIG-I can interact with its
O43
IMPORTANT DIFFERENCES IN THE IFN-I RESPONSE TO DNA AND RNA VIRUS PARTICLES
D. Hare1,*, K. Mossman1,2
1Pathology & Molecular Medicine, 2Biochemistry & Biomedical Sciences, McMaster University, Hamilton, Canada

Introduction: Type-I interferon (IFN-I) is produced following virus infection and signals the upregulation of IFN-stimulated genes (ISG) which collectively create an antiviral state in surrounding cells to limit virus replication. Transcription factors like IFN regulatory factor 3 and 7 (IRF3/7) and NF-kB are involved in the upregulation of IFN-I, but also directly upregulate certain ISGs. While recognition of either cytosolic DNA or double-stranded RNA (dsRNA) are believed to activate the same transcription factors and upregulate IFN-I, there are still unexplained differences in the antiviral response to different virus classes which complicate the existing model.

Methods: To compare antiviral response to DNA and RNA virus particles we used UV-inactivated human cytomegalovirus (HCMV) and Sendai virus (SeV) as representative DNA and RNA viruses. To more directly compare differences in signalling following DNA or dsRNA recognition we transfected cells with matched sequence DNA or dsRNA of viral origin. A variety of assays were used to observe protein signaling, induction of ISGs and antiviral state induction.

Results: UV-inactivated HCMV particles potently activated IRF3 and upregulated ISGs like ISG56 and ISG15 in fibroblasts, even when IFN-I signalling was blocked. In contrast, the antiviral response to UV-inactivated SeV particles was entirely IFN-dependent and associated with weak IRF3 activation. Transfection with either DNA or dsRNA was capable of activating IRF3 and NF-kB, but marked differences were seen in the nature of the antiviral response.

Conclusion: We are currently investigating factors that may be responsible for the differences we see between the antiviral response to HCMV and SeV particles. To do this we are expanding our experiments to include additional virus classes as well as utilizing a transcriptomic approach to characterize different antiviral gene profiles triggered by viruses in the presence or absence of IFN-signalling.

Disclosure of Interest: None Declared

O44
IL-27P28 IS AN ANTAGONIST OF B CELL RESPONSE DURING TOXOPLASMOSIS
J. Park1, J. H. DeLong1, C. Konrad1, E. D. T. Wojno2, C. A. Hunter1
1University of Pennsylvania, Philadelphia, 2Cornell University, Ithaca, United States

Introduction: IL-27 is a heterodimeric cytokine that consists of IL-27p28 and Ebi3 subunits and myeloid cells are major sources of IL-27. This heterodimer controls inflammation through T cell or B cell activation and proliferation. The IL-27p28 subunit has been shown to act as a low affinity receptor antagonist of cytokines such as IL-6 that signals through gp130. However, little is known about whether p28 alone impacts on the immune response on infection.

Methods: Therefore, transgenic mice in which T and B cells express IL-27p28 were infected with Toxoplasma gondii. Survival rate, parasitic burden, and immune cell responses were analyzed.

Results: Compared to control mice, transgensics were more susceptible to T. gondii associated with increased parasites in the CNS. Our analysis showed that while there were some inhibitory effects on the parasite specific T cell responses, the production of IFN-g was largely intact. However, the overexpression of IL-27p28 had a major inhibitory effect on germinal center formation and parasite-specific IgG secretion that is required for long resistance to this infection.

Conclusion: The current study supports the idea that IL-27p28, independent of its role as a component of IL-27,
Cytokines 2018 – Abstracts

has an important role in limiting antibody mediated diseases.

Disclosure of Interest: None Declared

O45
IL-36 CYTOKINES ALTER THE COMPOSITION OF THE INTESTINAL MICROBIOME TO PROTECT AGAINST OBESITY AND METABOLIC DYSFUNCTION

E. Giannoudaki1,2,*, Y. E. Hernandez-Santana1,2, K. Multaul1,2, S. L. Doyle1,2, E. Hams1, P. G. Fallon1, A. Mat3, D. O'Shea3, M. Kopf4, A. E. Hogan2,5, P. T. Walsh1,2
1Clinical Medicine, Trinity College Dublin, 2National Children's Research Centre, Our Lady's Children's Hospital Crumlin, 3St Vincent's University Hospital, University College Dublin, Dublin, Ireland, 4Institute of Molecular Health Sciences, ETH Zurich, Zurich, Switzerland, 5Biology, National University of Ireland, Maynooth, Ireland

Introduction: The IL-36 cytokines are novel members of the IL-1 family which have been implicated in autoinflammatory conditions, but unlike other members of the family, they have yet to be investigated for a potential role in obesity. The IL-36 subfamily consists of IL-36α, β and γ, which act as agonistic ligands of the IL-36 receptor, and the IL-36 receptor antagonist, which specifically inhibits IL-36 dependent responses. In this study the role of IL-36 cytokines in obesity and metabolic disease was examined.

Methods: The levels of the IL-36 cytokines were measured by ELISA in the serum of obese and lean individuals. Mice deficient for the IL-36 receptor antagonist (Il36rn−/−), with constitutively unregulated IL-36 signalling, were fed a high fat diet (HFD) for 8 weeks and weight gain and metabolic profile, as determined by glucose and insulin tolerance tests, were compared with that of wild type (wt) control mice. Mice on normal chow were also monitored for up to 12 months. The intestinal microbiome profile of Il36rn−/− mice was examined by 16S rDNA sequencing and qPCR. Colonic tissue was analysed by H&E, Alcian Blue/PAS mucin staining, qPCR, and ELISA of colon explant supernatants. Microbiome analysis was also performed on Il36rn−/− mice after cohousing with matched wt mice for 8 weeks, prior to exposure to HFD for a further 8 weeks and measurement of weight gain and metabolic function.

Results: A significant increase in serum levels of IL-36γ was observed in obese patients, which showed a striking association with improved glucose metabolism in patients with Type 2 diabetes. Il36rn−/− mice gained less weight and exhibited significantly improved metabolic health compared with wt mice when on HFD, or on normal chow diet. Intriguingly, the intestinal microbiome of Il36rn−/− mice was found to differ from control mice, with a dramatic increase in the abundance of mucus resident bacterial strain Akkermansia muciniphila, which has been implicated in protection from obesity and Type 2 diabetes. This outgrowth occurred in association with an increase in colonic goblet cell numbers and mucin expression, as well as IL-9 secretion in the colons of Il36rn−/− mice. Importantly, co-housing of Il36rn−/− mice with wt controls, led to an equalization of the components of the intestinal microbiome, and a loss in the observed outgrowth of A. muciniphila, which resulted in Il36rn−/− mice no longer being protected from obesity and metabolic dysfunction.

Conclusion: IL-36 cytokine signalling exerts protective effects on obesity and metabolic syndrome, at least in part, by influencing the microbiome and specifically by enhancing A. muciniphila abundance, through changes in the colonic mucosa.

Disclosure of Interest: None Declared

O46
PLACENTA-SPECIFIC 8 SUPPRESSES INTERFERON GAMMA PRODUCTION BY TH1 CELLS

C. D. Slade1,*, K. L. Reagin2, K. D. Klonowski2, W. T. Watford4
1Infectious Diseases, 2Cellular Biology, University of Georgia, Athens, United States

Introduction: CD4 T helper 1 (Th1) cells are induced via IL-12 stimulation and contribute to the elimination of intracellular pathogens through production of the inflammatory cytokine, interferon gamma (IFNγ). However, IFNγ production must be tightly controlled, because its dysregulation is known to contribute to the development of autoimmune diseases. Although some T cell-targeted treatments for autoimmune diseases have shown promise, low efficacy, off-target effects, and increased susceptibility to infections are routinely encountered, necessitating the identification of more precisely targeted therapeutic candidates. We identified placenta-specific 8 (Plac8) in an unbiased RNA-Seq screen for genes that are preferentially expressed by Th1 cells1. The goal of this study is to evaluate the role of Plac8 in regulating Th1 responses to gain insight into its therapeutic potential.

Methods: Naïve CD4 T cells (CD4+CD62L+CD44+) from C57BL6 mice were polarized into Th1, Th2, Th17 or iTReg recipient mice. Eight to ten we

Results: Independent polarization studies validated that Plac8 mRNA expression is highly and uniquely induced in Th1 cultures. IL-12 stimulation induced Plac8 mRNA
expression in CD4 T cells via delayed kinetics at 24 h, suggesting indirect regulation. In contrast, type I and type II interferons, known to amplify the Th1 program, induced Plac8 mRNA expression within 6 h. In addition, Plac8−/− CD4 T cells dramatically over-produced IFNγ protein in response to IL-12 stimulation, suggesting that Plac8 is induced as a negative regulator of the Th1 response. To address this hypothesis, a T cell transfer model of colitis was employed in which Th1 cells and IFNγ are known to contribute to pathology. Rag−/− mice that received Plac8−/− CD4 T cells had significantly greater weight loss and colonic inflammation compared to those receiving WT CD4 T cells. Specifically, Plac8 ablation led to increased accumulation and IFNγ production by CD4 T cells within spleens and mesenteric lymph nodes.

**Conclusion:** These data demonstrate that Plac8 is induced in Th1 cells to negatively regulate IFNγ production and thereby mediate feedback inhibition of the Th1 response. Future experiments will seek to determine how Plac8 leads to suppression of IFNγ and if this mechanism can be utilized therapeutically to alleviate the severity T cell-driven autoimmunity.

**Disclosure of Interest:** None Declared

---

**O47**

**EPIGENETIC LANDSCAPE OF FOXP3 ENHANCER SITES DURING THYMIC FOXP3+ TREG DEVELOPMENT OF CNS0- AND CNS3-DEFICIENT MICE**

R. Kawakami1, Y. Kitagawa1,2, K. Hirota3, H. Watanabe3, G. Kondo3, K. Chen1, N. Ohkura1,4, S. Sakaguchi1,2
1Laboratory of Experimental Immunology, Immunology Frontier Research Center, Osaka university, Suita city, 2Laboratory of Experimental Immunology, Institute for Frontier Life and Medical Science, 3Laboratory of Integrative Biological Science, Institute for Frontier Life and Medical Sciences, Kyoto university, Kyoto city, 4The Center of Medical Innovation and Translational Research, Osaka university, Suita city, Japan

**Introduction:** Regulatory T cells (Tregs) characterized by the master transcription factor Foxp3 predominantly develop in the thymus. In addition, the precise coordination of Treg-specific epigenetic modifications and chromatin conformation are required to stably exert their full suppressive activity. These regulatory elements, in general, accumulate within the evolutionarily highly-conserved non-coding sequences (CNSs). The contribution of CNS0, 1, 2, and 3 proximal of the Foxp3 gene is recently highlighted as binding sites of key transcription factors and chromatin modifiers for Treg development (Kitagawa, Nat Immunol 2017, Zheng, Nature 2010). However, it remains unclear to what extent these cis-regulatory elements contribute towards establishing the Treg lineage by transcriptional control over the Foxp3 gene. To this end, we evaluated the contribution of CNS0 and CNS3 to enhancer formation at the Foxp3 locus during early developmental stages of Treg differentiation.

**Methods:** H3K4me1/H3K27ac ChIP-seq and ATAC-seq were performed to examine genome-wide poised/active enhancer elements and chromatin accessibility. The kinetics of enhancer formation from bone marrow precursors to thymic Tregs were assessed in order to investigate the enhancer landscape related to functionality and development of Tregs. CNS0 and CNS3-deficient mice were generated using the CRISPR/Cas9 system to examine their specific roles in the delineation of Treg identity through the establishment of Treg-specific localized epigenetics at the Foxp3 locus.

**Results:** We found that a part of global Treg-specific active enhancer sites were active in Lin-c-kit+ hematopoietic progenitor cells in the bone marrow or in double negative thymocytes, while chromatin conformation of proximal regions remained closed as assessed by H3K4me1 ChIP-Seq and ATAC-Seq, respectively. Moreover, the two enhancer elements CNS0 and CNS3 at the Foxp3 locus were similarly found to be poised in HSCs and thymocytes at the double negative stage. We found that CNS0 and CNS3 regions were independently activated during thymocyte development. While CNS0- or CNS3-deficient mice were not lethal, the frequencies of Foxp3+ Tregs in these mice were significantly reduced. Consistently, the induction of Foxp3 in thymic Treg precursors by TCR stimulation was drastically impaired. Although the enhancer signal H3K27ac around the deleted regions of either CNS0 or CNS3 were reduced, the individual deletions did not affect the stability and function of mature Foxp3+ Tregs in vivo.

**Conclusion:** The CNS0 and CNS3 enhancer elements control the epigenetic landscape for Foxp3 gene regulation in the thymic Treg development, but were dispensable for the maintenance and stability of peripheral Tregs.

**Disclosure of Interest:** None Declared

---

**O48**

**HIPPO/MST SIGNALING COUPLES METABOLIC STATE AND FUNCTION OF CD8α+ DENDRITIC CELLS FOR CYTOTOXIC T-CELL PRIMING**

X. Du1, J. Wen1, Y. Wang1, P. Karnaus1, A. Khataian2, H. Tan3, Y. Li2, C. Guy4, T.-L. M. Nguyen1, Y. Dhungana1, G. Neale4, J. Peng3, J. Yi2, H. Chi4
1Department of Immunology, 2Department of Computational Biology, 3Department of Structural Biology and Developmental Neurobiology, 4Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children’s Research Hospital, Memphis, United States

**Introduction:** CD8α+ dendritic cells (DCs) present antigens to CD8+ T cells and play a pivotal role in activating cytotoxic T cell responses to viruses, bacteria and tumors. Although lineage-specific transcriptional regulators of CD8α+ DC development have been identified, the molecular pathways that selectively orchestrate CD8α+ DC function remain elusive. Moreover, metabolic reprogramming is important for DC development and
activation, but metabolic dependence and regulation of DC subsets are unknown.

**Methods**: A novel systems biology approach named NetBID was developed to predict the selective drivers of CD8α+ DCs by integrating transcriptomics, proteomics and phosphoproteomics data of CD8α+ DCs and CD8α+ DCs. The predicted results were verified by immunoblot analysis. Mice with DC-specific deletion of Mst1/Mst2 (Mst1/2), Lats1/Lats2 (Lats1/2), or Yap/Taz were generated to systematically dissect the roles of Hippo signaling in DCs. Homeostasis of T cells and DCs was examined by flow cytometry in the mice abovementioned. DC function was determined by classical antigen presentation assay in vitro and in vivo. Metabolic state and gene expression profiles of Mst1/2-deficient DCs were examined to investigate the underlying molecular mechanisms.

**Results**: NetBID analysis reveals a marked enrichment of the activities of Hippo pathway kinases in CD8α+ DCs relative to CD8α- DCs. DC-specific deletion of Mst1/2, but not Lats1/2 or Yap/Taz that mediate canonical Hippo signaling, disrupts homeostasis and function of CD8+ T cells and anti-tumor immunity. Mst1/2-deficient CD8α+ DCs are impaired in presenting extracellular proteins and cognate peptides to prime CD8+ T cells, while CD8α- DCs lacking Mst1/2 have largely normal function. Mechanistically, compared with CD8α- DCs, CD8α+ DCs show much stronger oxidative metabolism and critically depend upon Mst1/2 signaling to maintain bioenergetic activities and mitochondrial dynamics for functional capacities. Further, CD8α+ DCs selectively express IL-12 that depends upon Mst1/2 and the crosstalk with non-canonical NF-kB signaling.

**Conclusion**: Mst1/2 function as selective drivers of CD8α+ DC function by integrating metabolic activity and cytokine signaling.

**Disclosure of Interest**: None Declared

---

**O49 CYTOKINE AND TRANSCRIPTIONAL REGULATION OF STEM CELL-LIKE CD8 T CELLS**

**T. Wu**, P. Schwartzberg

**Introduction**: T cell exhaustion, characterized by poor effector function and reduced proliferation, is often seen during chronic infections and cancer. The molecular pathways that govern the long-term persistence of antigen-specific T cells during chronic infections and cancer remain elusive.

**Methods**: To study CD8 T cell responses during chronic viral infection, we infected WT and mutant mice with lymphocytic choriomeningitis virus (LCMV) clone 13. LCMV-specific CD8 T cells were identified by either tetramers or congenic markers expressed by adoptively transferred P14 TCR transgenic CD8 T cells. Single cell RNA-sequencing was performed with ~3000 P14 CD8 T cells per sample isolated from LCMV infected mice. PRISM and R were used to analyzed the data.

**Results**: We have identified a stem cell-like TCF1highTim3low CD8 population generated during chronic viral infection and cancer. This population can both self-renew and replenish the terminally-differentiated TCF1lowTim3high CD8 population, and is critical for sustained antiviral T cell response during chronic viral infection. The differentiation of stem cell-like CD8 T cells was enhanced through increasing TCF1-Bcl6 signaling or blocking type I interferon (IFN). We found that the differentiation of stem cell-like CD8 T cell is suppressed by cell-intrinsic type I IFN signaling as early as day 2 post-infection. Moreover, the lack of type I IFN signaling in the environment does not favor the differentiation of TCF1highTim3low CD8 T cells; hence the effects of IFNAR1 blockade on CD8 stem-like cell differentiation are predominantly cell-autonomous. To explore the transcriptional regulatory network that govern the differentiation of stem cell-like CD8 T cells, we used single cell RNA-sequencing and uncovered novel gene modules unique to stem cell-like CD8 T cells. Our functional analyses have identified pathways that are essential to stem cell-like CD8 T cell differentiation and long-term antiviral CD8 response during chronic viral infection.

**Conclusion**: Our results have shown that the differentiation of stem cell-like CD8 T cells and long-term antiviral CD8 T cell immunity are tightly controlled by cytokine and transcription factor signaling.

**Disclosure of Interest**: None Declared

---

**O50 IL-17 DRIVES THE BIO-ENERGETIC ACTIVITY OF LYMPH NODE FIBROBLASTIC RETICULAR CELLS TO PROMOTE EXPANSION DURING TH17 CELL ACTIVATION**


1Department of Medicine, 2Department of Pediatrics, 3UPMC Hillman Cancer Center, 4Renal-Electrolyte Division, University of Pittsburgh, Pittsburgh, 5Immune Activation Section, NIAID, NIH, Bethesda, United States

**Introduction**: Fibroblastic reticular cells (FRC) form the stromal support network of T cell zones, playing important roles in lymph node (LN) homeostasis as well as regulation of adaptive immunity. The inflammation that accompanies T cell priming causes rapid LN hypercellularity, which ultimately leads to FRC proliferation to provide continuing support to the enlarged inflamed LN. T cells are thought to be important for FRC expansion, but the signals driving this process have been unclear.

**Methods**: Mouse inguinal lymph nodes were isolated from mice immunized with MOG-CFA or treated with DSS to induce colitis. FRC number, gene expression, phenotype and metabolic status were assessed by flow cytometry, RNA-Seq, immunoblotting, and Seahorse assays.

**Results**: In absence of IL-17R signaling, FRCs undergo cell cycle arrest and ultimately apoptosis, along with signs
of nutrient stress including AMPK activation. Rather than directly promoting proliferation, IL-17 drove mitochondrial activity and increased glucose uptake by activated FTC. Enhanced glucose uptake in response to IL-17 was mediated through NF-kb which in turn regulated expression of the glucose transporter glut1, and Cpt1a, the rate-limiting enzyme for mitochondrial fatty acid oxidation. 

**Conclusion:** These data reveal that IL-17 produced by differentiating Th17 cells has a profound impact on LN stromal organization by promoting FTC expansion. This is achieved through a previously unexplored role of IL-17 in boosting the bio-energetic fitness of a stromal cell population. These findings provide new insight into mechanisms by which Th17 cells can mediate chronic inflammation and tissue remodeling in lymphoid organs.

**Disclosure of Interest:** None Declared

---

**P001**

**RUXOLITINIB SUPPRESSES INNATE AND ADAPTIVE IMMUNE ACTIVATION IN HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS THROUGH BOTH INTERFERON-GAMMA DEPENDENT AND INDEPENDENT MECHANISMS**

S. Albeiltuni1,*, K. Verbst1, P. E. Tedrick2, H. tillman1, K. E. nichols1

1Oncology, St. Jude Children’s Research Hospital, 2oncology, st.jude children's research hospital, memphis, United States

**Introduction:** Hemophagocytic lymphohistiocytosis (HLH) is an often-fatal cytokine storm syndrome characterized by severe hyper-inflammation driven by the uncontrolled proliferation of activated T lymphocytes and macrophages. Disease morbidity and mortality in HLH is largely a result of exacerbated production of pro-inflammatory cytokines, including interferon-gamma (IFNγ), interleukins (IL)-1, -2, -6, -12, -18 and Tumor Necrosis Factor-alpha (TNFα). Among these cytokines, IFNγ is thought to be the main driver of disease. However, as many of the cytokines amplified in HLH signal through the JAK-STAT pathway, including IFNγ, we hypothesized that targeting a broader range of cytokines with the JAK1/2 inhibitor ruxolitinib would be more efficacious than IFNγ neutralization alone for the treatment of HLH.

**Methods:** The efficacy of the JAK1/2 inhibitor ruxolitinib in comparison to IFNγ neutralization was tested in two pre-clinical models of HLH: LCMV-infected perforin-deficient mice to model “primary” or inherited HLH and administration of CpG+cIl10R to model “secondary” or non-inherited HLH. Mice were treated with ruxolitinib or IFNγ blocking antibody day 4-8. On day 9, mice were evaluated for HLH. In addition, survival of LCMV-infected perforin-deficient mice was assessed up to day 35 post-infection following discontinuation of either treatment on day 9 post-infection.

**Results:** HLH-associated cytopenias were ameliorated by either agent, but cellular inflammation was improved only by ruxolitinib. Consistently, ruxolitinib was also superior to αIFNγ in reducing the number and size of hepatic inflammatory foci and decreasing the numbers and cytokine-producing capacity of tissue-infiltrating CD8+ T cells and neutrophils. Surprisingly, ruxolitinib-induced reductions in pro-inflammatory cytokine levels and enhancements in survival were sustained despite discontinuation of the drug. However, such was not the case following discontinuation of αIFNγ. Fatality in these latter animals was reversed upon the simultaneous depletion of neutrophils.

**Conclusion:** These data reveal that the hematological parameters of HLH are IFNγ-dependent, while tissue immunopathology results from the activity of numerous cytokines that drive expansion and activation of CD8+ T cells and neutrophils. These data further support the incorporation of ruxolitinib into future HLH clinical trials.

**Disclosure of Interest:** None Declared

---

**P002**

**TRANSCRIPTIONAL PROFILES OF SYNOVIAL BIOPSIES PREDICT THERAPEUTIC RESPONSE TO BIOLOGICS IN RHEUMATOID ARTHRITIS.**

B. Cossins1,2,*, R. Andrews2, J. Twohig1,2, N. Williams3, G. Jones1,2, S. Jones1,2

1Department of Infection & Immunity, School of Medicine, 2Systems Immunity University Research Institute, 3Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff, United Kingdom

**Introduction:** Remission of disease and prevention of irreversible joint damage remain the ultimate objectives for inflammatory arthritis treatment. Current therapies are based on reducing inflammation and include inhibition of cytokines with biologics such as anti-tumour necrosis factor (TNFα) agents. Biologics show considerable efficacy in rheumatoid arthritis (RA) and improve both clinical outcome and the quality of life for patients. However not all patients respond to an individual drug, or a class of drug. For example, while anti-TNFα therapies have had a major impact on the treatment of inflammatory arthritis, a considerable proportion of patients show negligible improvement. In this regard, synovial histopathology in early and established RA is heterogeneous and joint inflammation is defined as fibroblast-, granulomatous- or lymphoid-rich synovitis. Thus, two major challenges exist: to (A) predict the course of RA in patients with undifferentiated early pathology, and (B) define the major pathways that drive disease in individual patients.

**Methods:** Bioinformatic analysis was conducted on transcriptomic datasets from RA patients (3x independent cohorts; n=49; n=20; n=12) with defined forms of synovial pathology, and biopsy samples taken before and after DMARD (methotrexate) or biologic (tocilizumab, rituximab) intervention. Computational approaches relevant to the stratification of joint pathology were combined with molecular pathway analysis to identify gene expression patterns that predict therapeutic effects on cytokine responses.
Results: Cytokines that signal through the Janus kinase and signal transducer and activator of transcription (Jak-STAT) pathway control gene expression, cell proliferation, differentiation and survival. Several biologics and small molecule inhibitors that target STAT-activating cytokines (e.g., the IL-6R blocker tocilizumab) or prevent Jak activation (e.g., tofacitinib) are now prescribed as alternate drugs to anti-TNFα therapies in RA. Computational approaches outlined here evaluated how cytokines that signal through the Jak-STAT pathway affect synovitis, and responses to biologic therapies. Adopting transcriptomic signatures reflective of IL-6, IFNγ, STAT1 and STAT3 activity we present gene profiles that reflect differences in response to therapy (stratified according to Good, Moderate or Poor response), clinical outcome (based on DAS28 scores and C-reactive protein) and the establishment of define forms of synovitis. A TNF gene signature was used as an internal control of inflammation. Analysis highlighted the detection of STAT1 signatures as reliable predictor of clinical outcome, and illustrated defined contributions for STAT3 in synovitis.

Conclusion: The clinical features of joint inflammation in rheumatoid arthritis are highly diverse. This may explain why some patients respond well to certain biological drug interventions, whilst others show no benefit to treatment. Methods to predict a response to therapy are currently lacking and our approach may provide an opportunity to improve patient diagnosis and stratification based on an analysis of cytokine signalling signatures.

Disclosure of Interest: None Declared

P003
THERAPEUTIC TARGETING OF THE LATENT TRANSCRIPTION FACTOR STAT3 IN INFLAMMATORY ARTHRITIS
A. Derrac Soria1, X. Liu1, B. Cossins1, R. Andrews1, B. Szomolay1, H. Yu2, M. Kortylewski2, G. W. Jones1, S. A. Jones1
1Systems Immunity Research Institute, Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, United Kingdom, 2City of Hope, Beckman Research Institute, Department of Comparative Medicine, Duarte, United States

Introduction: The latent transcription factor Signal Transducer and Activator of Transcription-3 (STAT3) is intrinsically linked with the development of synovitis in rheumatoid arthritis (RA) and contributes to the rapid progression of the disease. Biological drugs (e.g., tocilizumab) and small molecule inhibitors (e.g., tofacitinib) used in the treatment of RA often inhibit STAT3 activity as part of their mode of action. However, these are non-specific for STAT3, do not selectively target cell types responsible for disease development and may elicit adverse outcomes. Here, we investigate the efficacy and mode of action of a novel anti-cancer therapy in two forms of inflammatory arthritis – myeloid-rich (wild-type mice) and lymphoid-rich (Il27ra-deficient mice) synovitis. Moreover, we determine whether CpG-Stat3siRNA disrupts formation, maintenance and functional properties of ectopic lymphoid-like structures associated with lymphoid-rich synovitis.

Methods: On disease induction, antigen-induced arthritis (AIA) challenged mice were treated with a synthetically engineered Stat3siRNA linked to a Toll-like receptor (TLR)-9 agonistic CpG oligonucleotide (CpG-Stat3siRNA). This drug has a unique mode of action and selectively blocks STAT3 activity in cells expressing TLR9. Using RNA sequencing (RNA-seq) in combination with histological assessments, we explored holistic gene changes (day 3 and 10 post arthritis induction – reflecting early and established forms of the disease) in synovial tissues as a response to treatment and evaluated of CpG-Stat3siRNA therapy on defined features of synovial histopathology.

Results: Synovial histopathology showed that CpG-Stat3siRNA improves disease outcome in experimental inflammatory arthritis. More specifically, the infiltration of immune cells into the inflamed synovium and pannus hyperplasia was substantially reduced in treated mice as compared to the control groups. Bioinformatic analysis of synovial tissue from mice with myeloid-rich and lymphoid-rich pathology showed that CpG-Stat3siRNA therapy controlled defined aspects of the disease that were both common and unique to each form of synovitis. Molecular pathway analysis evaluated contribution from both STAT1 and STAT3 signalling and identified gene controlled through putative STAT1/STAT3 cross-regulation mechanisms.

Conclusion: CpG-Stat3siRNA is currently in development for certain lymphomas and some solid tumours. The outlined study represents the first attempt to reposition CpG-Stat3siRNA for the treatment of autoimmunity. The data provide evidence of the therapeutic potential of CpG-Stat3siRNA in inflammatory arthritis and may inform opportunities to test its application in other clinical indications, including infection, chronic inflammation, and autoimmunity.

Disclosure of Interest: None Declared

P004
PREDICTING TNFALPHA INHIBITOR TREATMENT RESPONSE USING CYTOKINES IN PATIENTS WITH RHEUMATOID ARTHRITIS
M. N. Lassere1, J. Gu1, S. Baker1
1Department of Rheumatology, St George Hospital, University of NSW, Sydney, Australia

Introduction: Tumour necrosis factor-alpha inhibitors (TNFαi) are the main biologics (b-MARDs) used to treat active rheumatoid arthritis (RA) in patients that have failed disease modifying treatment (DMARD). However, in 10% of patients with rheumatoid arthritis, TNFα inhibitors do not work at all. Patients are continued on this treatment for several months risking side-effects in the hope that the treatment will work. Another 40% of patients respond partially to this treatment and have to also be treated with other drugs such as methotrexate and prednisone in addition to treatment with TNFαi. Biomarkers offer an
opportunity to identify before starting or soon after starting treatment with TNFαi which patients will be responders and whether prednisone and other drugs can be reduced and optimise the risk-benefit of treatment. Therefore we undertook a series of experiments with the following aims: determine whether cytokine biomarkers will predict which patients with rheumatoid arthritis are: (a) sustained DMARD early treatment responders (b) sustained TNFαi early treatment responders, (c) TNFαi early treatment failures, in a longitudinal cohort of eight months duration.

**Methods:** We used the Millipore’s MILLIPLEX MAP Human Th17 Magnetic Bead kit for the simultaneous quantification of the following cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, GMCSF, IFNγ, MIP-3α, TNFα and TNFβ, γ, MIP-3α, TNFα and TNFβ. We evaluated 14 patients with RA starting on a DMARD and 26 patients with RA starting on a TNFαi after failing DMARDs. These cytokines were assayed monthly 2 or 3 months prior to starting a TNFαi to evaluate month-to-month cytokine variability and every month up to 15 months after initiation of treatment (mean 4.5 months, SD 3.2 months). RA disease activity was measured every month using the RA Disease Activity Score (DASCRP28) which includes joint counts, CRP and a patient-reported outcome of health status. All samples were blocked with Heteroblock to reduce rheumatoid factor and other heterophilic antibodies. Rheumatoid factor was measured before and after blocking. The same negative and positive controls were included across all plate runs. All assays were done in singlet to accommodate longitudinal samples. Mixing studies were undertaken to evaluate whether cytokine results could be analysed using quantitative statistics.

**Results:** We had 67 serum samples in the DMARD treated group (14 patients) and 202 serum samples in the TNFαi treated group (26 patients) because of the longitudinal study design. Using mixed effects linear regression to account for longitudinal data in a model that included all 25 cytokines, treatment-time and treatment type (DMARD or TNFαi/-DMARD), we found that in patients on DMARDs, IL-6, IL-1β, IL-28A, and TNFβ were associated with treatment response. However, in TNFαi treated patients, TNF-α, GM-CSF and IL-6 were associated with treatment response. Only p values <0.005 are reported given that 25 cytokines were included in the model.

**Conclusion:** In this study of treatment response comparing DMARDs and TNFαi in a longitudinal cohort of 26 patients with a total of 202 samples measuring TNFα and GM-CSF may predict early TNFαi responders. Although IL-6 was also associated with response, IL-6 is strongly associated with CRP, and CRP is an inexpensive laboratory test.

**Disclosure of Interest:** None Declared

---

**P005**

CCL17 BLOCKADE AS A THERAPY FOR OSTEOARTHRITIS PAIN AND DISEASE

*M.-C. Lee*1,*, R. Salem1, A. Achuthan1, A. Fleetwood1, J. Hamilton1, A. Cook1

1Medicine, The University of Melbourne, Parkville, Australia

**Introduction:** Osteoarthritis (OA) is a major cause of disability and chronic pain has been inadequately managed due to a poor understanding of the underlying mechanisms. We have previously demonstrated a new GM-CSF→Jmjd3→interferon regulatory factor 4 (IRF4)→chemokine (c-c motif) ligand 17 (CCL17) pathway, which is important for the development of inflammatory arthritis pain and disease. Tumour necrosis factor (TNF) can also be linked with this pathway. Here we investigated the involvement of the pathway in OA pain and disease development using a GM-CSF-dependent collagenase-induced OA model.

**Methods:** CiOA was induced in C57BL/6 wild-type (WT), Lr4+/-, Ccl17+/+, Ccr4-/-, Tnf-/- and GM-CSF-/- mice. Additionally, therapeutic targeting of CCL17, Jmd3 and cyclooxygenase 2 (COX-2) was evaluated. Development of pain (assessment of weight distribution) and OA disease (histologic scoring of synovitis, cartilage destruction and osteophyte size) were assessed. Synovial joint cells, including neutrophils, macrophages, fibroblasts and endothelial cells, were isolated (cell sorting) and gene expression analyzed (quantitative PCR).

**Results:** Studies in the gene-deficient mice indicated that IRF4, CCL17 and the CCL17 receptor, CCR4, but not TNF, were required for CiOA pain and optimal cartilage destruction and osteophyte size. Therapeutic neutralization of CCL17 and Jmd3 alleviated both pain and disease, whereas a COX-2 inhibitor only ameliorated pain. In the synovium, Ccl17 mRNA was only expressed by the macrophages in a GM-CSF- and IRF4-dependent manner. Ccr4 mRNA was expressed by various types of cells, including fibroblasts, while only fibroblasts expressed MMP3 and MMP13 in a GM-CSF-, IRF4- and CCL17-dependent manner.

**Conclusion:** The GM-CSF→Jmjd3→IRF4→CCL17 pathway is important for the development of experimental OA pain and disease. Therefore, CCL17 could be a potential therapeutic target for the treatment of both OA pain and disease in patients.

**Disclosure of Interest:** None Declared

---

**P006**

BARICITINIB INHIBITS B CELL ACTIVATION AND AMELIORATES MURINE LUPUS

*J. Lee*1,*, S. Jang1, S.-M. Hong1, S. Baek1, S.-H. Park1,2, S. Kwok1,2

1Rheumatism Research Center, Catholic Research Institute of Medical Science, 2Division of Rheumatology,
Cytokines 2018 – Abstracts

**P007**

**IDENTIFICATION AND CHARACTERIZATION OF A NOVEL MULTI-STAT INHIBITORY COMPOUND C01L_F03 WITH ANTI-ATHEROSCLEROTIC POTENTIAL**

M. Plens-Galaska1, M. Szela1, A. Collado2,3, P. Marques2,3, S. Vallejo4,5, M. Ramos-González4,5, J. Wesoly6, M. Jesus Sanz2,3, C. Peiro4,5, H. Blyussenn1

1Dep. of Human Molecular Genetics, Adam Mickiewicz University, Poznan, Poland, 2INCLIVA, University Clinic Hospital of Valencia, 3Dep. of Pharmacology, University of Valencia, 4Dep. of Pharmacology, Universidad Autónoma de Madrid, 5Hospital La Paz, Institute for Health Research, Madrid, Spain, 6Lab. of High Throughput Technologies, Adam Mickiewicz University, Poznan, Poland

**Introduction:** Cardiovascular diseases (CVDs) including atherosclerosis are currently the leading cause of death in developed countries. Key factors contributing to onset and progression of this disease include the pro-inflammatory cytokines: Interferon (IFN)α and IFNγ and Toll-like receptor 4 (TLR4). Together, they activate members of the Signal Transducer and Activator of Transcription (STAT) family. Searches for inhibitory compounds, exploring the STAT pTyr-SH2 interaction area yielded a variety of small molecules, often with unknown STAT-SH2 binding specificity. We hypothesized that multi-STAT-inhibitors simultaneously blocking STAT1, STAT2 and STAT3 activity and pro-inflammatory target gene expression may be a promising avenue for the treatment of CVD.

**Methods:** We developed a pipeline approach combining comparative *in silico* docking of STAT-SH2 models on multi-million compound libraries with *in vitro* STAT inhibitor validation. Studies were performed both at the protein and gene expression level with application of methods such as: Western blot, qPCR, ChIP qPCR and microarray analysis. In addition *ex vivo* studies were conducted assessing migration, adhesion and contraction capacity of vascular cells treated with a combination of inhibitory compound and inflammatory stimuli (IFNγ and LPS).

**Results:** Our approach allowed us to identify a new type of multi-STAT inhibitor- C01L_F03 which targets the SH2 domain of STAT1, 2 and 3 with equal affinity. The compound was primarily validated at the protein level in human microvascular endothelial cells (HMECs). Consequently C01LF03 at various concentrations inhibited phosphorylation of STAT1, STAT2 and STAT3, induced by treatment with IFNγ.

Moreover, we show that C01L_F03 is able to block interaction of multiple STATs with DNA. In a second set of experiments, we tested the genome-wide effect of the compound on gene expression in response to treatment with IFNγ and LPS in HMECs. Microarray data analysis in combination with promoter prediction studies allowed us to determine that C01L_F03 strictly inhibits STAT dependent expression of pro-inflammatory and pro-atherogenic genes. In agreement with this in *ex vivo* experiments we proved that C01L_F03 is able to block: endothelial cell (EC) migration, leukocytes adhesion to ECs and mesenteric artery segments contractility under the treatment with inflammatory stimuli.

**Conclusion:** We propose C01L_F03 as a member of new group of multi-STAT inhibitory compounds. What is more, based on our previous results we postulate that many of known STAT inhibitors claimed to be specific should belong to group of multi-STAT inhibitors like STATTIC and STX-0119. Since C01L_F03 seemed to be less potent than other inhibitory compounds therefore we propose potential ways of improving inhibitory capacity and their potency. Introducing additional steps such as chemical modifications, similarity screening seem to be promising tool in search of potent multi-STAT inhibitory compounds and the treatment of CVDs.

**Disclosure of Interest:** None Declared

---

**P008**

**TOWARDS THE STRUCTURE OF INTERLEUKIN-27, MEMBER OF THE IL-12 FAMILY.**

K. Skladanowska1,2, Y. Bloch1,2, S. Savvides1,2

1Department of Biochemistry and Microbiology, Ghent University, Gent, 2Unit for Structural Biology, VIB Center for Inflammation Research, Zwijnaarde, Belgium
Introduction: Interleukin 27 (IL-27) is a recently discovered member of the IL-12 family of cytokines and have emerged as intriguing molecular player as a result of its role in both pro- and anti-inflammatory responses in arthritis, inflammatory bowel disease, and cancer (Yoshida & Hunter, 2015). Such functional dichotomy is additionally fuelled by sharing of common features (GP130 receptor and Ebi3 cytokine) by IL-35 and IL-27 in terms of molecular organization and utilization of receptor modules (Collison et al., 2012).

Methods: The goal of our research project is to elucidate the structural and mechanistic basis of signalling complexes mediated by IL-27. In order to achieve this, milligram amounts of proteins need to be produced in HEK293 cells and subsequently purified via immobilised metal affinity chromatography (IMAC) as well as size exclusion chromatography (SEC). The purified proteins are used in structural studies via macromolecular crystallography as well as in a collection of biochemical, biophysical and cellular studies such as the bio-layer interferometry (BLI), isothermal titration calormetry (ITC) and proliferation assays.

Results: IL-27 is a heterodimeric cytokine consisting of an alpha-helical subunit IL-27p28 and a receptor-like subunit Ebi3. Unlike in the case of IL-12 and IL-23, these subunits are not covalently linked, which poses some difficulties during expression and purification. So far we have been able to express and purify murine IL-27p28 alone as well as synthetically linked murine Ebi3-p28 fusion protein in HEK293 cells. We are also able to produce milligram amounts of both receptor ectodomains (GP130 and IL27Ra). We also produced the short forms of the receptors - domains 1-3 (Ig-CHR) for GP130 and domains 1 and 2 (CHR) for IL-27Ra and tested their binding to the Ebi3:p28 fusion protein in BLI. Both receptors showed binding to the cytokine in the micromolar range.

Conclusion: More experiments are needed to validate the binding model of IL-27. Observed binding

Disclosure of Interest: None Declared

P008.A
A BISPECIFIC ANTIBODY STRATEGY TO TARGET MULTIPLE TYPE 2 CYKOTINES IN ASTHMA
1argenx, 2VIB-UGent Center for Inflammation Research, Zwijnaarde, Belgium

Introduction: Asthma is a chronic inflammatory airway disease in which innate and adaptive immune cells act together to cause eosinophilic inflammation, goblet cell metaplasia, and bronchial hyperreactivity. In clinical trials using biologicals against IL-4 receptor alpha (IL-4Ra) or IL-5, only a subset of patients with moderate-to-severe asthma responded favorably, suggesting that distinct pathophysiologic mechanisms are at play in subgroups of patients called endotypes. The effect of multiple cytokine blockade using bispecific antibodies has not been tested. We therefore sought to target simultaneously the IL-4, IL-5, and IL-13, and IL-5 signaling pathways with a novel IL-4Ra/IL-5-bispecific antibody in a murine house dust mite model of asthma.

Methods: Two monospecific antibodies neutralizing IL-4Ra and IL-5 were generated by using a llama-based antibody platform. Their heavy and light chains were then cotransfected in mammalian cells, resulting in a heterogeneous antibody mixture from which the bispecific antibody was isolated by using a dual anti-idiotypic purification process. C57BL/6J mice were finally sensitized and challenged to house dust mite extracts and treated during challenge with the antibodies.

Results: We successfully generated and characterized the monospecific and bispecific antibodies targeting IL-4Ra and IL-5. The monospecific antibodies could suppress eosinophilia, immunoglobulin E synthesis, or both, whereas only the IL-4Ra/IL-5-bispecific antibody and the combination of monospecific antibodies additionally inhibited goblet cell metaplasia and bronchial hyperreactivity.

Conclusion: Type 2 cytokines act synergistically to cause goblet cell metaplasia and bronchial hyperreactivity in house dust mite-exposed mice. These preclinical results show the feasibility of generating bispecific antibodies that target multiple cytokine signaling pathways as superior inhibitors of asthma features, including the difficult-to-treat goblet cell metaplasia.


P008.B
DIRECT INHIBITION OF IL-17A WITH SMALL MOLECULE COMPOUNDS
S. Nielsen1*, J. Andersson1, M. Bengtsson1, S. Cowland1, T. Franch1, S. Glad1, A. Gouliaev1, L. Moretti2, N. Narager2, S. Rast1, G. Smith1, L. Stasi1
1Nuevolution, Copenhagen, Denmark

Introduction: IL-17A is the key cytokine driving multiple diseases, including Psoriasis, Psoriatic arthritis, and Ankylosing spondylitis as supported by clinical data from the use of IL17A-directed antibodies. The development of small molecule IL-17A inhibitors might offer a safer and more convenient oral and/or topical alternative to current injectables.

Methods: Chemetics DNA-encoded libraries (DEL) were used to identify small molecule IL-17A inhibitors. Direct binding of DEL compounds to IL-17A was verified using SPR and thermal shift assay and inhibition of the IL-17A/IL-17RA interaction was investigated by AlphaScreen and in a panel of cell-based assays using a selection of IL-
17A target genes as readout. Structural information of the compound/IL-17A interaction was obtained using X-ray crystallography. In vivo efficacy of select compounds was investigated using the mouse collagen-induced arthritis (CIA) model.

Results: We screened 40 trillion DNA-encoded small molecule compounds against the human IL-17A protein and discovered three distinct chemical series capable of inhibiting the IL-17A/IL-17RA interaction in the nM range. Co-crystal structures of 2 series complexed with IL-17A showed distinct mechanisms of IL-17A binding for the two chemotypes, allowing further lead optimization to yield IL-17A inhibitors with low nM potency. In primary human keratinocytes, the inhibitors suppress IL-17A-induced and IL-17A/TNFα-co-induced gene expression in the low nM range. Finally, in a mouse CIA model, when dosed starting 28 days after immunization a lead inhibitor completely blocked the further progression of established arthritis and paralleled the efficacy attained with an IL-17A-neutralizing antibody.

Conclusion: We have developed first-in-class small molecule inhibitors of IL-17A. The compounds potently inhibit IL-17A-induced gene expression in cells and show similar efficacy to an IL-17A neutralizing antibody in a relevant disease model. Our inhibitors warrant further investigation as potential small molecule anti-IL-17A therapeutics.


P008.C
A COMPARISON OF THE ANTI-INFLAMMATORY EFFECTS OF COMBINED STATIN AND ANTIPATELET THERAPIES ON TNF-MEDIATED ACUTE INFLAMMATION IN VIVO
O. Cho,1 K.-Y. Park,1 Y.-J. Jang,1 H.-S. Kim,1 T.-H. Heo1 on behalf of Corresponding author
1The Catholic University Of Korea, Bucheon-si, Korea, Republic Of

Introduction: Background: Aberrant production of TNF causes acute and chronic disorders, and systemic inflammatory diseases. Due to the pleiotropic effects of statins and antiplatelet agents, these drugs have frequently been prescribed and have been studied in combination with other drugs. However, not all combinations exerted obvious beneficial effects compared with individual drugs. In this study, we aimed to compare the anti-inflammatory effects of monotherapy and combination therapy, or four different combination therapies including statins and antiplatelet agents on tumor necrosis factor (TNF)-mediated inflammation in vivo.

Methods: Methods: Mice were orally administered rosuvastatin alone, cilostazol alone, cilostazol and rosuvastatin (CILOR), cilostazol and pravastatin (CILOP), clopidogrel and pravastatin (CLOP), or clopidogrel and rosuvastatin (CLOR); then, acute inflammation was induced by injecting the animals with lipopolysaccharide (LPS) or TNF. Serum TNF levels, macrophage accumulation in the lesioned aortas, and mouse mortality were observed to compare the anti-inflammatory effects of the combination therapies.

Results: Results: In mice with LPS-induced acute inflammation, CILOR showed enhanced anti-inflammatory effect than that of each monotherapy, CILOP and CLOR substantially reduced the serum TNF levels and macrophage infiltration of aortic lesions compared with CLOP and CLOR. Moreover, among the four combinations, CILOP significantly improved the survival rate of mice with TNF-mediated acute lethal inflammation.

Conclusion: Conclusion: Combination therapy with antiplatelet and statin exerts beneficial effects in inhibiting both LPS- and TNF-driven inflammatory activities compared with monotherapies. The combination of cilostazol and statins exhibited substantially increased anti-inflammatory effects compared with the combination of clopidogrel and statins. In particular, CILOP exerts better anti-TNF effects on an in vivo acute inflammatory model; thus, a suitable combination therapy, such as CILOP, represents a potential remedy for TNF-related diseases.

Disclosure of Interest: None Declared

P009
GASTROINTESTINAL HELMINTH INFECTION IMPROVED INSULIN SENSETIVITY AND DECREASED SYSTEMIC INFLAMMATION IN A MOUSE MODEL OF T2D
Z. Agha,1*, R. Alhallaf1, L. Jones1, R. Eichenberger1, M. Field1, J. Sotillo1, A. Loukas1 on behalf of Zainabagha
1James Cook University, AITHM, Cairns, Australia

Introduction: The incidence of type 2 diabetes (T2D) is rapidly increasing worldwide and is becoming a major health problem particularly in western countries. Microorganisms, including parasitic worms and gut microbiota, have co-evolved with their host over millennia. This co-evolutionary relationship has established an immunological interaction that is essential for the formation and maintenance of a balanced immune system. Previous experimental studies using mouse models and human trials showed a protective effect of helminths and their excretory/secretory products against different inflammatory-mediated diseases, including T2D. It has already been suggested that helminth infections might protect against T2D by inducing a T-helper-2 polarization of the immune system. On the other hand, recent data suggest that many inflammatory diseases, and in
particular T2D, might be associated with gut microbiota alternations. Moreover, other studies have highlighted the role of helminth infections in altering the composition of the gut microbiota.

Methods: Male C57BL/6 wild-type (WT) mice were used. At the age of 5 weeks the control group were fed a normal control diet (NCD) and to induce T2D the mice were either fed a high glycaemic index diet (HGI) (SF03-30; speciality feeds, western Australia) or a high-fat diet (HFD) (SF07-066; speciality feeds, western Australia).

Results: We here demonstrated that infection with the parasitic nematode _Nipponstrongylus brasiliensis_ significantly reduced body weight, fasting blood glucose and oral glucose tolerance test in mouse models of T2D. We also found that this infection was associated with boosted type 2 immune responses measured by an increase in the eosinophil number. We further investigated the effect of this helminth infection on the gut microbiota composition. Interestingly, we found that _N. brasiliensis_ infection altered the gut microbiota composition, resulting in a general increase of bacteria belonging to phyla Firmicutes, Bacteroidetes and Actinobacteria, in particular orders; Clostridiales, Bacteroidales and Coriobacterials.

Conclusion: Our findings show that _N. brasiliensis_ infection is associated with changes in the gut microbiota and in local and systemic cell populations. These changes might restore gut homeostasis and improve systemic inflammation, suggesting that this helminth might be a novel therapeutic approach for preventing T2D.

Disclosure of Interest: None Declared

P010

MULTI-OMICS ANALYSIS REVEALS THE INTEGRATIVE ROLE OF IFNG ON THE MICROBIOME, LIPID METABOLISM, AND AUTOIMMUNITY IN A MURINE AUTOIMMUNE DISEASE MODEL

H. R. Bae^1, D. L. Hodge^1, S.-M. Jeon^2, J. C. Valencia^1, J. M. Fenimore^1, A. Dultzev^1, G. Trinchieri^1, H. A. Young^1

^1Cancer and Inflammation Program, National Cancer Institute, Frederick, United States, ^2Department of Food Science and Nutrition, Kyungpook National University, Daegu, Korea, Republic Of

Introduction: A close relationship between lipid metabolism and gut microbiota has been recognized in various human disorders including autoimmune diseases. However, the mechanisms are not understood yet.

Methods: We previously reported that the chronic expression of IFNγ develops female-dominant kidney and liver autoimmune diseases in a murine model, designated ARE-Del as a result of the replacement of the AU-rich element in 3' UTR region of IFNγ mRNA with random nucleotides. Here, we demonstrate how IFNγ impacts lipid metabolism and the gut microbiome to induce autoimmunity in the ARE-Del model based on genomic, metabolome, and microbiome data.

Results: First, the microbial profiles in the cecum from ARE-Del mice were changed and overwhelmed by _Clostridium_ strains. Consistent with microbiome data, metabolome analysis also revealed an impact on the microbiome as a microbial metabolite, p-cresol, was suppressed over 10 fold in ARE-Del mice. We next focused on sex-different metabolic changes and discovered notable differences in Tauro-conjugated bile acids and metabolites linked to fatty acid oxidation. In female ARE-Del mice, the relative level of Tauro-beta muricholic acid is specifically high; this metabolite is regulated by the gut microbiome and is an antagonist of FXR (farnesoid X receptor). Based on these findings we propose that IFNγ-mediated gut microbial changes, in turn, dysregulate lipid metabolism by FXR. Other central regulators for lipid metabolism, LXR, PPAR as well as FXR were also detected as top canonical pathways in RNA analysis of PBMC from female ARE-Del mice at the early stage of the disease. As rising evidence suggests the important role of LXR and PPAR in clearance of apoptotic cells and bacteria via autophagy and phagocytosis, we examined these processes in macrophages from female ARE-Del mice. Strikingly, the isolated macrophages had defective formation of autophagosomes upon mTOR inhibition. This finding was supported by gene expression data as autophagy-related genes were specifically downregulated along with an increase of macrophage markers such as MARCO in the kidneys from female ARE-Del mice.

Conclusion: Taken together, our data support the model where IFNγ dysregulates macrophage function, possibly via altered lipid metabolic pathways, which upon interaction with the gut microbiome, results in the induction of autoimmunity.

Disclosure of Interest: None Declared

P011

GENOMIC DISRUPTION OF INTERFERON RECEPTORS IN NOD MICE RESULTS IN AUTOIMMUNE DIABETES WITH LOSS OF ADAPTIVE TOLERANCE

T. Brodnicki^1, S. Akazawa^1, B. Krishnamurthy^1, G. Jhala^1, L. Mackin^1, S. Fynch^1, L. Hawkey^2, I. Smyth^2, P. Trivedi^1, K. Graham^1, T. Kay^1, H. Thomas^1

^1Immunology & Diabetes Unit, St Vincent's Institute, Fitzroy, ^2Australian Phenomics Network, ^3Anatomy & Developmental Biology, Monash University, Clayton, Australia

Introduction: Inflammatory cytokines, particularly the interferons, have been implicated in the pathogenesis of type 1 diabetes (T1D). Interferon-stimulated genes, including those that encode MHC proteins, are pathological hallmarks of the pancreatic islet during the development of T1D in both humans and the non-obese diabetic (NOD) mouse model. Various studies have also shown that interferons promote the destructive interaction between antigen-specific CD8+ T cells and the insulin-producing beta cell. Surprisingly, deficiency of individual
interferons or their receptors in NOD mice does not protect against the development of T1D, possibly due to signaling redundancy amongst the three receptors for type I, II and III interferons.

**Methods:** We used CRISPR/Cas9 genome editing to generate NOD mice in which one or different combinations of the three interferon receptor genes (Ifnar1, Ifngr1 and Ifnrl1) are disrupted. These mice were then characterized for disease pathogenesis including immune cell infiltration of the pancreatic islets and the onset of diabetes.

**Results:** Remarkably, while insulinis in the triple interferon receptor knockout NOD mice was delayed, these mice developed diabetes with the same cumulative incidence as wild-type NOD mice. This result indicated that the lack of protection observed in triple knockout NOD mice was not due to functional redundancy between the three receptors. The frequency of islet-specific autoreactive T cells was actually increased in these mice. Furthermore, their beta cells lack upregulation of PD-L1 expression, and they were resistant to diabetes acceleration by anti-PD-L1 antibody treatment, suggesting that normal interferon regulatory pathways are altered in these mice.

**Conclusion:** Collectively, these results indicate that, in addition to their proinflammatory role, interferons are also important for ‘adaptive tolerance’ that limits the action of pathogenic T cells. Hence, we observed a ‘neutral’ effect on progression of diabetes in NOD mice lacking interferon receptors. The apparent loss of interferon-dependent adaptive tolerance in these mice suggests interferon neutralization may not be effective in preventing autoimmune diseases such as type 1 diabetes.

**Disclosure of Interest:** None Declared

**P012**

**AN OCULAR SURFACE COMMENSAL AS A POSSIBLE PATHOBIONT IN AUTOINFLAMMATORY DISEASE**

A. J. St. Leger1,2, K. Raychaudhuri1, F. Almaghrabi1, I. J. Fuss3, W. Strober3, R. T. Goldbach-Mansky3, R. J. Bishop4, C. Okeagu4, M. J. Mattapallil4, R. R. Caspi1,∗

1Lab Immunol, NEI, NIH, Bethesda, 2Ophthalmology, Pittsburgh Univ, Pittsburgh, 3Lab Clin Immunol & Microbiol, NIAID, 4Consult Services Section, NEI, NIH, Bethesda, United States

**Introduction:** We recently demonstrated that the mouse ocular surface harbors a resident commensal flora and identified *Corynebacterium mastitidis* (*C. mast*) as a commensal that tunes mucosal immunity at the ocular surface by eliciting production of IL-17 from conjunctival γδ T cells (1). *C. mast* is also known to colonize humans. Muckle-Wells Syndrome (MWS) is one of several human autoinflammatory diseases known as Cryopyrin Associated Periodic Syndromes (CAPS). All are caused by gain-of-function mutations in the NLRP3 inflammasome gene, resulting in overproduction of IL-17, a strong stimulator of IL-17. Patients experience fever and arthralgia, neutrophilic dermatitis and severe recurrent conjunctivitis. We hypothesized that an aberrant immune response to commensal microbes at the ocular surface may be connected to the ocular disease in patients with NLRP3 inflammasome mutations.

**Methods:** We used a mouse model generated by a knock in (KI) of the human mutated NLRP3 inflammasome gene, cloned from a MWS patient. The mice, initially negative for *C. mast*, were ocularily colonized with the bacterium. *In vivo* and *in vitro* responses to the commensal were assessed by changes in transcriptome, production of IL-1 and IL-17, neutrophil infiltration and clinical appearance. We also measured the response of CAPS patients and healthy controls to *C. mast* lysate.

**Results:** We show that in a murine host with an overactive inflammasome function, the commensal *C. mast* behaves as a pathobiont and induces ocular inflammation. Mechanistic studies indicated that the NLRP3 inflammasome mutation acts via DC, and also intrinsically in conjunctival γδ T cells, to increase production of IL-1 as well as IL-17, resulting in enhanced recruitment of neutrophils and tissue pathology. Notably, PBMC of 3 patients with mutations affecting the NLRP3 inflammasome responded to *C. mast* lysate with robust IL-17 production, whereas 5 healthy controls had a minimal response.

**Conclusion:** We propose that overproduction of IL-1 and IL-17 at the ocular surface, caused by an aberrant immune response to commensal bacteria, may underlie the recurrent severe conjunctivitis that is characteristic of CAPS and similar NLRP3 inflammasome disorders. Our results may have implications for clinical treatment of ocular inflammation in these patients.

**Disclosure of Interest:** None Declared

**P013**

**NON-REdundant REQUIREMENT FOR CXCR3 SIGNALING FOR EFFECTIVE TREATMENT OF CNS Autoimmune Eye Disease with Type I Interferon**

J. Chen1,∗, W. Wang1, H. Zhou1, M. Zhou1, W. P. Chong1, I. Gery2, R. R. Caspi2

1State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China, 2Laboratory of Immunology, National Eye Institute, NIH, Bethesda, United States

**Introduction:** Type I interferons (IFNs) have shown therapeutic potential in treating central nervous system (CNS) autoimmune diseases, e.g. IFN-β for multiple sclerosis and IFN-α for uveitis. However, treatment is not always effective in patients, and the molecular mechanisms by which type I IFNs exert their immunomodulatory functions remain largely unknown.

**Methods:** To address this question we examined the modulatory effects of type I IFNs in mouse models of experimental autoimmune uveitis (EAU) and T cell-transfer model of experimental uveitis (Chen et al. J Immunol
Cytokines 2018 – Abstracts

006), as well as in PBMCs of patients with autoimmune uveitis. Autopathogenic T cells were detected and tracked in mice with a clonotypic antibody.

**Results:** Effective treatment with type I IFNs inhibited autopathogenic CD4+ T cell migration to effector sites in mice by upregulating expression of the cognate ligands CXCL9, CXCL10 and CXCL11, causing ligand-mediated downregulation of CXCR3 expression and effector T cell retention in the spleen. These effects of type I IFNs also required IFN-γ. In the absence of CXCR3, type I IFNs were ineffective in active EAU. In patients with uveitis, disease exacerbations correlated with reduced serum IFN-α concentrations. Importantly, type I IFNs inhibited CXCR3 expression and human effector T cell migration, and these parameters markedly correlated with IFN-α therapeutic efficacy in uveitis patients.

**Conclusion:** We demonstrate a non-redundant requirement for Gal-coupled CXCR3 in the immunomodulatory actions of type I IFNs that culminates in the suppression of human uveitis and EAU. Our findings provide new insights into the molecular basis of type I IFN therapy for CNS autoimmune eye diseases and identify CXCR3 as a critical biomarker for effective immunotherapy with type I IFNs.

**Disclosure of Interest:** None Declared

**P014**

IL-17A SIGNALING TO SCA-1+ CARDIAC FIBROBLASTS INDUCES GM-CSF AND PROMOTE DEVELOPMENT OF HEART FAILURE

D. Cihakova1* on behalf of Cihakova lab
1Johns Hopkins University, Baltimore, United States

**Introduction:** Heart failure is a global public health issue with high morbidity and mortality rates, affecting about 26 million people worldwide. Studies from our lab on myocarditis mouse model revealed that interleukin (IL)-17A plays an important role during post-injury cardiac inflammation and drives the development of heart failure. We found that IL-17A signaling to cardiac stroma cells, not to immune cells, is essential in the development of heart failure in an experimental autoimmune myocarditis (EAM) mouse model. We showed that IL-17A induced cardiac fibroblasts to produce myelotropic cytokines and chemokines, such as granulocyte macrophage colony-stimulating factor (GM-CSF) to modulate the immune responses during cardiac inflammation. In this study, we set to investigate whether all cardiac fibroblasts are able to produce GM-CSF or whether a specialized cardiac fibroblast subset is responsible for this pathogenic pathway that drives heart failure in both EAM and myocardial infarction (MI) mouse models. In addition, we show a specific causation of IL-7A signaling to cardiac fibroblasts Sca1+ subtype and development of heart failure.

**Methods:** Murine models of EAM and MI. To induce EAM, mice received subcutaneous immunizations on days 0 and 7 of 125 μg per mouse of myosin heavy chain α peptide (MyHCα614-620) in CFA. To induce MI, mice were subjected to permanent ligation of the left anterior descending coronary artery or to a sham operation without ligation. Mice hearts were analyzed by histology and by FACS. Human frozen endomyocardial biopsy samples were assayed by multiparameter FACS.

**In vitro plasticity experiments:** Human primary cardiac fibroblasts from Cell Applications, Inc (San Diego, CA) or primary mouse cardiac fibroblasts were cultured in vitro under different Th environments and their cytokine and chemokine production assessed by ELISA and qPCR.

**Results:** We identified the main GM-CSF producers during cardiac inflammation to be a subset of cardiac fibroblasts, the stem cell antigen-1 positive (Sca-1+) cardiac fibroblasts characterized by the marker profile CD45–CD31–CD29–PDGFRα–Sca-1+periostin+. Specific ablation of IL-17A signaling to Sca-1+ cardiac fibroblasts using Cre-lox system protected mice from post-infarct heart failure and resulted mortality. Moreover, Sca-1+ cardiac fibroblasts exhibited plasticity and produced GM-CSF and CCL2 in response to a Th17 microenvironment, while they were able to be repolarized to secrete eotaxin when placed in a Th2 microenvironment. Thus, Sca-1+ cardiac fibroblasts are the specialized cardiac fibroblast subpopulation that produces GM-CSF and drives heart failure. To confirm our findings in mice, we examined both in vitro cultured human primary cardiac fibroblasts and human heart failure patient endomyocardial biopsy samples for similar GM-CSF-expressing cardiac fibroblast subset. We characterized the human primary cardiac fibroblasts and found similar GM-CSF-expressing cardiac fibroblasts that express human mesenchymal stem cell markers, including CD29, CD73 and CD105. These cardiac fibroblasts are potent cytokine producers and exhibit similar functional plasticity in response to different microenvironments similar to the mouse Sca-1+ cardiac fibroblasts.

**Conclusion:** We identified a specific cardiac fibroblast subset that drives the development of heart failure through production of GM-CSF.

**Disclosure of Interest:** None Declared

**P015**

CONGENITAL MUTATION IN JAK1 LEADS TO PATHWAY- AND CELL-TYPE- SPECIFIC GAIN-OF-FUNCTION

C. N. Gruber1*, G. Evrony2, S. Buta1, D. Dunkin2,3, L. Jarchin2,3, J. Saland4, B. Webb2,5, B. Gelb2,5,6, D. Bogunovic2,6
1Microbiology, 2Pediatrics, 3Gastroenterology, 4Icahn School of Medicine at Mt. Sinai, New York, United States, 5Genetics and Genomic Sciences, 6Mindich Child Health and Development Institute, Icahn School of Medicine at Mt. Sinai, New York, United States

**Introduction:** Monogenic causes of complex immune disorders provide invaluable insight into the intricate regulation of the human immune system. Here, we describe a patient with severe, early-onset immunodysregulatory disease marked by membranous...
nephropathy, non-specific eosinophilic enteritis and chronic dermatitis. Whole-exome sequencing uncovered a de novo mutation (S703I) in JAK1, which mediates signal transduction from a remarkably broad range of cytokines by phosphorylation of STAT proteins.

Methods: We utilized genomic, molecular and multiparametric immunological tools to probe the role of S703I JAK1 mutation in vitro and ex vivo in order to investigate clinical dysfunction in vivo. Additionally, we tested JAK-inhibitor therapy in a JAK1−/− cell line reconstituted with WT or mutant JAK1 constructs.

Results: In vitro, ectopic expression with the mutant but not WT JAK1 allele demonstrated basal STAT1, STAT2 and STAT3 phosphorylation. This gain-of-function could be rescued by treatment with ruxolitinib or tofacitinib, two FDA-approved available JAK inhibitors. In line with the allele evaluation, CyTOF analysis of whole blood revealed heightened basal STAT1, STAT3 and STAT5 phosphorylation, each in unique immune cell subsets. Multiplex ELISA failed to detect inflammatory cytokines in plasma, suggesting intrinsic, constitutive JAK1 activity results from S703I. Furthermore, upon stimulation with JAK1-mediated cytokines (IFNγ, IL-2, IL-4, IL-6), patient leukocytes exhibited heightened phosphorylation of STAT1 and STAT3, but not STAT5 or STAT6, as compared to WT. Interestingly, patient cells exhibited non-canonical STAT phosphorylation following stimulation with IL-2 and IL-4, suggesting that S703I also confers promiscuity to the kinase domain. Natural Killer (NK) cells in the patient, which were skewed toward a CD56high/CD16low phenotype, displayed particularly strong hyper-responsive and promiscuous signaling. Lastly, single-cell-RNA-sequencing was employed to understand the transcriptomic impact of S703I JAK1 across PBMC subsets. Patient leukocytes demonstrated higher expression of target genes downstream of JAK1 as compared to healthy control cells, with NK cells again displaying exceptionally strong phenotypic signatures, specifically high expression of Interferon-Stimulated Genes.

Conclusion: These results indicate that S703I functions as a gain-of-function mutation in a cell type-, STAT- and cytokine-specific manner. This study enhances our molecular understanding of JAK-STAT autoregulation, while also highlighting a role for overactive JAK1 activity in multifactorial diseases previously not considered amenable to JAK-inhibitor therapy.

Disclosure of Interest: None Declared

P016
THE IMMUNE CHECKPOINT PROTEIN PDL1 IS AN ESSENTIAL DOWNSTREAM EFFECTOR OF THE INTERFERON-DRUG RESPONSE IN A MOUSE MODEL OF MULTIPLE SCLEROSIS.

D. Harari1, S. Reich-Zeligser2, A. Dov3, T. M. Salame3, N. Friedman2, G. Schreiber1

1Biomolecular Sciences, 2Immunology, 3Biological Services, The Weizmann Institute of Science, Rehovot, Israel

Introduction: Mouse knockout models of multiple sclerosis (MS) support the notion that endogenous signaling of type I Interferons (IFNs) play a direct role to reduce localized CNS neuro-inflammation, this which takes place in a PDL1-dependent manner 1-4. But drug-induced IFN therapy in MS may result in the activation of different PD1-dependent biological effectors to that resolved by knockout models, this being the subject of this study.

Methods: Here we used a transgenic mouse model which has been modified to respond to human type I IFNs. A high affinity (beta-like) IFN previously was shown to strongly curtail disease symptoms in a mouse MS model (MOG-induced EAE) 5. In this model we tested the effects of +/- IFN and PD-blockade using a neutralizing PDL1 antibody.

Results: IFN-injection severely curtailed the infiltration of peripheral immune cells into the CNS of EAE-induced mice, thus supporting the presence of a CNS-independent therapeutic activity. PDL1-blockade partially abrogated the therapeutic activity of IFN-injection therapy, supporting that PDL1 is an effector of the IFN-induced therapeutic response in EAE. Preliminary CYTOF analysis of MOG-induced splenocytes briefly extracted from the EAE mice shows that IFN-injection results a two-fold increase in splenic CD8+ cells compared to EAE controls, this effect which was partially abrogated by PDL1-blockade, whereas the CNS, IFN therapy blocked almost totally CD8-T-cell infiltration, this which was partially abrogated by PDL1-blockade.

Conclusion: PDL1 is an effector of the injected IFN-therapeutic response in EAE, this which might involve sequestration of pathogenic CD8+ cells en route to the CNS to peripheral lymphoid organs, advancing previously published findings of linear correlation for increased PDL1 expression in IFN-induced splenic cells and reduction in EAE disease symptoms 5. Our data indicates that in EAE, the IFN/PDL1 axis has additional effects to that resolved by previous IFNAR and IFNβ KO studies.

Disclosure of Interest: None Declared

P017
COMMON CYTOKINE NETWORKS LINK THE DEVELOPMENT, MAINTENANCE AND ACTIVITY OF TERTIARY LYMPHOID STRUCTURES ACROSS AUTOIMMUNITY, CANCER AND INFECTION.

D. Hill1,*, A. Cardus2, D. Lucchesi3, L. Yu4, B. Cossins1, E. Pontarini5, L. McLeod1, R. Andrews1, N. Williams1, C. Pitzalis2, M. Bombardier2, B. Jenkins3, S. Jones2, G. Jones2

1Division of Infection & Immunity, School of Medicine, Cardiff University, Cardiff, 2Experimental Medicine and Rheumatology, Queen Mary University of London, London, United Kingdom, 3Centre for Innate Immunity and
Introduction: Lymph node-like follicles called tertiary lymphoid structures (TLS) often develop in inflamed tissues affected by autoimmunity, cancer and infection. TLS range from co-localised aggregates of T- and B-cells to highly segregated and functional structures displaying germinal centre reactions. Despite a prominent influence on the progression of many human diseases, the mechanisms that underpin the development, maintenance and activity of TLS remain ill-defined.

Methods: To identify cross-disease mechanisms involved in TLS regulation, mouse models of inflammatory arthritis, gastric cancer and viral-induced sialadenitis were used. Whole tissue RNA-sequencing, immunohistochemical analysis, flow cytometry and antibody blockade were used to identify cytokine-regulated pathways involved in TLS control. The clinical significance of these pathways were evaluated in stratified patient cohorts with TLS-associated pathology.

Results: Interleukin-27 inhibits the development of TLS in inflammatory arthritis and a viral-inducible model of sialadenitis. Whole tissue RNA-sequencing of the inflamed synovium reveals that IL-27 organises the synovial infiltrate through suppressing genes involved in leukocyte activation, effector T cell function, B cell maturation, immunometabolism and chemotaxis. Gene set enrichment analysis has identified a prominent pathogenic T helper (Th)-17 cell signature in TLS-associated arthritis, which is confirmed by flow cytometry of joint-infiltrating T cells and immunohistochemical analysis. Interestingly, a robust Th17 response is also linked with the development of TLS in a spontaneous model of inflammation-associated gastric cancer in gp130Y757F:Y757F mice. While genetic ablation of IL-17 in gp130Y757F:Y757F mice showed that the early formation of lymphoid aggregates does not require IL-17, the development of mature and functional TLS was IL-17-dependent. Antibody blockade of IL-17 similarly disrupted TLS development in a viral-inducible model of sialadenitis, by inhibiting the infiltration of IL-22-producing T helper cells required for TLS development. Notably, rheumatoid arthritis patients with synovial TLS are enriched for the expression of Th17-associated genes (e.g., IL-17, IL-21), highlighting the significance of the Th17 axis in clinical disease. We also find that TLS and Th17 gene signatures are associated with advanced clinical disease in intestinal-type gastric cancer but are not prognostic of improved patient survival.

Conclusion: These studies highlight a cross-disease role for the Th17 effector programme in orchestrating TLS development and activity, and presents a therapeutic opportunity to improve the stratification and treatment of diseases where TLS feature.

Disclosure of Interest: None Declared
assessed four histological parameters, including leukocyte infiltration, pannus formation, cartilage damage, and bone destruction. In addition, we determined the concentration of pro-inflammatory cytokines in serum.

**Results:** The arthritic scores of DBA/1J mice that were injected by the enzyme were lower than those of PBS-treated DBA/1J mice. Histological evaluation showed significant reductions of histological severities in DAB/1J mice injected by the enzyme. Serum concentration of two pro-inflammatory cytokines was significantly decreased in DBA/1J mice injected by the enzyme. We found that the in vivo injection ameliorated clinical conditions of CIA and decreased the extent of inflammation. To understand the effect of the enzyme on immune responses, we examined whether the enzyme treatment affected the differentiation of dendritic cells (DCs) that were in vitro induced from bone marrow cells with GM-CSF. As the expression of DC markers were comparable with or without the enzyme treatment, the modification of terminal sugars was unlikely associated with DC differentiation.

**Conclusion:** These data suggest that the enzyme reduces inflammation caused by immune responses and may be a new target for RA.

**Disclosure of Interest:** None Declared

**P020**

**THE HEDGEHOG PATHWAY SUPPRESSES CD4 T CELL PATHOGENICITY DURING NEUROINFLAMMATION**

R. Kapoor1,*, M. C. Miller1, T. Ochoa1, L. Dragin1, L. Cheslow1, J. I. Alvarez1

1Pathobiology, University Of Pennsylvania, Philadelphia, United States

**Introduction:** During neuroinflammation, the concerted actions of the nervous and immune systems affect disease outcome. Despite significant advances in the field, our understanding of the mechanisms regulating this interplay is incomplete. Astrocytes are recognized as important players in the regulation of the inflammatory responses in diseases like multiple sclerosis (MS). We have uncovered a novel and critical role for astrocyte-secreted Sonic Hedgehog (Shh) in inducing and maintaining CNS immune privilege properties while also limiting neuroinflammation. However, the mechanisms by which Shh regulates immune cell function under neuroinflammatory conditions remain ill-defined.

**Methods:** To address the impact of Shh on T cells, we first differentiate human memory T cells (CD4+CD45RO+) into Th1 and Th17 populations in conditions stimulating the Hh pathway and determine the impact of Shh on cytokine production and immune activation state. To address the role of the Hh pathway on antigen specific responses, we study the effect of Shh on the phenotype of encephalitogenic T cells and define the CNS-centric role of Shh inducing experimental autoimmune encephalomyelitis in mice lacking the Hh receptor smoothened (smo) on T cells (CD4-Cre; smo<sup>−/−</sup>)

**Results:** On human CD4+CD45RO+ T cells, Shh had a profound impact antagonizing the inflammatory program of Th1 cells by reducing the expression of IFN-γ, GM-CSF, TNF-α and IL-17 as well as upregulating the expression of the anti-inflammatory cytokine IL-10. While the Hh pathway activation modestly regulated the Th17 program. In rodents, Shh induced a significant increase in IL-10 expression by murine encephalitogenic CD4 T cells. To address the effect of Hh stimulation within the CNS we induced EAE induction in mice lacking the Hh receptor smoothened (smo) on T cells (CD4-Cre; smo<sup>−/−</sup>). Hh deficiency in the T cell compartment resulted in earlier onset of disease and higher clinical scores characterized by exacerbated immune cell infiltration, demyelination and axonal pathology. This phenotype was associated with increase expression of IFN-γ, GM-CSF, TNF-α as well as a profound reduction in IL-10 expression on CD4+ T cells. Interestingly, such effect was unique to the CNS as peripheral Hh-deficient CD4+ T cells expressed comparable levels of IFN-γ, GM-CSF, TNF-α and IL-10 than controls. Our findings indicate that in response to inflammation, activation of the Hh signaling dampens the neuroinflammatory status of pathogenic Th1 cells.

**Conclusion:** Collectively, these data support a critical role for Shh signaling in the crosstalk between the CNS and T cells to antagonize neuroinflammation and enhance neuroprotective mechanisms.

**Disclosure of Interest:** None Declared

**P020.A**

**MONOCYTES DRIVE TYPE I INTERFERONOPATHY IN PATIENTS WITH MUTATIONS IN ISG15**

M. Martin-Fernandez1,2,3,1, C. Gruber1,2,3, S. Buta1,2,3, A. Nieto-Patlan4, X. Qiu1,2,3, J. Desa5, E. Ferre5, A. K. Sood6, A. Alakeef7, R. Halwani8,9, J. Bustamante4,10,11, M. Hernandez4, F. Alshohime12, J.-L. Casanova4,10,13,14,15, M. Lionakis5, D. Bogunovic1,2,3

1Microbiology, 2Pediatrics, 3The Mindich Child Health and Development Institute, Icahn School Of Medicine At Mount Sinai, New York, United States, 4Paris Descartes University, Imagine Institute, Inserm U1163, Paris, France, 5Fungal Pathogenesis Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, 6Center for Environmental Medicine, Asthma and Lung Biology, University of North Carolina at Chapel Hill, Chapel Hill, United States, 7Dermatology, Department of Dermatology, King Saud University Medical City, King Saud University,, 8Pediatrics, King Faisal Specialist Hospital and Research Center, 9Immunology Research Laboratory, College of Medicine, King Saud University, Riyadh, Saudi Arabia, 10Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM, U1163, 11Center for the Study of Primary Immunodeficiencies, AP-HP, Necker Hospital for Sick Children, Paris, France, 12Pediatrics, King Saud University Medical City, College of Medicine, King Saud University, Riyadh, Saudi Arabia, 13St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University,
Introduction: Type I interferonopathies comprise a group of Mendelian disorders characterized by a persistent Type I IFN-mediated inflammation and the resulting increase in the expression of IFN-stimulated genes (ISGs). The perturbation in the ability to regulate inflammation is a well-described cause of type I interferonopathies, as shown in patients with deficiencies in USP18 and ISG15, two negative regulators of IFN signaling pathway. Here we report a novel cell type specificity in the development of Type I Interferonopathy in patients from three families with different biallelic mutations in ISG15: Two patients from two related consanguineous families (P1 and P2) from Saudi Arabia and a child with a compound heterozygous mutation from USA (P3).

Methods: We obtained fibroblasts from P1 and P2 and whole blood from P3. Whole blood was subjected to CyTOF analysis. PBMCs were also isolated for single cell RNA sequencing (scRNAseq). ISG (IFI1T, MX1, RSAD2) expression analysis was performed by qRT-PCR in fibroblasts stimulated with 1000 U/mL of IFNα2b.

Results: We investigated three patients presenting with type I interferonopathy features. P1 and P2 developed also BCG-itis following vaccination. P3 presented with severe skin inflammation and was not BCG vaccinated. Whole exome sequencing revealed that P1 and P2 have a homozygous mutation in a splicing site in ISG15 gene (c.4-1G>A), which results in exon 2 skipping and lack of protein expression. P3 carries compound heterozygous mutations c.310G>A (p.V104M) and c.352C>T (p.Q118*) in the ISG15 gene. Both mutations of P3 lead to impaired protein expression despite normal mRNA levels. Ex vivo, all three patients have steady state IFN-1 signature in whole blood. In vitro, P1 and P2’s fibroblasts displayed enhanced ISGs accumulation in response to IFN challenge. Immunophenotyping did not reveal any significant differences between P3 and healthy control in cell type distribution. Qualitatively, CD14+CD16+ monocytes exhibited significantly higher expression of Siglec-1 in the patient. Single cell RNAseq analysis of patient PBMCs demonstrated a specific set of ISGs also upregulated in patient’s monocytes specifically.

Conclusion: We identified three patients from two families with different biallelic mutations in ISG15, which resulted in the development of Type I Interferonopathy. Together the findings of this work suggest that the inflammatory phenotype observed in these patients is driven by monocytes.

Disclosure of Interest: None Declared

P020.C
INHIBITION OF ERAD PLAYERS P97 AND SEC61 MODULATES PROINFLAMMATORY CYTOKINES PRODUCTION IN PRIMARY T CELLS
A. P. Yeola1,*, P. I. A. D. Mercy1, J. Baillargeon1, M. Rangachari1,2
1Centre de recherche de CHU de Québec, 2Molecular Medicine, Université Laval, Quebec city, Canada

Introduction: ER-associated degradation (ERAD) is a protein quality system that removes misfolded,
mismatched proteins in the ER by selectively dislocating them into the cytosol where they are subsequently degraded by the cytosolic ubiquitin proteasome system (UPS). Sec61 plays an important role in the import of newly synthesized proteins into the endoplasmic reticulum (ER), while the p97 translocon is crucial for the reverse transport of misfolded proteins to the cytosol. While the role of sec61 and p97 has been investigated extensively in neurodegenerative diseases and cancer, their function in autoimmune disease, and in T cell function in particular, are incompletely understood.

Previous studies have shown that mycolactone-mediated Sec61 blockade has immediate inhibitory effects on the production of cytokines in immune cells. However, the suppression of cytokines by sec61 inhibitor is not completely explored in MS. Our aim is to investigate the effect of ER translocation inhibitors in different T cell subsets.

**Methods:** We isolated CD4+ T cells and differentiate them in effector Th1 and Th17 cells. At day 5, we treated cells with different concentrations of these inhibitors for 4hr (2, 4, 6, 8, 10, 12 μM), with respective concentrations of DMSO (vehicle control). We assessed their production of inflammatory cytokines (IFN-γ, IL-17A, IL-2, TNF-α) by flow cytometry.

**Results:** The present study provides evidence that Apratoxin A (sec61 inhibitor) as well as NMS873 and Eeyarestatin I (ErsI, p97 inhibitors), suppresses the pro-inflammatory cytokines in Th1 and Th17 CD4+ T cells. When Th1 cells treated in vitro with ErsI, we found the surprising change in the cytokine profile in all concentrations compare to controls. ErsI decreases IFN-γ expression (p<0.0001) significantly. In Th17 ErsI does not change IL-17A production significantly. However, NMS873 IFN-γ expression (p<0.0001) significantly in Th1 cells and IL-17A (p<0.0001) in Th17 cells. Interestingly, we have observed that sec61 inhibitor Apratoxin A dramatically reduces the IFN-γ, IL-2 as well as TNF-α (p<0.0001) secretion in Th1 cells.

**Conclusion:** These data suggest that ER translocation is critical for the differentiation of Th1 and Th17 cells. In future work, we will test its significance in autoimmunity by using adaptive transfer models of Experimental autoimmune encephalomyelitis (EAE), which recapitulates the immune aspects of multiple sclerosis (MS).

**Disclosure of Interest:** None Declared

**P021**

**MOLECULAR MECHANISM OF IL4-INDUCED CCL17 PRODUCTION IN HUMAN MONOCYTES AND MURINE MACROPHAGES**

A. Achuthan1, A. Hsu1, T. Lupancu1, M.-C. Lee2, A. Fleetwood3, A. Cook1, J. Hamilton1

1Department of Medicine, RMH, The University of Melbourne, Parkville, Australia

**Introduction:** Interleukin 4 (IL4) is generally viewed as a Th2 cytokine capable of polarizing macrophages into an anti-inflammatory phenotype, while granulocyte macrophage-colony stimulating factor (GM-CSF), on the other hand, is often viewed as a proinflammatory cytokine with part of this function due to its action on monocytes/macrophages. Paradoxically, these two cytokines act additively to enhance in vitro the differentiation of dendritic cells from precursors such as monocytes. One upregulated marker of an IL4-polarized M2 macrophage is the chemokine (C-C motif) ligand 17 (CCL17), which we have recently reported to be induced by GM-CSF in monocytes/macrophages in an interferon regulatory factor 4 (IRF4)-dependent manner.

**Methods:** Gene and protein expression were measured by quantitative PCR and Western blotting, respectively. Secreted protein in culture medium was by ELISA. Genes were silenced using siRNA technology. Enrichment of histones and transcription factors at the gene loci was determined by chromatin immunoprecipitation.

**Results:** IL4 also induces CCL17 production by acting through IRF4 in human monocytes and murine macrophages. Further, IL4 upregulates IRF4 expression at the epigenetic level by enhancing the expression and activity of jumonji domain-containing protein 3 (JMJ3D) demethylase. Intriguingly, silencing the signal transducer and activator of transcription 6 (STAT6) gene led to a decrease in not only CCL17 formation, but also in that of its upstream regulators, JMJ3D and IRF4. Moreover, IL4 treatment of human monocytes resulted in an increased association of STAT6 to the promoter regions of the CCL17, IRF4 and JMJ3D genes.

**Conclusion:** In spite of their vastly different functions, IL4 and GM-CSF appear to share elements of a common signaling pathway in regulating CCL17 production in human monocytes and murine macrophages.

**Disclosure of Interest:** None Declared

**P022**

**B CELL SPECIFIC IL-6 PRODUCTION PLAYS A PARADOXICAL ROLE IN B CELL DIFFERENTIATION.**

M. Akkaya1,2, S. K. Pierce1

1NIAID, NIH, Rockville, United States

**Introduction:** During acute immune responses, IL-6 is secreted by many different immune cells and plays a major role in plasma cell differentiation. B cells secrete IL-6 in response to TLR agonists or CD40L stimulation yet the significance of B cell specific IL-6 secretion in the progression of immune response is largely unknown.

**Methods:** In order to identify the role of B cell mediated IL-6 secretion in B cell functions, we carried out in vitro B cell stimulation assays using mouse splenic B cells purified from either WT or IL6 KO mice. Furthermore, to understand the role of B cell specific IL-6 secretion in the development of overall immune response, we generated bone marrow chimeric mice by transferring bone marrow from B cell deficient mice mixed with bone marrow from either IL-6 KO or WT mice at 9:1 ratio in order to obtain B cell specific IL-6 KO mice and control mice respectively. Upon reconstitution, these mice were challenged with a T
cell dependent antigen adjuvanted with either alum or alum plus TLR9 agonist CpG.

Results: Here we show that, among different TLR agonists, TLR9 agonist CpG induces the highest magnitude of IL-6 secretion which is antagonized by B cell receptor signaling. However IL-6 KO B cells responded normally to CpG in terms of proliferation, prevention of activation induced cell death and activation marker expression in vitro. On the other hand, using chimeric mouse models, we showed that B cell specific IL-6 deficiency resulted in weaker germinal center responses, lower number of antigen specific plasma cells, lower titers of specific antibodies and less antibody affinity maturation upon immunization with alum adsorbed T dependent antigen NP-CGG. Paradoxically, the same responses enhanced when NPCGG is adjuvanted with CpG.

Conclusion: These results suggest that, in the absence of TLR agonists, B cell mediated IL-6 secretion is driven by B-T interactions and works in favor of T dependent responses. However, in the presence of TLR agonists, B cell specific IL-6 production favors early commitment towards T independent plasma cell generation and thus plays role in dampening germinal center formation and affinity maturation.

Disclosure of Interest: None Declared

P023
ACTIVE TYPE I IFN SIGNALING IN ABSENCE OF DETECTABLE CYTOKINE
J. Altman1,*, J. Taft1, D. Bogunovic1
1Microbiology, Icahn School of Medicine at Mount Sinai, New York, United States

Introduction: Interferon stimulated gene 15 (ISG15) and ubiquitin specific peptidase 18 (USP18) are induced following type I IFN (IFN) signaling, thus they fall into the broader category of interferon stimulated genes (ISGs). USP18 is stabilized by ISG15 and binds to IFN receptor 2 (IFNAR2) to inhibit IFN signaling. Cells derived from human patients deficient in either ISG15 or USP18 exhibit 5-50 fold increased levels of ISGs (e.g. MX1 and IFIT1) compared to control cells up to 5 days post initial IFN priming when no cytokine can be detected in the supernatant.

Methods: In order to determine what could be mediating this persistence we assayed for nascent RNA transcription in the absence of the cognate cytokine. Additionally, we probed for interferon stimulated gene factor 3 (ISGF3) at this late time point post IFN priming using western blotting for its protein components. Kinase activity was evaluated using chemical Janus kinase (JAK) inhibitors, namely Cerdulatinib and Ruxolitinib.

Results: IFN blocking antibodies given after the 12hr IFN prime exhibited no effect on ISG transcription, proving no residual soluble IFN was mediating the ISG persistence. Nascent RNA capture illustrated that patient, but not control cells exhibit active transcription of select ISGs 48-72hrs after IFN priming. In line with this, phosphorylated ISGF3 (pISGF3) was detected in patient cell lines 48hrs after IFN priming, but not in control cells. Furthermore, chemical inhibition of the JAKs ablated this late acting pISGF3 indicating that the kinases are still active and signaling. Taken together, these data demonstrate that active IFN signaling is occurring in the absence of any detectable cytokine.

Conclusion: Our data implicate an active role for USP18 in deactivating IFNAR post IFN departure and that this novel function of USP18 likely plays a role in the pathophysiology of these type I interferonopathy patients.

Disclosure of Interest: None Declared

P024
THE RNA-BINDING PROTEIN ARID5A ORCHESTRATES IL-17-MEDIATED INFLAMMATION THROUGH POST-TRANSCRIPTIONAL CONTROL OF MRNA
N. Amatya1,*, J. A. Cruz1, F. E. Y. Aggor1, E. E. Childs1, A. V. Garg1, A. Berman2, U. Atasoy3, S. Gaffen1
1Department of Medicine, 2Department of Biological Sciences, University Of Pittsburgh, Pittsburgh, 3Division of Allergy and Immunology, University of Michigan, Ann Arbor, United States

Introduction: Interleukin-17A (IL-17) is a proinflammatory cytokine essential for clearance of microbial pathogens, but conversely drives immunopathology in autoimmune settings. Although IL-17 is consistently found to be a modest activator of gene transcription in experimental settings, its biological impact in vivo is profound. In addition to de novo transcription, IL-17 also upregulates inflammatory gene expression by stabilizing their transcripts. mRNA stabilization is a central, but still poorly understood facet of IL-17 signaling. Here, we report an RNA binding protein Arid5a (AT-rich interactive domain-containing protein 5A) as a novel mediator of IL-17-induced stabilization of inflammatory mRNAs.

Methods: To assess the role of Arid5a in IL-17 signaling, we performed gene knockdown using siRNA in the murine stromal cell line ST2 and primary MEFs. Following IL-17 treatment, expression of IL-17 target genes was analyzed using qRT-PCR, ELISA and western blot. mRNA half-life of IL-17 target genes was assessed after actinomycin D treatment. Arid5a-binding transcripts were identified via RNA immunoprecipitation. Co-immunoprecipitation assays were performed to identify adaptor proteins that associate with Arid5a.

Results: We found that IL-17 stimulation upregulated Arid5a expression, whereupon Arid5a was rapidly recruited to TRAF2. Arid5a stabilized multiple IL-17-induced cytokine mRNA transcripts, including Il6, Cxcl1 and Cxcl5, by binding to their 3’ untranslated regions (UTR). In some cases, Arid5a counteracted mRNA degradation mediated by the endoribonuclease MCPIP1 (Regnase-1). Additionally, Arid5a enhanced IL-17-induced expression of the transcription factors IkBζ (Nfk biz) and C/EBPβ (Cebpb), which could then transactivate IL-17-dependent promoters. Arid5a bound to Nfk biz or Cebpb
transcripts, but only stabilized transcripts of Nfkbiz but not of Cebpb. Surprisingly, Arid5a facilitated translation of both C/EBPβ and ikBζ.

**Conclusion:** Our findings demonstrate novel role of Arid5a in controlling downstream IL-17 signaling and reveal a new function for Arid5a in mediating mRNA translation. Understanding the mechanisms of Arid5a-mRNA interaction could lay the ground work for designing RNA-based inhibitors for the treatment of IL-17 related autoimmune diseases.

**Disclosure of Interest:** None Declared

**P025**

**IL-33 STIMULATING HUMAN MAST CELL CHEMOKINE VIA MAPK ACTIVATION, AND INHIBITION BY TETRAMETHOXYLUTEOLIN**

M. Bawazeer1, T. Theocharides2

1Program in Pharmacology and Experimental Therapeutics, 2Department of Immunology, School of Medicine, Tufts University, Boston, United States

**Introduction:** Mast cells (MCs) are hematopoietic cells that mature in all tissues and are known to be stimulated by allergic triggers such as IgE/anti-IgE, which are augmented by the cytokine IL-33. Upon stimulation, MCs release pre-formed mediators (histamine, β-hexosaminidase, and tryptase), as well as newly synthesized cytokines (TNF, IL-6, and IL-13) and chemokines [CXCL8 (IL-8), CCL5 (RANTES), and CCL2 (MCP-1)]. SP and IL-33 synergistically stimulate TNF release from human cultured MCs, with IL-33 evidencing the most potent effect. IL-33 activates both NF-κB and MAPK-AP-1 signaling; it is important to note that both pathways are essential for cytokine production. The naturally occurring flavonoid, 3’4’5,7-tetramethoxyxyluteolin (methoxyxyluteolin) has potent anti-inflammatory properties in vitro. The intent of this work is to investigate the action of IL-33 on chemokine activation in human cultured LAD2 MCs and the effect of methoxyxyluteolin on this activation.

**Methods:** LAD2 MCs were stimulated with IL-33 (1-100 ng/ml) for 24 hours, following which, chemokine (CXCL8, CCL5, CCL4, CCL3, and CCL2) secretions were measured by ELISA and their gene expression was assayed by qRT-PCR after 6 hours of stimulation. Then, the LADs MCs were treated with IL-33 (10 ng/ml) or left untreated for 30 minutes for phospho-kinase array. The intracellular components that showed greater expression in treated cells were confirmed by western blot. For the inhibitory demonstration, LAD2 MCs were pre-incubated with methoxyxyluteolin (10, 50, 100 μM) for 2 or 24 hours then stimulated by IL-33 (10 ng/ml) for 24 hours to examine chemokine secretion or for 6 hours to examine their gene expression. Using western blot, the expressions of phosphorylated p38α MAPK, JNK, Erk were compared in cells pre-incubated with methoxyxyluteolin (50 μM) for 2 hours, then stimulated for 10 minutes with IL-33 (10 ng/ml) and the stimulated condition without methoxyxyluteolin.

**Results:** IL-33 showed significant dose-response increase in secretion of CXCL8, CCL5, CCL4, CCL3, and CCL2, as well as dose-response increase in their gene expression. IL-33 stimulation causes activation of mitogen-activated protein kinase (MAPK) components, as shown by phosphorylation of p38α MAPK and JNK. Inhibition of these responses by compounds known to inhibit each, inhibited chemokine secretion, which verifies the role of MAP kinase in chemokine secretion. IL-33 stimulated responses were significantly inhibited by 2 hours of pre-incubation with methoxyxyluteolin with 30%>70% inhibition for all the investigated chemokines except CXCL8. The inhibition is not via MAP kinase inhibition as phosphorylated p38α MAPK and JNK expression are not changed by methoxyxyluteolin compared to IL-33 without methoxyxyluteolin. Further experiment will be conducted looking at methoxyxyluteolin effect downstream MAPK pathway.

**Conclusion:** IL-33 plays a major role in secretion of various chemokines from human MCs, which can be inhibited by methoxyxyluteolin. This work will lead to an understanding of chemokine regulation in human MCs, and development of a novel treatment for inflammatory and allergic diseases.

**Disclosure of Interest:** None Declared

**P026**

**A NOVEL MECHANISM OF IMMUNE RESPONSE REGULATION IN HUMAN PLASMACYTOID DENDRITIC CELLS BY TOLL-LIKE RECEPTOR 10**

P. Deb1,2, S. Singh1, N. Hess2, R. Tapping3, P. Fitzgerald-Bocarsly4

1Pathology and laboratory medicine, Rutgers University, Newark, 2University of Wisconsin Madison, Madison, 3Microbiology, University of Illinois, Urbana-Champaign, Urbana-Champaign, United States

**Introduction:** Plasmacytoid dendritic cells (pDCs) are the most potent producer of type-i interferon and play a central role in innate immunity. The innate sensing of pathogens by pDCs and subsequent triggering of an immune response is orchestrated by toll-like receptors (TLRs). Unlike other TLRs, the role of TLR10 in pDCs has not been characterized. Here, we have interrogated if human pDCs express TLR10, how that expression compares to other immune cells, and how the functionality of pDCs is affected upon antibody-mediated engagement of TLR10.

**Methods:** PBMCs were isolated from heparinized blood from healthy volunteers via ficoll-hypaque gradient centrifugation. The expression of TLR10 was measured in pDCs compared to other immune cells via flow cytometry. pDCs were isolated from PBMCs via magnetic bead-based negative selection. Gene expression of TLR10 in pDCs was measured via qRT-PCR at a resting state and following viral and cytokine stimulation. Purified pDCs and PBMCs were pre-incubated with anti-TLR10 antibody and stimulated with herpes simplex 1 (HSV1), flu, and sendai virus. The effect of TLR10 engagement was measured by comparing cytokine production and expression of co-
stimulatory markers by pDCs upon pre-incubation with an anti-TLR10 antibody or matching isotype via flow cytometry and ELISA. Phospho-flow was employed to see the phosphorylation of various transcription factors in pDCs upon TLR10 engagement. Amnis ImageStream was used to see how TLR10 engagement affects virus uptake and nuclear localization of transcription factors.

Results: We observed that primary human pDCs, B cells, and monocytes constitutively express TLR10. Treatment with different viral stimuli failed to alter TLR10 expression on pDCs. Upon pre-incubation with an anti-TLR10 antibody, production of cytokines including interferon-α, tumor necrosis factor-α, interleukin-6, and interferon-γ was downregulated in response to stimulation with DNA and RNA viruses. Similar effects were seen in monocytes. Expression of co-stimulatory and activation markers on pDCs including CD40, CD80, CD83, and CD86 was not modulated by TLR10-engagement. Upon further investigation into the possible mechanism, we documented phosphorylation of signal transducer and activator of transcription 3 (STAT3) upon antibody-mediated engagement of TLR10 leading to induction of inhibitory molecule suppressor of cytokine signaling 3 (SOCS3) expression. TLR10 engagement did not affect pDCs’ ability to uptake viruses. However, upon TLR10-engagement, nuclear translocation of interferon regulatory factor 7 (IRF7) was hindered in pDCs, suggesting the possible mechanism of downregulation of cytokine expression.

Conclusion: Our data provide the evidence that TLR10 is constitutively expressed on the surface of human pDCs and works as a regulator of their innate response. Our findings indicate the potential of harnessing the function of pDCs by antibody-mediated targeting of TLR10 that may open a new therapeutic avenue for autoimmune disorders.

Disclosure of Interest: None Declared

P027 MECHANISM OF GP130 ACTIVATION AND REGULATION OF ITS DOWNSTREAM SIGNALLING
F. Dehkhoda1*, N. Durisic2, Y. Chhabra3, A. Brooks4
1Diamantina Institute, The University of Queensland, Woolloongabba, 2Queensland Brain Institute, The University of Queensland, St Lucia, Australia

Introduction: GP130 is the founding member of the tall cytokine receptors and serves as a signal transducing subunit for several cytokines including IL-6, IL-11, IL-27, LIFR, and others. The GP130 family of cytokines are diverse with key roles in immune responses, neural growth, maintenance of stem cell pluripotency and cardiovascular system through signalling via IL-6, CNTF, LIF, and CT-1 [1]. In addition, GP130 has important roles in cancer and inflammation biology as illustrated in mouse models harbouring mutations in the suppressors of cytokine signalling (SOCS) binding region of GP130 resulting in hyper-active signal transducers and activators of transcription (STAT)1 and 3 signalling promoting gastric cancer incidences. Moreover, small in-frame deletions in domain 2 of GP130 have been identified in 60% of patients with inflammatory hepatocellular adenomas (IHCA) making GP130 D2 mutants hepatic oncogenes [2]. Although numerous studies have investigated the structure of the GP130 receptor extracellular complex, little is known about its activation mechanism and regulation of signal transduction.

Methods: Single particle tracking by super resolution microscopy with photo-activated localization microscopy (PALM) of GP130 molecules was performed on ligand stimulated and non-stimulated HEK293 cells to analyse diffusion properties and oligomeric state of the receptors on the cell membrane. To analyse the signal transducing orientation of GP130, the extracellular domain of receptor was swapped with the leucine zipper dimerisation domain of c-jun transcription factor to generate GP130 dimers on the cell surface of the pro-B cell line, Ba/F3.

Results: The effect of length, charge, and rotation of the GP130 extracellular juxtamembrane region was investigated resulting in identification of active and inactive receptor configurations. Variable rotations of the GP130 transmembrane and intracellular domains induced by alanine insertions mimicking cytokine induced activation led to differential activation of JAK/STAT and MAPK signalling pathways and generated distinct proliferative responses in Ba/F3 cells. These models with FRET (foster resonance energy transfer) reporters fused after the JAK binding Box1-2 region were generated and movements of the intracellular domains of GP130 with associated JAK kinases were assessed.

Conclusion: This is the first study aimed at determining the precise movements of GP130 receptor upon activation. Herein, we demonstrate that receptor orientation at the transmembrane domain induced by alanine insertions results in differential receptor activation, FRET efficiencies (arising from distinct receptor intracellular domain movements), and induction of downstream signalling in active and inactive receptor models.

Disclosure of Interest: None Declared

P028 CRTAM IS REGULATED BY IL-6 AND IL-27 IN INFLAMMATORY ARTHRITIS
A. Derrac Soria1,*, X. Liu2, A. Cardus Figueras4, J. Twohig1, R. Andrews1, B. Jenkins2, R. Benson3, C. Prendergast3, P. Garside2, G. W. Jones4, S. A. Jones1
1Systems Immunity University Research Institute, Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, United Kingdom, 2Centre for Innate Immunity & Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia, 3Institute of Infection, Immunology and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

Introduction: Recent experiments from our laboratory identified a series of genes that are negatively regulated by interleukin (IL)-6 in activated naïve CD4 T-cells.
Transcriptomic analysis identified Crtam as a gene significantly inhibited by IL-6 during in vitro T cell activation with anti-CD3/CD28 co-stimulatory antibodies. Class-I MHC-restricted T-cell associated molecule (CRTAM) is an activation-induced surface receptor that regulates T-cell development and proliferation. CRTAM+CD4+ T-cells secrete IFN-γ and express cytotoxic T-cell-related genes. Although recent studies highlight the importance of CRTAM in regulating T-cell effector characteristics, the lymphokines involved in controlling CRTAM expression remain unknown. Here, using an unbiased transcriptomic approach, we investigated the role of IL-6 and IL-27 on CRTAM expression in CD4 T-cells and examined the impact of CRTAM+CD4+ T-cells in inflammatory arthritis.

**Methods:** Naïve CD4 T-cells were stimulated with plate-bound anti-CD3 and anti-CD28 co-stimulatory antibodies in the presence of IL-6 or IL-27. Changes in CRTAM together with markers of T-cell activation, cytokine release and proliferative survival were analysed by flow cytometry, qPCR, chromatin immunoprecipitation (ChIP), and ELISA. The in vivo regulation of CRTAM was monitored by immunohistochemistry and immunofluorescence of joint sections from antigen-induced arthritis (AIA) challenged wild-type, Il6ra−/− and Il27ra−/−mice.

**Results:** Activation of naïve CD4 T-cells caused a temporal increase in CRTAM surface expression. Maximal expression was observed 18hrs post-stimulation. Treatment with IL-6 and IL-27 significantly impaired CRTAM expression (50-60%). This form of suppression is controlled by gp130 activation of STAT3, which promotes Zeb1 expression as a transcriptional repressor of CRTAM. Open access repository datasets from patients with rheumatoid arthritis show that CRTAM and its ligand CADM1 is highly expressed in the inflamed synovium. Importantly, Cadm1 was not controlled by IL-6 or IL-27. Synovial CRTAM expression was particularly evident in AIA models where the leukocyte infiltrate contributed to joint pathology – myeloid-rich (wild-type mice) and lymphoid-rich (Il27ra−/−) synovitis. Indeed, synovial CRTAM was a more prominent feature of lymphoid-rich synovitis, and its pattern of expression suggested a potential involvement in the local control of adaptive immune responses.

**Conclusion:** Our findings highlight the regulatory effects of IL-6 and IL-27 on CRTAM expression in naïve CD4 T-cells and provide the first evidence of CRTAM+CD4+ T-cells within the inflamed synovium. Selective CRTAM expression correlates with joint pathology and therefore, may represent a novel biomarker of T cell-driven pathology in inflammatory arthritis.

**Disclosure of Interest:** None Declared

---

**P029**

**INTERFERON INCREASES TGF-B-INDUCED CASPASE 8 ACTIVATION AND APOPTOSIS**

F. ElAsmi1,2, L. Dianoux1, M. K. Chelbi-Alix1

1Paris Descartes University, INSERM UMR-1124, Paris, France

**Introduction:** PML, an interferon-stimulated gene product, is essential for the formation of nuclear matrix-associated organelles named PML nuclear bodies (NBs) that act as a platform for post-translational modification and protein degradation. PML NBs harbor transiently and permanently localized proteins and have been associated with the regulation of several cellular functions including apoptosis. There are seven isoforms of PML, six nuclear (PML-VI) and one cytoplasmic (PML-VII), which are encoded by a single gene via alternative RNA splicing. It has been reported that murine PML-null primary cells are resistant to TGF-β-induced apoptosis and that cytoplasmic PML is an essential activator of TGF-β signaling. It has been reported that caspase 8 activation is an essential mediator of apoptosis induced by TGF-β. In addition, caspase 8 was shown to be SUMOylated at lysine 156, and this modification was associated with its nuclear localization and without impairing its processing.

**Methods:** Here we show that interferon (IFN) α potentiated TGF-b-mediated apoptosis in human cells.

**Results:** IFNα treatment or ectopic expression of PMLIV, but not PMLIII, promoted TGF-β-induced caspase 8 activation. In response to TGF-β, both PMLIII and PMLIV were conjugated to SUMO and shifted from the nucleoplasm to the nuclear matrix, however only PMLIV interacted with caspase 8. This process was followed by the recruitment of caspase 8 within PML NBs resulting in a caspase-dependent PML degradation and PML NB disruption.

**Conclusion:** Taken together, these findings highlight the important function of PML NBs in the increase by IFNα of TGF-β-induced apoptosis.

**Disclosure of Interest:** None Declared

---

**P030**

**THE ROLE OF AUTOCRINE MEDIATORS IN THE METABOLIC REPROGRAMMING OF LPS ACTIVATED MACROPHAGES**

A. J. Fleetwood1,2, D. P. De Souza3, M. K. S. Lee4, A. Achuthan5, M.-C. Lee6, D. Tull7, A. D. Cook1, A. J. Murphy3, J. A. Hamilton1,4

1Medicine, The University Of Melbourne, 2Bio21 Molecular Science and Biotechnology Institute, Metabolomics Australia, 3Haematopoiesis and Leukocyte Biology, Baker IDI Heart and Diabetes Institute, 4Australian Institute for Musculoskeletal Science , The University Of Melbourne, Melbourne, Australia

**Introduction:** Metabolic reprogramming and its links with macrophage polarisation and effector function have been keenly studied of late. For example, macrophages activated with the bacterial cell wall component lipopolysaccharide (LPS), so called “M1” macrophages, have been shown to commit to glycolysis, display enhanced fatty acid synthesis and have a truncated Krebs cycle (tricarboxylic acid cycle) leading to the accumulation
of numerous intermediates. These intermediates have now been implicated in contributing to the inflammatory phenotype and function of these cells. The factors driving these metabolic changes are incompletely understood.

**Methods:** Metabolic reprogramming was measured in human macrophages and bone marrow-derived macrophages (generated from wild-type and gene-deficient mice). Gene expression (RT² Profiler PCR Arrays for Glucose Metabolism), changes in the oxygen consumption rate (OCR), extracellular acidification rate (ECAR) and spare respiratory capacity (SRC) were measured by Seahorse XF-e96 bioanalyzer. Intracellular metabolites were extracted and measured by Shimadzu GC-QQQ analysis.

**Results:** Macrophages stimulated with LPS produce autocrine mediators that are crucial to their commitment to glycolysis, reduced mitochondrial oxidative phosphorylation (OXPHOS) and fatty acid synthesis.

**Conclusion:** We describe a profound role for autocrine mediators in the metabolic reprogramming and polarization of macrophages.

**Disclosure of Interest:** None Declared

---

**P031**

**DIFFERENTIAL INFLAMMATORY RESPONSES TO TYPE I AND III INTERFERONS ARE REGULATED BY INTERFERON REGULATORY FACTOR 1**

A. Forero‡, S. Ozarkar‡, C. Heng-Lee‡, L. So‡, E. A. Hemann‡, M. Nadjsombati‡, R. Green‡, S. N. Sarkar‡, J. von Moltke‡, M. Gale Jr.‡, R. Savan‡

‡UNIVERSITY OF WASHINGTON, Seattle, United States

**Introduction:** Type I and III interferons (IFNs) are critical antiviral molecules thought to activate similar signaling cascades resulting in the induction of vastly overlapping IFN-stimulated genes. However, recent studies have challenged this notion by showing that type I, but not type III IFNs (IFNA), promote strong inflammatory responses. The molecular mechanisms explaining their distinct responses and functional consequences are not fully understood.

**Methods:** In this study, we take an integrative approach to evaluate the transcriptional and translational mechanism downstream of IFN-sensing. We apply genome-wide gene expression analysis and evaluate the in vivo consequences of type I or type III IFN treatment.

**Results:** In this study, we have shown that the differential responses to the two IFN families is primarily regulated at the transcriptional level. We demonstrate that the transcription factor interferon regulatory factor 1 (IRF1) is induced exclusively by type I IFN, identify a unique set of IRF1-dependent genes, and show that IRF1 serves to facilitate the type I IFN-mediated early antiviral protective responses. Induction of IRF1 by IFNA occurred only upon IFNLR1 overexpression, demonstrating that receptor abundance determines the threshold of STAT1-driven IRF1 expression. The absence of robust IRF1 induction likely results in the delay in IFN-stimulated gene expression, dampening of chemokine production, and immune cell recruitment observed after murine IFNA intranasal inoculation. Lastly, we identify IFNA-responsive kinases and transcription factors that further diversify the biological activities of type I and type III IFN. Overall our data show that type III IFNs are specific elicitors of antiviral immunity versus the ubiquitous inflammatory program typical of type I IFNs.

**Conclusion:** This study suggests that type III IFNs are predominantly an innate IFN that control viral spread at the site of the infection and restrict damage by limiting inflammatory responses and initiating epithelial repair, while the transient induction of inflammatory responses by type I IFNs serve to recruit adaptive immune effectors to the site of infection.

**Disclosure of Interest:** None Declared

---

**P032**

**DNA METHYLATION IMPAIRS MONOCYTE FUNCTION IN TUBERCULOSIS LEADING TO DISEASE PROGRESSION**

F. A. Zambuzi1, M. S. Espindola1, L. S. Soares1, L. J. Galvão-Lima1, R. C. Castro1, F. G. Frantz1,* on behalf of Laboratory of Immunology and Epigenetic

1Faculdade de Ciências Farmacêuticas de Ribeirão Preto, University Of São Paulo, Ribeirão Preto, Brazil

**Introduction:** Despite treatment and cure, Tuberculosis (TB) is still considered a world health public problem. It is known that interaction between bacteria and host immune mechanisms results in changes in gene expression, which could be a result of epigenetic changes, as DNA methylation. Recent studies suggest that alterations in host epigenome could be promoted by Mtb infection and influence immune cells function. In this context, we aimed to determine the methylation profile of monocytes from tuberculosis patients and correlate with immune function of these cells.

**Methods:** Monocytes were isolated from active TB patients (APTB) with treatment-time less than one month (n=10) and nontuberculosis controls (CTRL) (n=16) - Ethics Committee approval FMRP-USP (Protocol #6481/2013). The monocytes were used to evaluate the global DNA methylation content and immune function, through cytokine and ROS production after heat killed Mtb challenge.

**Results:** When we evaluated the group of tuberculosis patients according to their lung injury degree, we found an increased methylation in those with more severe disease. Also, it was possible to observe that monocytes from patients with increased methylation profile, presented a reduction in secretion of anti-inflammatory cytokine, IL-10 and increased pro-inflammatory IL-12, indicating an impairment in monocytes ability to regulate the excess of inflammation, which mediates the lung injury often observed in these patients.

**Conclusion:** Hence, we suggested that global DNA methylation content might act as a clinic prognostic toll for active tuberculosis disease.

**Disclosure of Interest:** None Declared
P033 CRANIOSYNOSTOSIS IS CAUSED BY DEFECTIVE MATURATION OF THE INTERLEUKIN-11 RECEPTOR
C. Garbers1, M. Agthe1, J. Bruegge2, Y. Garbers2, M. Wandel3, B. Kespohl4, P. Arnold5, C. M. Flynn1, J. Lokau4, S. Aparicio-Sieg mund4, C. Bretscher4, S. Rose-John1, G. H. Waetzig1, T. Putoczki5, J. Groetzinger1
1Institute of Biochemistry, 2Institute of Psychology, 3Institute of Anatomy, Kiel University, 4CONARIS Research Institute AG, Kiel, Germany, 5The Walter and Eliza Hall Institute for Medical Research, Victoria, Australia

Introduction: The development of the mammalian skull is a complex biological process. Alterations of skull development result in a human phenotype called craniosynostosis, which is caused by premature closure of the sutures which connect the cranial bones. Recently, several craniosynostosis patients with missense mutations within the gene encoding the interleukin-11 receptor (IL-11R) have been described. As IL-11 is a cytokine that has fundamental functions in bone remodeling, we investigated the molecular mechanisms that caused craniosynostosis in these patients.

Methods: Using different biochemical and biological approaches, we characterized several IL-11R patient mutations and analyzed expression, maturation, cell-surface expression and biological activity of these IL-11R variants. Using molecular modeling and saturation mutagenesis, we identify structural traits within the IL-11R that are required for folding and maturation of the receptor. We further use different genetically modified mouse strains to analyze skull formation in vivo.

Results: We find that the analyzed patient mutations prevent maturation of the IL-11R, resulting in endoplasmic reticulum (ER) retention and diminished cell-surface appearance. We identify the disruption of a conserved tryptophan-arginine zipper within the third domain of the IL-11R as the underlying cause of the defective maturation. In vivo, we identify IL-11 classic signaling via the membrane-bound receptor as the crucial pathway for normal skull development in mice.

Conclusion: Our results show that craniosynostosis patient mutations impair maturation of the IL-11R, which results in reduced IL-11R cell-surface appearance and thus lack of IL-11 signaling.

Disclosure of Interest: None Declared

P034 MANIPULATING IL-10 IMMUNO-MODULATORY PROPERTIES BY FINE-TUNING ITS RECEPTOR BINDING PROPERTIES
C. Gorby1, P. Fyfe1, J. Martinez-Fabregas1, E. Pohler1, S. Wilmes1, I. Moraga-Gonzalez1
1Division of Cell Signalling and Immunology, School of Life Sciences, University of Dundee, Dundee, United Kingdom

Introduction: Interleukin-10 (IL-10) is a key immuno-regulatory cytokine with strong immunosuppressive effects. Because of its potency as an anti-inflammatory agent, it was hoped that this cytokine could be utilised therapeutically in the treatment of auto-immune diseases. However, several clinical trials performed with IL-10 have shown only mild benefits in sub-sets of patients, highlighting the complex biology of this cytokine. IL-10 elicits its bioactivities by engaging a surface receptor comprised of two IL-10Rα and two IL-10Rβ chains, leading to the activation of the JAK1/TYK2/STAT3 signalling pathway. While the structure of IL-10 and its separate receptor components have been reported, IL-10 in complex with the complete receptor set has yet to be crystallised due to its weak affinity for the Rβ subunit (1).

Methods: Here we have taken advantage of protein engineering methodologies to generate IL-10 mutants with increased affinity for the Rβ subunit. A monomeric form of IL-10 (2) was used as a template for error-prone PCR to introduce random mutations. Yeast surface display was then used to screen IL-10 mutants and to select for those with increased affinity for the Rβ subunit.

Results: This has yielded IL-10 mutants with apparent IL-10Rβ binding affinity in the nM range. Many of these mutations appear on the A and D helices of IL-10, the region previously predicted to be the IL-10Rβ binding site and closely correlate with amino acids modelled to be important in IL-10Rβ binding (3). These mutants were recombimantly expressed and used to examine the link between ligand-receptor complex stability and bioactivities in the IL-10 system. Preliminary results show that increased affinity for the IL-10Rβ subunit leads to a more potent activation of STAT1 and STAT3 in human T cells, even when the mutants are used in a monomeric conformation.

Conclusion: These newly engineered IL-10 mutants open new avenues to treat anti-inflammatory syndromes and provide tools to ultimately crystallise IL-10 in complex with the complete receptor set, giving a more comprehensive understanding of the mechanisms through which this cytokine exerts its effects.

Disclosure of Interest: None Declared

P035 REGULATION OF IL-17A EXPRESSION DURING TOXOPLASMA GONDII INFECTION IN RETINAL CELLS
F. Fahmi-Bittich1, V. Greigert2, A. W. Pfaff1, E. Candolfi1, J. Brunet1
1Institut De Parasitologie Et Pathologie Tropicale, Université De Strasbourg, Strasbourg, 2Hôpitaux Civils de Colmar, Colmar, France

Introduction: Toxoplasmosis is caused by an obligate intracellular protozoan, *Toxoplasma gondii*, which is capable of modulating various signaling pathways of the host cell in order to multiply and to evade the immune response. However, severe clinical forms like ocular toxoplasmosis are explained by an excessive inflammatory response. The inflammatory cytokine IL-17A has been identified as a marker of disease severity. South
American patients have more severe forms of ocular toxoplasmosis than European patients, due to the existence of more virulent *T. gondii* strains in South America. This difference in the virulence of the strains leads to markedly different cytokine profiles. However, the mechanisms of IL-17A regulation *in vitro* are still unknown.

**Methods:** The experiments were performed with cultures of trophoblastic cells: BeWo and retinal cells: microglia, Müller and astrocytes. We used different strains of *T. gondii*: RH ∆ KU80, RH ROP16 KO, PRU and LEF. We evaluated the activity of IL-17A promoter after *T. gondii* infection using Luciferase assay. In parallel, we assessed the expression of IL-17A protein in infected and uninfected cells using flow cytometry.

**Results:** We observed that the infection induced activation of IL-17A promoter. This activation seemed to depend on cell type and parasite strains. The virulent strain (RH) showed an activation pick at 6h post-infection, the avirulent strain (PRU) was responsible for late activation (24h post-infection) whereas the intermediate virulence strain (LEF) showed a constant activation from 3h post-infection. On the other hand, infection of BeWo cells with RH ROP16 KO resulted in the inactivation of IL-17A promoter. Finally, we showed that UHRF1 binds to the IL-17A gene promoter and modulates its activity during toxoplasma infection. We observed, using siRNA-UHRF1 that the expression of IL-17A increases when UHRF1 protein expression is neutralized.

**Conclusion:** By this work, we show that the regulation of the IL-17A promoter in infected cells varies according to cell type and parasite strain. This strain-dependent regulation is modulated by the polymorphic protein ROP16. Furthermore, the transcription factor UHRF1 is overexpressed in cells infected with *T. gondii* via the action of ROP16 and other parasite proteins, but the mechanisms of action are still unknown. In addition, our preliminary results show that the activation of the IL-17A promoter can be controlled by epigenetic modulation that may involve histone modifications, by acetylation or methylation. These epigenetic phenomena could be due to the recruitment of enzymes such as HDACs, DNMTs and G9a via UHRF1.

**Disclosure of Interest:** None Declared

---

**P036**

**STAT3 SUPPRESSES PRIMARY TUMOUR GROWTH, BUT INCREASES METASTASIS IN MYC-DRIVEN SMALL CELL LUNG CANCER**

A. C. Guanizo¹, J. Chen¹, S. Jayasekara¹, D. N. Watkins², D. J. Gough¹

¹Centre for Cancer Research, Hudson Institute of Medical Research, Clayton; ²Garvan Institute of Medical Research, Darlinghurst, Australia

**Introduction:** Signal transducer and activator of transcription 3 (STAT3) is a transcription factor critical for cellular growth, differentiation, immune function, metabolism and survival. Aberrant STAT3 activation has been observed in approximately 50% of human cancers, and has been linked to tumour progression and therapeutic resistance. One key target gene of STAT3 is *MYC* which is a potent transcription factor with oncogenic potential as observed in at least 40% of tumours. The STAT3-dependent induction of *MYC* expression in tumours is a key event in STAT3-driven tumourigenesis. It would therefore, be expected that STAT3 is redundant in tumours with *MYC* amplification in which *MYC* expression is not responsive to STAT3.

**Methods:** To determine the requirement for STAT3 in MYC-driven tumours, we generated a Myc-driven mouse model of small cell lung cancer (SCLC). In this model, *Myc* is expressed from the Rosa26 locus following delivery of adenoviral Cre into the airways via inhalation. These mice were crossed with Stat3 conditional mice resulting in simultaneous overexpression of *Myc* and loss of *Stat3*. *MYC* amplification is observed in up to 30% of SCLC patients, however this is always in combination with loss-of-function mutations in *pRb* and *Tp53*. Therefore, we also generated more clinically relevant mouse models in which *Myc* is overexpressed and *Tp53* and *pRb1* are deleted (RPM mice) with or without *Stat3* loss. We performed immunohistochemical analysis on the primary and metastatic tumours that formed in these mouse models.

**Results:** Our data show that Stat3 loss corresponds to a decrease in primary tumour burden in both the Myc-amplified and the RPM mice. However, we observed a significant increase in the liver metastasis in RPM mice following Stat3 deletion. This is accompanied by a significant increase in lipid deposition in the liver. Analysis of primary tumour cell lines derived from our mouse models shows that the deletion of Stat3 leads to increased expression of enzymes involved in lipogenesis. Finally, the deletion of Stat3 from metastatic human SCLC cell lines leads to accelerated growth and earlier tumour onset in xenografted animals.

**Conclusion:** Together, our data show that STAT3 is crucial for the onset of primary tumours in MYC-driven SCLC. However, STAT3 concomitantly increases lipogenesis and liver metastasis.

**Disclosure of Interest:** None Declared

---

**P037**

**MOLECULAR MECHANISM OF CYTOKINE RECEPTOR ACTIVATION AND DYSREGULATION BY ONCOGENIC MUTATIONS**

S. Wilmes¹,², M. Hafer³; I. Moraga¹,³,⁴, J. Vuorio⁵, J. Tucker⁶, S. Löchte⁷, S. Hubbard⁷, I. Vattulainen⁵, K. C. Garcia³,⁴, I. Hitchcock³, J. Piehler²

¹Division of Cell Signaling and Immunology, University of Dundee, Dundee, United Kingdom; ²Division of Biophysics, University of Osnabrück, Osnabrück, Germany; ³Howard Hughes Medical Institute; ⁴Department of Molecular and Cellular Physiology, and Department of Structural Biology, Stanford University School of Medicine, Stanford, United States; ⁵Department of Physics, University of Helsinki, Helsinki, Finland; ⁶Department of Biology, University of York, York, United Kingdom; ⁷Skirball Institute & Department of Biochemistry and...
Introduction: The mechanism of cytokine receptor activation and its dysregulation by mutations has remained controversially debated. Paradigmatic members of homodimeric class I cytokine receptor family such as growth hormone (GHR), erythropoietin (EpoR) and thrombopoietin (TpoR) are currently believed to be pre-dimerized and activated by ligand-induced conformational changes. However, the precise mechanism, which triggers Jak activation of pre-formed dimers, has remained speculative. We have tackled this fundamental question by quantitative single molecule imaging in combination with mutational studies and molecular dynamics simulation, yielding a consistent molecular mechanism of cytokine receptor activation by ligand-induced dimerization.

Methods: In order to resolve the spatiotemporal organization of homodimeric cytokine receptors at physiological expression levels, we have established dual-color single molecule imaging techniques. Using highly efficient posttranslational labeling techniques, monitoring diffusion and interaction of individual receptors in the plasma membrane of live cells with high spatial and temporal resolution. Receptor dimerization was reliably quantified by dual color co-tracking-analysis and complemented by single molecule FRET.

Results: Diffusion and interaction analysis at single molecule levels revealed that GHR, EpoR and TpoR are not pre-dimerized. Upon ligand stimulation, efficient receptor dimerization was detectable. Detailed quantification of receptor dimerization revealed stabilization of ligand-induced dimers by Jak2 that is mediated by its pseudokinase (PK) domain. Strikingly, the constitutively active Jak2-V617F mutation located within the PK domain stimulated ligand-independent receptor dimerization of GHR, EpoR and TpoR. Likewise, the constitutively active TpoR mutant W515L was pre-dimerized in absence of ligand. A comprehensive energy landscape obtained from these quantitative dimerization analyses revealed multiple, synergistic, weak interactions within receptor dimers that are readily triggered by the binding energy of the ligand to assemble receptor dimers. By mutational analysis, we have identified the interface of PK-PK interactions in the signaling complex. Molecular dynamics simulation of the entire transmembrane and cytosolic part of the signaling complex pinpoint a model of Jak activation by allosteric interactions between the PK domains.

Conclusion: Our quantitative measurements and molecular models reconcile previous data to yield the first conclusive molecular model of Jak activation by cytokine receptors and explain how individual disease-related mutations can constitutively activate signaling.

Disclosure of Interest: None Declared

P039
THE GESTATIONAL CYTOKINE LEUKEMIA INHIBITORY FACTOR (LIF) CAN DEACTIVATE GAMMA-INTERFERON (IFNG)-TREATED MACROPHAGES, PROMOTING TROPHOBLAST MIGRATION AND INVASION IN VITRO

J. Hamelin-Morissette1,*, C. Vaillancourt2, C. Reyes-Moreno1
1Medical biology, University of Québec at Trois-Rivières, Trois-Rivières, 2Institut Armand-Frappier, INRS, Laval, Canada

Introduction: The immune system of the uterine endometrium is exceptional in its ability to protect the mucosa from a variety of pathogens while being supportive to a developing semi-allogeneic embryo [1]. However, aberrant activation of inflammatory pathways in macrophages at the maternal-fetal interface can affect...
trophoblast survival and function and potentially induce pregnancy complications such as early embryo loss in humans [1]. In mice, the pleiotropic cytokine LIF is essential for embryo implantation [2]. Moreover, LIF seems to play an immune-regulating role in both mice and human [3]. However, the ability of LIF to modulate the activation of leukocytes remains unclear. This study aimed to elucidate the molecular mechanism by which LIF modulates macrophage (Mφ) activation by the pro-inflammatory cytokine gamma-Interferon (IFNγ). This cytokine is especially important for the remodeling of uterine vasculature, but prolonged production throughout pregnancy is associated with spontaneous abortion, intrauterine growth restriction and pre-eclampsia [4].

Methods: A coculture of peripheral blood monocyte-derived Mφs and immortalized trophoblastic cells was used to evaluate the effect of a treatment with LIF in the deactivation of IFNγ-treated Mφs. Briefly, Mφs were activated with IFNγ for 3h, and treated with LIF for 18h. Cells were then washed and cultivated for 48h before the addition of trophoblast cells to the upper compartment of a Matrigel-coated Boyden chamber. To elucidate the molecular mechanism by which LIF counteracts IFNγ effects in Mφs, Stat1 phosphorylation, cell motility and matrix metalloprotease-9 (MMP-9) gene expression was assessed.

Results: Our results indicate that LIF treatment reverses the inhibitory effect of IFNγ-activated Mφs on trophoblast cells invasion. Moreover, LIF increases Mφ cell motility and reactivates cell motility when Mφs were immobilized by IFNγ. The mechanism may be in part explained by the ability of LIF to induce the expression of MMP-9. The molecular mechanism inhibition of IFNγ-triggered response in Mφs includes a decrease in phosphorylation levels of Stat1 transcription factor after LIF treatment.

Conclusion: In this context, we propose LIF as an important modulator of the gestational immune response, thereby supporting the survival and differentiation of the placenta.

Disclosure of Interest: None Declared

P040
THE INFLUENCE OF IFNL4 SINGLE NUCLEOTIDE POLYMORPHISMS ON IFNL3 EXPRESSION
R. Hartmann1,*, M. Mohlenberg1, E. Terczynska-Dyla 1
1Molecular Biology and Genetics, University of Aarhus, Aarhus, Denmark

Introduction: Hepatitis C virus (HCV) is a chronic infection of the liver and despite recent significant improvement in the therapeutic options HCV is still a global health burden. The chronic infection leads to progressive hepatic fibrosis, cirrhosis and liver cancer. Both spontaneous- and treatment-induced clearance of HCV depend on host genetic background and in particular on a genetic variation rs368234815 within the interferon lambda 4 gene (IFNL4), which destroys the reading frame of IFNL4. IFNL4 belongs to the family of type III IFNs together with IFNA1, IFNA2 and IFNA3. Recombinant IFNA4 shows a clear antiviral action against both HCV and coronaviruses and induces a classical IFN signature in human primary cells. However, despite its clear antiviral nature, the ability to express IFNA4 appears to be a disadvantage during HCV infection. Since the discovery of the association between genetic variations within IFNL loci and the HCV clearance (as well as liver fibrosis and inflammation) it has been debated whether it is in fact IFNL4 or IFNL3 that are causative.

Methods: We are using CRISPR/Cas9 technology and reporter assays.

Results: The activity of the IFN-λ protein affects viral clearance and thus strongly suggest that this is the causative gene, but its involvement in liver fibrosis and inflammation is less clear and expression of IFNL4 mRNA in liver from HCV infected patients are low. Those observations fueled a second hypothesis, where the genetic variation found within the IFNL4 gene would affect transcription of the neighboring IFNL3 gene. Therefore, we sought to investigate if the SNPs located within the IFNL4 gene could influence the transcriptional activity of IFNL3 in a model system. We have cloned 6kb upstream of the IFNL3 translation start site, generated luciferase-based reporter constructs encoding variations of rs368234815, rs12979860 or rs117648444 SNPs and tested their transcriptional activity.

Conclusion: Our data demonstrate that none of the tested SNPs influences the transcriptional level of IFNL3 in our model system. Neither have we observed an effect of the common haplotypes formed by the aforementioned SNPs. By using a CRISPR/Cas9 technology, we created cell lines which only differs at these specific SNP and the effect of the SNPs on IFNL3 transcription is currently being investigated.

Disclosure of Interest: None Declared

P041
HISTONE H2A.Z SUPPRESSION OF INTERFERON-STIMULATED TRANSCRIPTION AND ANTIVIRAL IMMUNITY IS MODULATED BY GCN5 AND BRD2
C. M. Horvath1,*, N. Au-Yeung1
1Molecular Biosciences, Northwestern University, Evanston, United States

Introduction: Most IFN actions are mediated by transcriptional responses that drive the simultaneous expression of hundreds of IFN-stimulated gene (ISG) loci, producing a wide range of products that combine to create a cellular “antiviral state” that prevents virus entry, interferes with cellular and viral RNA transcription, stability, and translation, and thwarts virus replication. Type I interferon (IFN)-stimulated gene (ISG) expression requires interaction between transcription factor complex, ISGF3, and target gene promoters to initiate transcription and protection against infection.

Methods: Little is known about how chromatin structure and nucleosome dynamics influence ISGF3 promoter engagement, transcriptional activity and innate immunity. To uncover chromatin regulatory features of this antiviral immune response, IFN-induced nucleosome
and histone dynamics of human ISG loci were examined. To investigate ISGF3-mediated interactions with native chromatin, the chromatin architecture and nucleosome organization of ISGs was characterized using chromatin immunoprecipitation sequencing (ChiP-Seq) and targeted high-resolution nucleosome position analysis.

**Results:** ISGF3 recruitment after IFN stimulation was accompanied by nucleosome reorganization at promoters and gene bodies. IFN stimulation induced loss of core histones H2B, H3, and H4, as well as H2A.Z at ISG promoters. A strong correlation was found between H2A.Z occupancy and ISGF3 target sites, and IFN-stimulated H2A.Z removal requires STAT1, STAT2, and IRF9. Neither INO80 nor SWI/SNF participate in IFN-driven H2A.Z eviction, but GCN5 and BRD2 are required. Interference with H2A.Z expression enhanced ISGF3 recruitment to ISG promoters, ISG mRNA expression, and IFN-stimulated antiviral immunity. These results reveal dynamic nucleosome remodeling associated with IFN stimulated transcription, and indicate a negative regulatory role for H2A.Z nucleosomes in innate antiviral immune signal transduction.

**Conclusion:** These findings indicate H2A.Z-nucleosomes at ISG promoters restrict optimal ISGF3 engagement, and modulate the biological response to IFN.

**Disclosure of Interest:** None Declared

---

**P042**

**ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE REGULATES MITOCHONDRIAL METABOLIC CHANGES CRITICAL TO THE PRODUCTION OF TYPE I INTERFERON BY PRIMARY HUMAN PLASMACYTOID DENDRITIC CELLS**

H. J. Hurley1,2*, P. Fitzgerald-Bocarsly1,2  
1Pathology, Rutgers New Jersey Medical School, 2School of Graduate Studies, Rutgers University, Newark, United States

**Introduction:** Plasmacytoid dendritic cells (pDC) play a crucial role in innate viral immunity as the most potent producers of type I interferons (IFN-α) in the human body. However, the metabolic regulation of IFN-α production in such vast quantity remains poorly understood. Adenosine monophosphate-activated protein kinase (AMPK) is a master regulator of catabolic metabolism [1]. It is this process that fuels mitochondrial oxidation, a process that we have observed is necessary for production of IFN-α by human primary pDC.

**Methods:** To investigate the role of metabolic pathways in IFN-α production of pDC, we employed flow cytometric methods and extracellular flux analysis (EFA) to measure changes in metabolic activity 6 h post TLR7 or -9 ligation with influenza virus (Flu) and herpes simplex virus (HSV). We implemented a panel of inhibitors targeting mitochondrial metabolism to determine its necessity in IFN-α production: oligomycin (inhibits ATP synthase), FCCP (a proton ionophore), rotenone (inhibits complex I), or antimycin A (inhibits complex III). To the same end, we used dorsomorphin, a highly specific inhibitor of AMPK [2].

**Results:** Basal oxygen consumption rate (OCR) and ATP synthase-related OCR of purified pDC both significantly increased following Flu or HSV stimulation, indicating an increase in the rate of oxidative phosphorylation (OXPHOS) in the mitochondrion. Likewise, mitochondrial membrane potential (MMP) significantly increased under the same conditions. Inhibiting these metabolic changes through a variety of molecular mechanisms all independently abrogated IFN-α production by pDC as observed by intracellular flow cytometry and ELISA. Dorsomorphin inhibited IFN-α production and the associated increase in MMP in pDC as observed by intracellular flow cytometry with MitoTracker Orange staining. Cell viability was unaffected by all treatments, suggesting a necessity of mitochondrial oxidation in the production of IFN-α, and a further necessity of AMPK in the associated upregulation of OXPHOS.

We did not observe significant inhibition of IFN-α production in pDC after treatment with GSK 2837808A, a lactate dehydrogenase inhibitor, which indicates that aerobic glycolysis is not necessary for IFN-α production by pDC. We also did not see an increase in glycolysis-associated proton efflux by EFA in Flu or HSV-stimulated pDC at 6 h.

**Conclusion:** We have demonstrated that mitochondrial oxidation is necessary for the innate antiviral response of pDC, the inference being that the increase in ATP production provides energy for the translation of large amounts of IFN-α. It follows that this change in metabolism is regulated by AMPK, possibly initiated by an increase in AMP concentration during cell stress.

**Disclosure of Interest:** None Declared

---

**P043**

**IL-27 TRIGGERS AUTOPHAGY IN MACROPHAGES VIA A MTOR AND LC3-INDEPENDENT MANNER**

S. Laverdure1, Z. Wang2, K. Nagashima2, H. C. Lane3, T. Imamichi1,  
1LIHRi, 2EML, LEIDOS BIOMEDICAL/FNLCR, Frederick, 3LI/NIAID, NIH, Bethesda, United States

**Introduction:** IL-27 is a member of the IL-12 cytokine family composed of IL-12, IL-23, IL-35, and IL-39. It is mainly produced by antigen presenting cells such as macrophages and dendritic cells in response to immune activation, including following infection by viruses or bacteria (1). We have previously shown that IL-27 was produced by primary human macrophages following treatment by a potent cervical cancer vaccine, HPV (human papillomavirus)-like particles (2) and that recombinant IL-27-treated cells (both macrophages, dendritic cells and CD4+ T-lymphocytes) were becoming resistant to HIV-1 infection (2-4). Specifically, this HIV-1 restriction was tied to Spectrin Beta Non-Erythrocytic 1 (SPTBN1) inhibition during monocyte-to-macrophage differentiation through a Tak1-dependent pathway (3). In
this study, we assessed impact of IL-27 on autophagy induction in macrophages.  

**Methods:** Freshly isolated CD14+ monocytes from healthy donors were differentiated into macrophages for 7 days using either 10% human AB serum, generating AB-MAC, or a combination of AB serum and IL-27 (100ng/mL, R&D systems), thereby creating ABI-MAC. Autophagosome staining was performed using the Cyto-ID® reagent (Enzo Life Sciences) and detected by a Zeiss AxioObserver motorized microscope at a 10x magnification. Image analysis was performed using the Fiji software. The mTOR and LC3 activation were detected by western blot.

**Results:** IL-27 triggers autophagy induction in human AB serum-induced macrophages but not in M-CSF-induced macrophages, while other IL-12 cytokine family members did not have such effect on these cells. Staining of autophagosome indicated that IL-27 was able to induce autophagy both in terminally differentiated macrophages, causing a 2 to 5-fold increase in autophagosome accumulation, as well as during monocyte-to-macrophage differentiation, where IL-27 effect was even stronger (generating a 20-fold increase in autophagosome formation). Autophagosome formation was confirmed by electron microscopy: IL-27-treatment induced double membrane vesicles in cytoplasm. Furthermore, we also determined that two key autophagy proteins, mTOR and LC3, were not involved in this phenomenon: while we were able to assess autophagosome accumulation in IL-27-treated cells via electron microscopy and immunofluorescence, this was not linked to either mTOR dephosphorylation nor LC3 lipidation, implying that IL-27 induces autophagy through a novel non-canonical pathway. Finally, we established that blocking IL-27 signaling during macrophage differentiation using both Ruxolitinib or Tofacitinib, respectively JAK1/2 and JAK3 inhibitors, successfully inhibited autophagy triggering together with STAT3 phosphorylation.

**Conclusion:** In this study, we demonstrated for the first time that IL-27 triggers autophagy induction in human AB serum-induced macrophages, but not in M-CSF-induced macrophages. This induction was not linked to either mTOR dephosphorylation nor LC3 lipidation, implying that IL-27 induces autophagy through a novel non-canonical pathway. (Funded by NCI Contract No. HHSN261200800001E).

**Disclosure of Interest:** S. Laverdure Shareholder of: None, Grant / Research support from: None, Consultant for: None, Employee of: None, Paid Instructor for: None, Speakers Bureau of: None, Z. Wang Shareholder of: None, Grant / Research support from: None, Consultant for: None, Employee of: None, Paid Instructor for: None, Speakers Bureau of: None, H. C. Lane Shareholder of: None, Grant / Research support from: None, Consultant for: None, Employee of: None, Paid Instructor for: None, Speakers Bureau of: None, T. Imamichi Shareholder of: None, Grant / Research support from: None, Consultant for: None, Employee of: None, Paid Instructor for: None, Speakers Bureau of: None

**P044 REGULATION OF ENDOGENOUS INTERLEUKIN-36 ACTIVITY**  
A. Jaafar.  
1Infection, Immunity and cardiovascular disease, University Of Sheffield, Sheffield, United Kingdom

**Introduction:** Interleukin-36 cytokines belong to the IL-1 family. IL-36 cytokines comprise three agonists, IL-36α, IL-36β, IL-36γ, and antagonist IL-36Ra. IL-36 cytokines use receptor IL-36R (IL-1RL2) and the IL-1/IL-33 co-receptor IL-1RacP. We intend to identify processes that lie between the activation of the IL-36 genes and activation of the cytokines through post translational proteolysis.

**Methods: Methodology:** untransformed Keratinocyte cell line (HaCaT), and epidermoid carcinoma cell line (A431) were used. RNA was extracted from HaCaT cells after being treated with 5nM IL-1α, 5nM IL-36α, 10ng/ml TNF and low serum media as a control while A431 cells were treated with 67ng/ml phorbol ester (PMA), 20ng/ml TNF and 67ng/ml PMA, or 20ng/ml TNF. The period of stimulation of A431 and HaCaT cells was 6 to 7 hrs, and triplicates were used for each activator. Cells were starved with low serum medium before being stimulated. The expression of IL36a, IL-36b, IL-36g, IL-1Rl2 and IL-36Ra mRNA in HaCaT cells and A431 cells was examined by RT-PCR. IL-36g protein in the A431 was detected by western blotting. The level of mRNA expression was assessed by RT-qPCR. As a surrogate of activation NFkB signalling, ELISA assays were used to detect secreted IL-8 in cells supernatants.

**Results:** We used rhIL-1α, rhN6-IL-36a and rhTNF at close to saturating concentrations to activate the IL-36 genes. IL-36 mRNAs were detected in both HaCaT and A431. Analysis by RT-PCR showed that IL-36b, IL-36g, IL-1RL2 mRNAs were expressed in the KC line HaCaT in monolayer, while IL-36α was barely detectable. QPCR was used to assess induction in the A431 cells, IL-36α and IL-36b were slightly induced, but IL-36g expression was increased. Western blotting showed that IL-36g protein was upregulated in A431 in response to combination of PMA and TNF or flagelline and TNF. In HaCaT, the inducer cytokines were effective in the order TNF>IL-1>IL-36. In A431 combination of PMA and TNF was more effective as an inducer than PMA or TNF alone. HaCaT also responded to inducers of IL-8/CXCL8 secretion in the order TNF>IL-1>IL-36.

**Conclusion:** Conclusion: NFκB activating stimuli induce expression of IL-36g at the mRNA level in HaCaT to an extent that reflects the extent to which the same activators stimulate IL-8 release from cells. It seems likely that this reflects the abundance or activity of receptors of TNF, L-1 and IL-36 on the HaCaT cells. Unprocessed IL-36g protein would be detected inn western blotting in TNF stimulated.
HaCaT and more abundantly in A431. A431 responded cooperatively to t a combination of PMA and TNF in activating expression of IL-36g protein. PMA (67ng/ml) proved a potent activator of IL-36g, IL-36b and IL-36g expression whereas IL-36a and IL-36b were not activated by 20ng/ml TNF alone, levels that cause strong IL-8 release. Both IL-36b and IL-36g mRNAs were co-operatively induced by a combination of 67ng/ml PMA and 20ng/ml TNF

Disclosure of Interest: None Declared

P045

METFORMIN ENHANCES ANTI-INFLAMMATION EFFECTS OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS BY ACTIVATING AMPK/MTOR SIGNALING: THERAPEUTIC POTENTIAL TO MRL/LPR LUPUS MODEL

S. Jang¹, J. Lee¹, S.-M. Hong¹, S. Kwok¹, S.-H. Park¹ ² ¹Rheumatism Research Center, Catholic Research Institute of Medical Science, ²Division of Rheumatology, Department of Internal Medicine, School of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

Introduction: Metformin is originally introduced as a biguanide antibiotic medication, has an anti-inflammatory effect via activating AMP-activated protein kinase (AMPK). Human adipose-derived mesenchymal stem cells (Ad-MSC) retards their immunosuppressive capacity in various inflammatory conditions. The lupus mouse model MRL/MpJ (MRL/lpr) recapitulates a few clinical manifestations and immune dysregulation observed in human SLE.

Methods: To examine metformin-treated Ad-MSC showed more potent inhibition of SLE development through the modulation of AMPK / mTOR signaling in MRL/lpr mice. We investigated the expression of IL-10, indoleamine 2,3-dioxygenase (IDO1) and TGFβ and the alteration of mTOR signaling were regulated by metformin pretreatment. MRL/lpr mice received lateral tail vein injections of 1 × 10⁶ metformin-treated Ad-MSCs or vehicle (PBS).

Results: Metformin-treated Ad-MSC increased mRNA synthesis of IL10, IDO1, and TGFβ compared with those of intrinsic Ad-MSC. The expression of p-AMPK, p-mTOR, p-Raptor and p-STAT3 was inhibited by metformin. Intravenously injected metformin-treated Ad-MSC reduced the splenomegaly, lymphadenopathy and they showed decreased the level of anti-dsDNA antibodies and pro-inflammatory cytokines in serum of MRL/lpr mice, while the administration of Ad-MSC showed insignificant effect. Altered level of mTOR in spleen and kidney and prevented renal pathology was observed of MRL/lpr mice injected with metformin-treated Ad-MSC.

Conclusion: Metformin can optimize the immunomodulatory potential of Ad-MSC, suggesting a promising strategy of MSC in SLE treatment.

Disclosure of Interest: None Declared

P046

THE OPPOSING EFFECTS OF IFN-GAMMA VS. IFN-ALPHA ON IL-12 AND TNF SECRETION BY HUMAN PRIMARY MONOCYTES

A. Muglia Thomaz da Silva Amancio¹, L. Mittereder¹, L. Carletti¹, A. Sher¹, D. Jankovic¹ ² ¹LPD, NIAID, NIH, Bethesda, United States

Introduction: When primary human monocytes are stimulated with live Toxoplasma gondii tachyzoites, a minor population of patrolling CD16⁺CD14⁺ but not CD16⁻CD14⁺ classical monocytes secrete the proinflammatory cytokines TNF and IL-12. In the present study we have examined the effects of conditioning human monocytes with either IFN-γ or IFN-α as well as different temporal combinations of the two IFN species on the cytokine response triggered by T. gondii.

Methods: Peripheral blood monocytes were obtained from healthy volunteers by counterflow centrifugal elutriation at the NIH Blood Bank under Institutional Review Board-approved protocols of both the NIAID and the Department of Transfusion Medicine. Monocyte subsets were purified using Milenyi CD16- and CD14-columns. Unfractionated or purified monocyte populations were primed with IFN-γ (10 ng/ml), IFN-α (10 ng/ml) or both before exposure to T. gondii tachyzoites. In addition, the effect of prior incubation with one IFN and followed by addition of the other was also analyzed. Tachyzoites, of the RH-B type I strain, were propagated in a human foreskin fibroblast cell line. Before assay, the parasites were washed, filtered through a polycarbonate 3-μm membrane and counted. The expression of cytokine mRNA and protein was assayed by RT-PCR and ELISA, respectively.

Results: We show that priming with IFN-γ increases the secretion of proinflammatory cytokines by human monocytes in the response to T. gondii. Surprisingly, this augmented responsiveness was entirely due to the stimulatory effect of IFN-γ on the CD16⁻CD14⁺ non-responder population. Despite comparable levels of IFNγR expression between the two subsets, IFN-γ failed to increase the cytokine response of CD16⁺ monocytes. In direct contrast to IFN-γ, priming with IFN-α failed to promote the responses of CD16⁺ monocytes and, moreover, inhibited the response of the CD16⁻ subset as well as IFN-γ-primed CD16⁺ monocytes. Furthermore, when human monocytes were first exposed to IFN-α they become refractory to the stimulatory effects of IFN-γ. The latter inhibitory effect of IFN-α was independent of IL-10 induction and was IFN-γ specific, since subsequent stimulation with Rapamycin was able to overcome the inhibitory effect of type I IFN priming.

Conclusion: Taken together our results demonstrate that the IFN-γ-priming selectively targets the CD16⁺ monocyte subset and reveal the antagonistic effects of IFN-γ and IFN-α on proinflammatory cytokine secretion by human primary monocytes in response to T. gondii tachyzoites. These findings strongly suggest that in clinical conditions characterized by increased steady state levels of type I IFN the ability of human monocytes to respond to microbial challenge may be “sabotaged”.

Disclosure of Interest: None Declared
This work was supported by the Intramural Research Program of the NIAID, NIH.

Disclosure of Interest: None Declared

P047
CROHN’S DISEASE-ASSOCIATED EPIGENETIC READER SP140 ORCHESTRATES IMMUNE CELL IDENTITY THROUGH REPRESSION OF CHROMATIN ACCESSIBILITY
K. Jeffrey, H. Amatullah, S. Digumarthi

1Medicine, MASSACHUSETTS GENERAL HOSPITAL, HARVARD MEDICAL SCHOOL, Boston, United States

Introduction: Dysregulated epigenetic enzymes are sentinel events in cancer, making proteins that “write”, “erase” and “read” the epigenome some of the most intently pursued targets in drug discovery today. Despite this, virtually nothing is known about how altered epigenetic enzymes contribute to immunological disorders. Speckled Protein 140 (SP140) is an epigenetic “reader” enzyme restricted to the immune system. Single nucleotide polymorphisms (SNPs) within SP140 associate with 3 immunological diseases: Crohn’s Disease (CD), Multiple Sclerosis and Chronic Lymphocytic Leukemia. We found that SP140 mutations result in a loss of this epigenetic enzyme in CD patients (1). We also identified SP140 as an orchestrator of macrophage identity and function through occupancy and repression of lineage-inappropriate genes such as HOX genes bearing repressive H3K27me3 (1). Loss of SP140 in CD patients stratified them by loss of macrophage identity genes and suppressed innate immune signatures in Toll-like receptor (TLR)4 or TLR3 stimulated peripheral blood mononuclear cells (PBMCs)(1). Depletion of Sp140 in the hematopoietic system of mice exacerbated DSS-colitis (1) establishing that this epigenetic enzyme is critical to intestinal health. The specific mechanism by which SP140 modulates chromatin and transcription to maintain identity of immune cells is unknown.

Methods: We determined the protein interactors of SP140 using Mass Spectrometry. Co-immunoprecipitation validated the top SP140 interacting hits in HEK293, THP1 monocytes and EBV-B cells from patients with and without SP140 SNPs. We examined Topoisomerase activity, double stranded breaks (marked by gamma-H2AX) and chromatin accessibility using Assays for Transposase-Accessible Chromatin using sequencing (ATACseq) in human THP-1 and mouse immortalized macrophages with a depletion of SP140 and in EBV-B cells from patients with and without SP140 SNPs.

Results: The top SP140 interacting proteins are involved in DNA unwinding (Topoisomerase I (TOP1), II alpha (TOP2A) and II beta (TOP2B); DNA-PK and SUPT16H (FACT complex 160 subunit) which were all confirmed by co-IP experiments. Deletion of SP140 resulted in increased activity of Topoisomerases and overexpression of SP140 repressed Topoisomerase activity. Moreover, patient cells bearing SP140 SNPs lost the interaction of SP140 with Topoisomerases and displayed increased Topoisomerase activity, increased gamma-H2AX and increased chromatin accessibility.

Conclusion: SP140 is an immune-restricted epigenetic reader protein that exists in protein complexes containing topoisomerases and other proteins involved in chromatin remodeling. SP140 suppresses these DNA unwinding mechanisms at loci of lineage inappropriate genes to maintain cell identity. Furthermore, this function of SP140 is lost in patients harboring SP140 SNPs. Thus, the epigenetic reader SP140 is critical for maintenance of immune cell identity through repression of chromatin accessibility to prevent expression of unwanted genes, such as lineage-inappropriate genes. Loss of SP140 due to genetic variation compromises chromatin dynamics and immune cell identity and contributes to immunological disease.

Disclosure of Interest: None Declared

P048
CD28 DIMER INTERFACE CONTROLS B7/CD28 COSTIMULATORY RECEPTOR ENGAGEMENT AND INFLAMMATORY CYTOKINE STORM ELICITED BY INFLUENZA A VIRUSES

1Biochemistry and Molecular Biology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel, 2Institute for Antiviral Research, Utah State University, Logan, Utah, United States

Introduction: The inflammatory cytokine response is indispensable for protective immunity, yet bacterial and viral infections often elicit a vastly excessive response harmful to the host. Lethality of influenza virus is associated with an inflammatory cytokine storm. Increasingly, pathogen resistance has characterized antiviral agents, such as neuraminidase inhibitors. Host-oriented therapeutics aimed at controlling and preventing the cytokine storm carry the advantage of being both broad-acting and less likely to be circumvented by virus mutation.


Results: Here, we focused on attenuating the host inflammatory response to infection. Full T-cell activation requires interaction between costimulatory receptors B7 and CD28. We show that an octapeptide mimetic of the human CD28 homodimer interface protects mice from lethal pandemic H1N1 and avian H5N1 influenza A virus infection in synergy with an antiviral drug dosed sub-therapeutically, while dampening excessive induction of inflammatory cytokines in the lungs, restoring arterial oxygen saturation and promoting recovery from severe weight loss induced by virus infection. Derived from the
self-adhesive CD28 dimer interface, the peptide binds CD28 directly. Within the extracellular domain of CD28, a β-barrel, the homodimer interface is positioned at the opposite pole from the remotely located binding site for the B7 co-ligands. Yet, our finding is that the CD28 mimic peptide inhibits formation of both B7-2(CD86)/CD28 and B7-1(CD80)/CD28 intercellular synapses and attenuates excessive signaling through CD28 for inflammatory cytokine induction, while leaving a basal response intact. **Conclusion:** These results demonstrate the protective potential against severe infection of attenuating B7/CD28 engagement and pro-inflammatory signaling through the CD28 homodimer interface. This highlights a novel regulatory role for the CD28 dimer interface in inflammatory signaling and renders it a therapeutic target against systemic inflammatory responses. Indeed, the CD28 dimer interface mimetic octapeptide is now advancing in a US Phase 3 clinical trial against necrotizing soft-tissue infections (‘flesh-eating bacteria’), for which no drug exists currently.

**Disclosure of Interest:** R. Kaempfer Grant / Research support from: NIAID grants UC1AI067231 and 2U54AI057168, Z. Rotfogel: None Declared, A. Popugailo: None Declared, D. Hillman: None Declared, R. Levy: None Declared, G. Arad: None Declared, T. Shpilka: None Declared, D. Barnard Grant / Research support from: NIAID DMID Virology Branch contract N01-AI-15435.

**P048.A**

**GLOBAL LANDSCAPE OF MOUSE AND HUMAN CYTOKINE TRANSCRIPTIONAL REGULATION**

S. Carrasco Pro1, A. Dafonte Imedio2, C. Santoso2, K. A. Gar1, J. Sewell1, M. Martinez2, R. Sereda2, S. Mehta2, J. Fuxman Bass1,2,*

1Bioinformatics, 2Biology, Boston University, Boston, United States

**Introduction:** Cytokines genes are highly regulated at the transcriptional level by combinations of transcription factors (TFs) that recruit cofactors and the transcriptional machinery. Although great progress has been made in the last decades in the study of cytokine transcriptional regulation, the known interactions between TFs and cytokine regulatory regions are scattered across the literature, limiting the ability to browse for these interactions and precluding systems level analyses. **Methods:** Here, we mined through three decades of studies to generate a comprehensive database reporting 843 and 647 interactions between TFs and cytokine genes, in human and mouse respectively. We mined the XML files from ~26 million articles available in Medline for studies mentioning a cytokine, a TF, and an experimental assay. The resulting 6,878 articles, together with 815 articles annotated in databases such as TRRUST and InnateDB, were manually curated to determine whether experimental evidence for the interactions was provided. The resulting database contains 1,552 interactions: 843 in human, 647 in mouse, and 62 from other species. To visualize this complex cytokine gene regulatory network (GRN) we developed CytReg (https://cytreg.bu.edu), a web tool where interactions can be browsed by species, TFs, cytokines, assay types, and TF expression patterns across different cell types. **Results:** By integrating CytReg with other functional datasets, we determined general principles governing the transcriptional regulation of cytokine genes. We show a correlation between TF connectivity in the cytokine GRN and immune phenotypes and diseases. We observe that the balance between pathogen/stress-activated TFs and tissue-specific TFs is shifted towards the former for interferons and pro-inflammatory cytokines and we provide a model for cooperative and plastic recruitment of cofactors to cytokine promoters. Using this cytokine GRN, we also provide a blueprint for further studies of cytokine misregulation in disease and identify novel TF-disease associations. Finally, we discuss biases and the completeness of the literature-derived cytokine GRN, and provide predictions for novel interactions which we validate using enhanced yeast one-hybrid assays and reporter assays in human cells. **Conclusion:** Overall, this comprehensive resource provides a framework for the rational design of future cytokine gene regulation studies. **Disclosure of Interest:** None Declared

**P048.B**

**ANCHOMANES DIFFORMIS; A POTENTIAL THERAPEUTIC AGENT TO INCREASED INFLAMMATION IN TYPE II DIABETES AND COMPLICATIONS**

T. D. Alabi1,2,*, N. L. Brooks2, O. O. Oguntibeju1 on behalf of Phytomedicine & Phytochemistry Group

1Biomedical Sciences, 2Wellness Sciences, Cape Peninsula University of Technology, Capetown, South Africa

**Introduction:** Persistent hyperglycemia has been known to cause enhanced generation of reactive oxygen species in diabetes. Several inflammatory cytokines are induced by oxidative stress and their release further increases oxidative stress, making them important factors in the development and progression of diabetes mellitus and its complications. Thus, interaction between oxidative stress and inflammatory mediators has been implicated in the development of diabetic complications such as nephropathy and cardiomyopathy. The present study investigated the modulatory potential of Anchomanes difformis on cytokines in the heart and serum in type 2 diabetes using animal model. **Methods:** Two weeks of fructose (10%) administration, followed by single intraperitoneal injection of streptozotocin (40mg/kg) was used to induce type 2 diabetes in male Wistar rats. Leaves extract (aqueous) of Anchomanes difformis (200 and 400mg/kg) was administered orally to the rats for six weeks. Blood glucose concentrations before and after interventions were determined. Interleukin (IL)-6, IL-10, IL-18, MCP-1 and TNFα were measured in the heart, and IL-18, MCP-1 in the
serum. Lipid peroxidation was evaluated by determining TBARS concentrations in the serum. Expression of NFkB/p65 was evaluated in the heart.

**Results:** *Anchomanes difformis* administration significantly (p<0.05) lowered the production of IL-18 and TNFα, and increased IL-10 and IL-6 significantly (p<0.05) concentration in the heart of diabetic rats. Concentrations of MCP-1 reduced in the heart of diabetic-treated rats, when compared to non-treated though not significant (p<0.005), also no significant difference was observed in the serum. However, serum levels of IL-18 was reduced in diabetic treated rats as comparable to normal rats. Lipid peroxidation decreased significantly (p<0.05) in the serum of diabetic rats treated with the *Anchomanes difformis* when compared with diabetic non-treated. Increased expression of NFkB/p65 was observed in the diabetic rats, but treatment with *Anchomanes difformis* significantly reduced its expression.

**Conclusion:** *Anchomanes difformis* is a new, promising therapeutic agent that can be explored in the treatment of pathological conditions associated with immune responses and oxidative stress as evidenced by its anti-inflammatory and anti-oxidative abilities.

**Disclosure of Interest:** None Declared

---

**P048.C**

**COMPREHENSIVE MAPPING OF THE HUMAN CYTOKINE GENE REGULATORY NETWORK**


1Biology, 2Bioinformatics, Boston University, 3Infectious Diseases, Boston University School of Medicine, Boston, United States

**Introduction:** The immune system has evolved a complex cytokine gene regulatory network (GRN) that orchestrates a multitude of cellular responses during development, homeostasis, and mounting immune responses. Transcriptional dysregulation of cytokine genes has been linked to diseases including autoimmunity, immunodeficiency, susceptibility to infections, and cancer. Thus, delineating the cytokine GRN presents an opportunity to understand how immune responses are mounted, which may ultimately lead to the development of new therapeutic targets to modulate cytokine expression in diseases.

**Methods:** To identify the transcription factors (TFs) that bind to the promoters of cytokine genes, we used the enhanced yeast one-hybrid (eY1H) platform – a high-throughput method that can map the binding of 1,086 (out of ~1,500) human TFs in a single experiment. Further, we evaluated the interactions identified by luciferase reporter assays in HEK293T cells. Finally, we investigated the regulatory effects of several TFs in the network by siRNA TF-knockdowns and RT-qPCR of cytokine mRNA levels in primary human macrophages.

**Results:** Using the eY1H platform, we delineated a comprehensive human cytokine GRN comprised of 1,087 interactions between 242 TFs and 81 cytokine promoters.

In comparison, the current literature-derived cytokine GRN comprises 843 interactions. To determine the quality of the network generated, we compared the eY1H interactions to interactions reported by chromatin immunoprecipitation and to interactions predicted by motif analyses, and found a significant overlap. Additionally, we found that TFs that bind to cytokine promoters are generally enriched in immune tissues and have themselves been associated with immune disorders in mouse and humans. More importantly, we observed a 70% validation rate when we tested 220 of the eY1H interactions by luciferase reporter assays in HEK293T cells, confirming the quality of the cytokine GRN we generated. Finally, we knocked down several TFs in primary human macrophages and found novel cytokine targets, including for TFs which are currently absent in the literature-derived cytokine GRN.

**Conclusion:** Altogether, this work greatly expands our current knowledge of human cytokine regulation and provides a blueprint to modulate cytokine expression in disease.

**Disclosure of Interest:** None Declared

---

**P048.D**

**DISCOVERY AND SCREENING OF PROTEIN BIOMARKERS WITH THE FIREPLEX® TECHNOLOGY PLATFORM**


1Abcam Inc., Cambridge, United States

**Introduction:** In patients and animal models, molecular biomarkers are used as indicators of normal and pathogenic processes. In drug discovery and screening pipelines, molecular biomarkers are used to assess the mechanism of action, efficacy, and toxicity of lead compounds. To address the need for rapid and sensitive quantitation of protein biomarkers, we have developed the FirePlex Technology Platform. Utilizing patented hydrogel particles and a three-region encoding design, FirePlex Immunoassays allow for true, in-well multiplexing, providing flexible and customizable quantification of analytes.

**Methods:** To facilitate biomarker discovery studies, we offer our standard-throughput FirePlex Immunoassays, which enable quantitation of up to 75 protein analytes per sample, from only 12.5 μl of input. These assays demonstrate 5 logs of dynamic range and sub-picogram/ml sensitivity, allowing for highly sensitive quantitation of analytes in serum, plasma, cell culture supernatant, urine, and saliva. Assays are run in 96well plate format, with readout on standard flow cytometers.

**Results:** For drug discovery and screening studies, we offer our high-throughput FirePlex Immunoassays (FirePlex-HT) for quantitation of up to 10 protein analytes per sample from only 6.25 μl biofluid input, in 384-well plate format. FirePlex-HT assays provide 3-4 logs dynamic...
range, demonstrate 1-100 pg/ml sensitivity, and have been validated in serum, plasma, and cell culture supernatant. The two-step workflow, no-wash assay format, and readout on high-content imagers limit hands-on time and are amenable to automation, thus making FirePlex-HT ideally suited for high-throughput screening studies.

**Conclusion:** Here we present data from studies investigating cytokine profiling in human and rodent samples using the FirePlex immunoassays, and introduce the simplified workflow of the FirePlex-HT immunoassays with data demonstrating the performance for quantifying key cytokines in multiplex, in biological samples. Together, this novel combination of multiplexed, high-sensitivity assays and bioinformatics tools enables rapid quantitation of protein biomarker signatures in biofluid specimens.

**Disclosure of Interest:** None Declared

**P049**

**INTERFERON SIGNALING CONZFS INTRINSIC RESISTANCE TO ANDROGEN DEPRIVATION THERAPY IN METASTATIC PROSTATE CANCER**

S. Agarwal1*, K. McGowen1, F. Elloumi1, M. Cam1, M. Beshiri1, E. Corey2, K. Kelly1

1National Cancer Institute, bethesda, 2university of washington, Seattle, United States

**Introduction:** Prostate cancer (PCa) is the second leading cause of male cancer-related death. Androgen receptor (AR) signaling drives PCa growth, and androgen deprivation therapy (ADT) has been the standard treatment. Most patients relapse within 2-3 years of ADT with a metastatic castration-resistant prostate cancer (CRPC). CRPC has a luminal cell phenotype, and a frequent class (~35%) is composed of dual TP53 and PTEN loss.

**Methods:** Using the Pten/Tp53-null mouse PCA model and organoid cultures, which allow growth of luminal stem cells (LSCs), we previously have shown that LSC organoids are intrinsically resistant to ADT. To identify resistance mechanisms in LSCs, we performed RNAseq on LSC organoids from wild-type(WT) prostate and Pten/Tp53-null tumor (pre- and post- ADT).

**Results:** Interestingly, we found no difference in gene expression between LSC organoids from pre- and post-ADT tumors, suggesting that Pten/Tp53 null LSCs are intrinsically resistant. Several pathways were upregulated in tumor LSC organoids including AR signaling, lipid metabolism and inflammation. Of note, we observed the most significant enrichment of Interferon(IFN) signaling in tumor LSC organoids relative to WT organoids.

IFN signaling is mainly anti-proliferative, however, expression of a subset of IFN genes protects stem cells and contributes to intrinsic drug resistance. Since, STATs (STAT1-5) regulate IFN genes we analyzed their expression in LSC organoids and prostate tissue by real-time PCR, western blot, and immunofluorescence, and identified STAT1 as a master regulator. shRNA knockdown of STAT1 decreased the self-renewal ability of tumor LSC organoids thus indicating a pro-survival role of STAT1.

Additionally, 13/45 patient-derived xenografts (PDX) of CRPC, express high IFN signaling and STAT1 depletion in 5 tested PDXs significantly reduced self-renewing ability of PDX derived organoids. Interestingly, STAT1 depletion synergized with ADT to inhibit the growth of PDX organoids ex vivo. Thus, STAT1-regulated IFN signaling may contribute to treatment resistance.

We further analyzed human CRPC datasets to determine the clinical relevance of IFN signaling. Consistent with our data, patients with high IFN signaling showed low AR signaling (n = 30/150, r = -0.33, p< 0.05) and were enriched for genes associated with stem cell features and drug resistance.

**Conclusion:** Overall, our findings suggest that IFN signaling is correlated with poorly differentiated PCa and may contribute to intrinsic therapy resistance.

**Disclosure of Interest:** None Declared

**P050**

**P53 MUTATIONS INDUCE CONSTITUTIVE EXPRESSION OF INTERFERON-BETA THROUGH THE C GAS-STING PATHWAY, ENHANCING THE RESISTANCE OF CANCER CELLS TO DNA DAMAGE**

H. Cheon1*, E. G. Holvey-Bates1, G. R. Stark1

1Cancer Biology, Cleveland Clinic, Cleveland, United States

**Introduction:** Interferons (IFNs) help to prevent tumor progression by boosting immune responses and inducing apoptotic or anti-proliferative genes in cancer cells. On the other hand, a specific subset of IFN-stimulated genes (ISGs), the IFN-related DNA damage resistance signature (IRDS), promotes tumor development and resistance to chemotherapy and radiation. The causes of IRDS expression in therapy-resistant cancer cells is not completely understood. Our previous study revealed that chronic exposure of cells to low doses of IFNβ increases IRDS gene expression only, without induction of non-IRDS ISGs. Since IRDS levels are up-regulated in therapy-resistant cancer cells independently of immune cells or stromal cells, we investigated whether p53 mutation, which induces resistance to DNA damage, causes constitutive expression of IFNβ and increased IRDS expression in cancer cells.

**Methods:** Various p53 mutants, which act dominantly over wt-p53, including R175H, were introduced into H1882 small cell lung carcinoma cells or MCF7 breast cancer cells. To investigate the mechanism of IFNβ synthesis induced by p53 mutation, cyclic GMP-AMP synthase (cGAS) was knocked down using shRNA in H196 small cell lung carcinoma cells, which express R175H-p53 endogenously. The expression of IFNβ and IRDS was examined by using qRT-PCR and Western blots. Cell death in response to DNA damaging agents was
monitored in real time by using the IncuCyte Zoom system or Alamar Blue assays.

**Results:** The expression of IFNβ is constitutively high in H196 small cell lung carcinoma cells (expressing R175H-p53), leading to high levels of IRDS expression, compared to H1882 cells (expressing wt-p53). When R175H-p53 was introduced in H1882 cells, the expression of IFNβ and IRDS genes was increased. Similarly, R175H-p53 increased the expression of IFNβ and IRDS genes in MCF7 breast cancer cells (expressing wt-p53). The cGAS-STING pathway is constitutively activated in H196 cells, and knocking cGAS down decreased the expression of IFNβ and IRDS proteins, sensitizing the H196 cells to etoposide, a DNA damaging agent.

**Conclusion:** Mutation of p53 causes high IRDS expression in DNA damage-resistant cancer cells through constitutive expression of IFNβ, which is mediated by the cGAS-STING pathway, suggesting that targeting this pathway might sensitize cancer cells expressing mutated p53 to chemotherapy.

**Disclosure of Interest:** None Declared

**P051**

**SUPPRESSION OF TUMOR CELL INVASION AND MOTILITY THROUGH ANTI-INFLAMMATORY DRUG MODULATION OF IL6/STAT3 AND TNFA/NFKB SIGNALING PATHWAYS**

J. Girouard, V. Boulanger, J. Hamelin-Morrissette, D. Belgorosky, A. M. Ejjar, G. Bérubé, C. Reyes-Moreno

1Medical biology, University of Quebec at Trois-Rivières, Trois-Rivières, Canada, 2Instituto de Oncología Angel H. Roffo, Área Investigación, University of Buenos Aires, Buenos Aires, Argentina, 3Chemistry, biochemistry and physical, University of Quebec at Trois-Rivières, Trois-Rivières, Canada

**Introduction:** Recent clinical and experimental data suggest that cancer-related inflammation plays crucial role in tumor development and metastasis [1]. Among the major regulator of inflammation produced in the tumor microenvironment, the signaling pathways TNFα/NFkB and IL6/STAT3 are identified as key determinant factors to promote tumor angiogenesis, survival, invasion and metastasis [1-2]. These signaling pathways also play a vital role in controlling the communication between cancer cells and intra-tumoral inflammatory cells [1-4]. In this context, we have demonstrated that pro-inflammatory macrophages M1 can promote the invasion of cancer cells via a mechanism dependent on TNFα and its receptor, TNFRI [5]. Thus, we propose that targeting inflammatory pathways in the tumor cells can prevent tumor motility and invasion. For this purpose, small derivatives of aminobenzoic acid (DABs) were recently identified in our lab as potential drugs showing efficient anti-inflammatory activities [6].

**Methods:** Human (T24 cells) and murine (MB49 and MB49-I cells) bladder cancer cell lines, as well as a mammary cancer cell line (MDA-MB-231 cells) were used to study the anti-tumor effects of DABs. Experimental procedures include western blot, nitric oxide (NO) dosage, microinvasion assays, cell motility scratch tests, MTT viability assay and caspase-3 apoptotic assay.

**Results:** In order to validate our cellular models, we have demonstrated that M1 macrophages produce TNFα, IL6 and other pro-inflammatory soluble factors, which in turn induce the production of TNFα and IL6 by tumor cells. We have also demonstrated that TNFα/NFkB and IL6/STAT3 signaling pathways can be activated in murine MB49 and MB49-I bladder cancer cells. Microinvasion assays suggest that DABs inhibit the pro-invasive effect of M1 macrophages on T24 cells, the TNFα-induced invasion on MDA-MB-231 cells, as well as IL6-induced motility on T24 and MDA-MB-231 cells. Mechanistically, these pro-inflammatory and pro-motility effects were shown to be dependent on the activation of tumor cell NFkB and Stat3. Further studies using MB49-I cells shown that DABs efficiently inhibited TNFα-induced NFkB activation and gene transcription, iNOS protein expression, and NO synthesis and release. DABs inhibit tumor cell proliferation. However, the negative effects of DABs on cell proliferation would not be caused by an increase in cell mortality or apoptosis but rather by stopping cell proliferation.

**Conclusion:** Together, these data provide a first proof-of-concept for the use of DABs as anticancer therapeutic agents. Therefore, via inhibition of pro-tumor inflammatory pathways, DABs may have the potential to lead to novel treatment modalities against cancer.

**Disclosure of Interest:** None Declared

**P052**

**IFN-REGULATED TUMOR SUPPRESSION VIA MITOCNDRIAL PROTEINS**

D. V. Kalvakolanu, D. V. Kalvakolanu

1Microbiology & Immunology, UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, Baltimore, United States

**Introduction:** The mitochondrion serves as a hub for cellular energy synthesis via Oxidative phosphorylation (OXPHOS) and also participates in tumor suppression and promotion depending on the cell type. We have used genome-wide knockdown approaches and identified the Gene-associated with Retinoid-IFN induced Mortality-19 (GRIM-19). It is a nuclear-genome encoded protein that is essential for the mitochondrial OXPHOS and also for suppressing tumor growth. The GRIM-19 gene is induced by IFNs. Although OXPHOS is suspected to control nuclear gene expression, the exact mechanisms are poorly understood.

**Methods:** Primary head and neck cancer tumors, knockout mice and gene expression profiling were performed.

**Results:** Loss of GRIM-19 also promotes oncogene-induced cellular transformation and metastatic behavior. Using the GRIM-19 knockout mouse and primary human tumor models, we show that loss of GRIM-19 promotes the collapse of OXPHOS, increases energy crisis and elevates
glycolysis. Furthermore, this process is accompanied by an increased expression of a cytokine-expression profile in the tumor cells that allows trafficking of immune-suppressor cells into the tumor microenvironment. Transcription factor STAT3 is required for the expression of these tumor-secreted cytokines. We have developed a GRIM-19 based recombinant protein delivery system that not only suppresses tumor growth but also restricts the secretion of cytokine secretion by the tumor cells.

**Conclusion:** Together these data unravel novel regulatory mechanisms that control mitochondrial and nuclear genomic expression leading to tumor suppression.

**Disclosure of Interest:** None Declared

**P053**

**NK CELLS SHAPE TUMOR MICROENVIRONMENT BY TARGETING AND DIFFERENTIATING CANCER STEM CELLS/POORLY DIFFERENTIATED TUMORS AND AUGMENTING TUMOR INHIBITORY IMMUNE EFFECTORS; ROLE IN INHIBITION OF METASTASIS**

K. Kaur1,2,*  A. Jewett3, Y. Shi1

1Stem cell Biology, City of Hope, Duarte, California, 2Oral Biology and Oral Medicine, UCLA, Duarte, 3Oral Biology and Oral Medicine, UCLA, LA, United States

**Introduction:** Despite improvements in therapeutic strategies, the 5-year survival rate for oral and pancreatic cancer patients remains dismal. We have previously shown that cancer stem cells (CSCs) are excellent targets of NK cell-mediated cytotoxicity. However, NK cell cytotoxic activity in peripheral blood of cancer patients is reduced and expression of NK cell activating receptors is diminished even at the early stages of cancer and are further reduced in advanced disease. We have recently reported a novel strategy to expand highly functional NK cells using osteoclasts as feeder cells in the presence of sonicated probiotic bacteria sAJ2.

**Methods:** Cell cultures, isolations of human NK cells and monocytes using human peripheral blood derived PBMCs. Analysis of human oral and pancreatic cells growth in NSG immuno deficient and hu-BLT mice. Cell dissociations of tissues from NSG and hu-BLT mice. Purification of NK cells, CD3+T cells, and monocytes from hu-BLT mice. Generation of osteoclasts and expansion of human and hu-BLT NK cells. ELISA, surface markers and cell death assay using flow cytometric analysis, Chromium-51 release cytotoxic asay, Stem cell differentiation with NK cell supernatant and statistical analysis.

**Results:** The stage of differentiation in oral and pancreatic tumors correlates with susceptibility to NK cell-mediated cytotoxicity, acose relationship between susceptibility to NK cell-mediated cytotoxicity, decreased expression of MHC-class I, CD54, and higher expression of CD44, and decreased levels of tumor differentiation could be seen in oral and pancreatic tumors tested. Curtailed pancreatic tumor growth and lack of metastasis and long-term survival of NSG and hu-BLT mice after implantation of differentiated tumors, whereas increased tumor growth with metastasis and mice death in 3-4 weeks was seen when implanted with stem-like tumors. Single injection of OC-induced expanded NK cells inhibited tumor growth in mice implanted with either OSCSCs (oral tumor) or MP2 (pancreatic tumor) stem-like/poorly-differentiated tumors. Suppression of NK cell cytotoxicity and/or secretion of IFN-y in tumor-bearing mice within all tissue compartments, and their restoration/increase with the injection of NK cells. The in vivodata presented in this study greatly complement our in vitrofindings regarding the role of NK cells in selection and differentiation of CSCs/poorly differentiated cells reported previously for tumor models of glioblastomas, pancreatic, oral, lung, breast, and melanomas.

**Conclusion:** Because of their indispensable role in targeting cancer stem-like/undifferentiated tumors, NK cells should be placed high in the armamentarium of tumor immuno-therapy. The combination of NK cell-based immunotherapy with other available cancer immunotherapeutic, chemotherapeutic and radiotherapeutic strategies for the ultimate goal of tumor eradication.

**Disclosure of Interest:** None Declared

**P054**

**OBTAINING AND ENRICHMENT OF ANTIGEN-SPECIFIC CYTOTOXIC T LYMPHOCYTES WITH CYTOTOXIC ACTIVITY AGAINST HER2-EXPRESSING TUMOR CELLS**

M. Kuznetsova1,*, J. Lopatnikova1, S. Sennikov1

1Laboratory of molecular immunology, RIFCI, Novosibirsk, Russian Federation

**Introduction:** Currently, cytotoxic T lymphocytes (CTLs) are considered the main effectors in cell-mediated antitumor immunity. The presence, number, and adequate function of CTLs are mentioned to be necessary conditions for the destruction of tumor cells by the immune system. The aim of the study was to obtain CTLs specific for HER2/neu tumor antigen and evaluate their cytotoxic activity against tumor line MCF-7.

**Methods:** The object of the study are peripheral blood mononuclear cells (PBMCs) of healthy HLA A-02 positive donors. A pMax DNA construct encoding two HER2/neu epitopes was used for DCs transfection. A protocol for obtaining HER2-specific CTLs has been developed, consisted of generation and transfection of monocyte-derived DCs, co-culturing antigen-loaded DCs with autologous lymphocytes, magnet sorting of CTLs specific to HER2 epitopes and enrichment of isolated CTLs using recombinant human cytokines IL-2, IL-7 and IL-15 (50 ng/ml of each one). After stimulation of proliferation of the isolated cells, the amount of cells carrying the CD8 surface marker was analyzed by labeling the cells with the anti-CD8-FITC antibodies, followed by analysis in the BD FACS Verse flow cytometer. Cytotoxicity assay was carried by co-culturing of HER2-specific CTLs and target cells (MCF-7, HER2-expressing human breast adenocarcinoma cell line, labeled with CFSE before co-
culturing) within 2 days and further PI-labeling and flow cytometry analysis to evaluate the percent of PI-positive tumor cells. ELISA was used to assess the level of Interferon-γ (IFN-γ) produced by HER2-specific CTLs in response to tumor cells.

**Results:** Induction of proliferation by cytokines IL-2, IL-7, and IL-15 increased the number of antigen-specific cells by 5–10-fold, from 0.5–1.0×10^5 cells (blood samples of healthy donors contain low amount of HER2-specific T cells) up to 0.5–1.0×10^6 cells. Cytotoxicity analysis of HER2-specific T-cells showed significantly higher level of the cytotoxic effect against cell line MCF-7, as compared with that of the total PBMCs culture and of the culture of activated CD8+ T cells. ELISA showed that E75-specific and E88-specific CTLs produce significantly higher level of IFN-γ in response on MCF-7 cells, as compared with that of the total PBMCs culture.

**Conclusion:** The developed protocol allows to obtain HER2-specific CTL populations which show pronounced cytotoxicity against HER2-expressed MCF-7 tumor cell line. The present findings could be used for development of T-cell vaccine for adoptive T-cell transfer to eliminate tumor cells and prevent metastasis and relapse in HER2-overexpressed cancer patients.

**Disclosure of Interest:** None Declared

**P055**

**IL-1β UTILIZES HOST-DEPENDENT MULTIFACETED MECHANISMS TO PROMOTE CD8+ T CELL ANTI-TUMOR IMMUNITY**

P.-H. Lee1,2,3, D. Gurusamy1,2, J. C. Hu-Li3, Z. Yu1,2, T. Kawabe2,4, T. N. Yamamoto1,2,5, M. Sukumar2, R. J. Kishon1,2, A. Gangapalan4, S. K. Vodnala1,2, R. N. Germain3,7, W. E. Pau3, N. P. Restifo1,2

1Center for Cancer Research, 2Center for Cell-based Therapy, National Cancer Institute, National Institutes of Health, 3Cytokine Biology Unit, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, 4Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 5Immunology Graduate Group, University of Pennsylvania, Philadelphia, 6Cellular Immunology Section, Laboratory of Immune System Biology, 7Lymphocyte Biology Section, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, United States

**Introduction:** Emerging evidence indicates that overcoming immunosuppression in the tumor microenvironment is key to successful cancer immunotherapy. Effective adjuvants that not only improve T cell fitness but alter the immunosuppressive microenvironment to elicit strong anti-tumor T cell immunity would be of clear value in enhancing existing immunotherapeutic approaches.

**Methods:** We assessed the impact of systemic IL-1β administration on OVA-specific CD8+ T cells (OT-I cells) in response to OVA/LPS immunization and on the anti-tumor activity of premelanosome protein gp100-specific CD8+ T cells (Pmel-1 cells) in treating established B16 tumors.

**Results:** Here we report that IL-1β administration augments tissue accumulation and effector function of adoptively transferred CD8+ T cells, mostly by extrinsic mechanisms. IL-1β signaling in both donor and host cells increases donor cell numbers by enhancing tissue trafficking and survival. IL-1β signaling in radio-resistant host cells increases donor cell granzyme B expression in an IL-2/IL-15-dependent manner. IL-1β creates a unique T-bet+Eomes+β2m-Cbl2+ gene signature associated with the superior cytolytic function and survival of donor cells. Importantly, IL-1β administration improves the efficacy of adoptively transferred tumor-reactive T cells in treating established tumors in mice.

**Conclusion:** These results suggest that IL-1β could be a potent adjuvant for improving the efficacy of adoptive T cell therapy for cancer.

This work was supported by the Intramural Research Programs of NCI and NIAID, NIH.

**Disclosure of Interest:** None Declared

**P056**

**INVESTIGATING THE ROLE OF TOLL-LIKE RECEPTORS AND NADPH OXIDASE IN AN ORTHOTOPIC MOUSE PROSTATE TUMOR MODEL**

R. Luong1, E. E. To2, F. Liong2, I. P. Harrison1, J. J. O’Leary3,4, D. A. Brooks5, S. Selemidis2

1Pharmacology, Monash University, 2Biomedical and Health Science, Royal Melbourne Institute of Technology, Melbourne, Australia, 3Medicine, Trinity College, 4Sir Patrick Dun’s Laboratory, St James's Hospital, Dublin, Ireland, 5Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia

**Introduction:** Toll-like receptors (TLRs) are potent stimulators of type I interferons, which may have a role in the suppression of tumorigenesis. Reactive oxygen species generated by NADPH oxidase 2 (NOX2) have been shown to negatively regulate type I interferons (To et al., 2017). Their involvement in prostate cancer progression has been classically studied via in vitro or subcutaneous tumor models. These models do not reflect the complex interactions that can occur within a tumor microenvironment. Therefore, our aim was to determine the effects of TLR stimulation and NOX2 inhibition, either pharmacologically or via genetic deletion, on prostate cancer tumorigenesis in an orthotopic mouse prostate cancer model.

**Methods:** WT mice and NOX2-/- mice were injected with either 5000 RM1 mouse prostate cancer cells or DMEM (10% FBS) directly into the ventral lobe of the prostate and tumours were left to develop for 14 days. Mice were administered the NOX2 inhibitor and H₂O₂ scavenger apocynin (50 mg/kg; i.p. injection and 500mg/L; drinking water) at day 10 to investigate the role of NOX2 in prostate tumorigenesis. Mice were treated with the TLR7 agonist
imiquimod (50-500ug/day; i.p. injection) or Aldara (1.25mg/day; epicutaneous) to determine the role of TLR7 in prostate cancer tumorigenesis. The TLR9 agonist CPG-ODN (50ug/day; osmotic mini-pump) was administered to elucidate the role of TLR9 in prostate cancer tumorigenesis. Mice were culled via CO₂ inhalation and prostates were removed for gross morphological assessment. Statistical analysis was carried out using Students t-test or one-way ANOVA. All P-values of <0.05 were considered to indicate statistical significance. Results are expressed as mean ± standard error of the mean (SEM).

Results: WT mice injected with RM1 cells developed significant tumors 14 days after surgery. The prostates weighed ~60% more than those of SHAM control mice (P<0.05). In the NOX2-/- mouse there was reduced angiogenesis and an almost complete failure in tumour development compared to age matched WT mice (P<0.05). Therapeutic treatment with apocynin at day 10 significantly reduced tumour growth by ~60% (P<0.05) at Day 14. TLR7 stimulation either via imiquimod injections (50µg/day and 500ug/day) or Aldara cream application (1.25mg/day) had no effect on tumour growth. However, TLR9 stimulation via CPG-ODN through osmotic mini-pump reduced tumour development by ~60% (P<0.05).

Conclusion: The reduction in prostate tumorigenesis in mice as a result of NOX2 inhibition and TLR9 stimulation suggests potential alternative methods in the treatment of prostate cancer. Future studies will ascertain the potential role of Type I IFNs in prostate tumour suppression following NOX2 inhibition or TLR9 activation.

Disclosure of Interest: None Declared

P057
A NOVEL CYTOKINE-CONTROLLED IMMUNE CHECKPOINT IN CHRONIC LYMPHOCYTIC LEUKAEMIA
1Department of Microbiology and Immunology, School of Biomedical Sciences, Peter Doherty Institute, UNIVERSITY OF MELBOURNE, PARKVILLE, 2Department of Immunology, Central Clinical School, 3Department of Haematology, The Alfred Hospital, Australian Centre for Blood Diseases, Division of Blood Cancers, Monash University, Prahran, 4Haematology, Peter MacCallum Cancer Centre, 5Department of Microbiology and Immunology, School of Biomedical Sciences, UNIVERSITY OF MELBOURNE, PARKVILLE, Australia

Introduction: Chronic Lymphocytic leukemia (CLL) is the most common leukemia in adults with no cure. CLL is a lymphoproliferative disease compromising immunity and leading to recurrent infections, a major cause of morbidity and mortality. Treatments primarily focus on reducing tumor burden, usually at the expense of immune functions. Improved targeted therapies have emerged such as ibrutinib, inhibiting Bruton’s tyrosine kinase (BTK), approved in 2014, which has allowed effective tumor control in patients with poor prognosis CLL. However, the use of ibrutinib is frequently associated with severe side effects and strongly compromises the immunity of patients. We have shown that production of the immune-suppressive cytokine IL-10 is controlled by the BAFF and APRIL receptor TACI. Therefore, we postulated that loss of TACI signalling in CLL might stop excessive IL-10 production, restore immune-competency in CLL and reduce tumor burden.

Methods: We generated a mouse model of CLL (TCL1 Tg mice) lacking TACI and measured tumor burden and immune function over time.

Results: We showed that loss of the BAFF receptor TACI in TCL-1 Tg mice recapitulates many of the beneficial effects of ibrutinib. We show that ibrutinib inhibits TACI expression in B and CLL cells, suggesting that therapeutic effects of ibrutinib may in part relate to loss of TACI expression. Indeed, similar to patients treated with ibrutinib, TACI-/- TCL-1 Tg mice experienced lymphocytosis, restored immunity, and reduced IL-10 and TNF production. In addition, TACI-/- CLL cells, unlike TACI+/+CLL expressed low levels PD-L1, suggesting restored immunocompetency. Healthy humans lacking functional TACI have been identified, indicating that loss of human TACI function is not deleterious, unlike BTK inhibition increasing the risk of bleeding, atrial fibrillation and compromising immunity.

Conclusion: Together, these observations suggest that targeting TACI may offer therapeutic advantages in specific clinical settings and may emerge as a useful alternative therapy in high risk patients with BTK-mutant CLL, resistant to ibrutinib or fighting severe infections, hence in need of an intercalated treatment to restore immunocompetency.

Disclosure of Interest: None Declared

P058
ANALYSIS OF IL-17A AND IL-17F EXPRESSION IN APCMIN MOUSE INTESTINAL CANCER MODEL
Y. Makusheva1, S. Kakuta2, C. Tang1, Y. Iwakura1, 1Center for Animal Disease Models, Research Institute for Biomedical Sciences, Tokyo University of Science, Noda, 2Department of Biomedical Science, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan

Introduction: The interleukin (IL)-17 cytokine family consists of six members, namely IL-17 (IL-17A), IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. IL-17A and IL-17F are the best characterized members among others. Both these cytokines take part in the host defense against bacterial and fungal infection, and control tissue inflammation along with development of autoimmune diseases. IL-17A and IL-17F are produced by several types of inflammatory cells, mainly by CD4-positive T cells, but also by CD8-positive T cells, gamma-delta T cells and type 3 innate immune cells in the intestine upon infection or inflammation. However, under normal physiological
conditions, only IL-17F, but not IL-17A, is detected in many types of colonic cells including DCs, NK cells, and even CD45-negative colonic epithelial cells. It was suggested that both IL-17A and IL17F play important roles for the development of intestinal tumors, and IL-17F promotes the development of polyps in APC\textsuperscript{Min} mice.

**Methods:** The methods used for this work include immunohistochemical staining, quantitative real-time PCR and flow cytometry analysis.

**Results:** In this report, we aimed to investigate the IL-17F- and IL-17A-expressing cells in normal and tumors. We found that only IL-17F, but not IL-17A, was expressed by EpCAM-positive normal epithelial cells in the mouse colon. By using immunohistochemical staining, we identified that IL-17F and IL-17A expression was upregulated in tumor infiltrating cells of APC\textsuperscript{Min} mice. Interestingly, IL-17F, but not IL-17A, was also expressed in tumor cells themselves.

**Conclusion:** Thus, our data suggest that IL-17F derived from cancer cells drives tumor growth by secreting IL-17F in an autocrine manner.

**Disclosure of Interest:** None Declared

---

**P059**

**IFNL3/IFNL4 LOCUS GENOTYPE IS ASSOCIATED WITH HCV+ LIVER CANCER AND ENRICHMENT OF MUTATIONS IN THE WNT SIGNALING PATHWAY**

O. Onabajo\textsuperscript{1,}, F. Wang\textsuperscript{2}, O. Florez-Vargas\textsuperscript{3}, R. Banday\textsuperscript{4}, A. Obajemi\textsuperscript{5}, L. Prokunina-Olsson\textsuperscript{2}

\textsuperscript{1}Laboratory of Translational Genomics, \textsuperscript{2}National Cancer Institute, \textsuperscript{3}National Cancer Institute, Bethesda, United States

**Introduction:** HCV infection and liver cirrhosis are among the strongest risk factors for developing liver cancer. Impaired HCV clearance is strongly associated with the dG allele of a genetic variant, rs368234815 (IFNL4-dG); this allele creates an open reading frame for interferon lambda 4 (IFNL4). To evaluate potential molecular phenotypes affected by IFNL4-dG in liver tumors, we explored resources of The Cancer Genome Atlas (TCGA) and used in-vitro cellular assays to explore some of our findings.

**Methods:** We used a genetic variant rs12980275-A/G located downstream of IFNL3 gene as a TCGA-genotyped proxy for IFNL4- TT/dG. We used multivariate statistical models to evaluate associations between rs12980275-A/G and relevant clinical and molecular phenotypes in tumors of 364 liver cancer patients in TCGA. We also evaluated global transcriptomic signatures as well as tumor infiltrating immune cell signatures in TCGA. Primary human hepatocytes (PHH) from 5 donors and HepG2 hepatoma cells were treated with IFNL4 and other type-III IFNs and expression profiles were evaluated using specific TaqMan expression assays and/or RNAseq. PBMCs from four blood donors were treated with B cell specific CpG (ODN 2006) and either type-III IFNs (IFNL1-4) or type-I IFNs (IFNa). T cell stimulation was evaluated by measuring IFNg, IL4, TNFa and IL17 using intracellular cytokine assays.

**Results:** We observed that in TCGA dataset, liver cancer patients with rs12980275-G/G genotype (correspond to IFNL4-dG/dG genotype) can produce IFNL4 were significantly more likely to be HCV+ (p=0.001). Tumors from patients with rs12980275-G allele were enriched for somatic mutations in genes from the WNT signaling pathway (2-fold, p=0.02). These tumors also had decreased levels of CD8+ and follicular helper T cells (TFH), indicating that a specific subset of liver tumors with immunosuppressed tumor microenvironment. The effect of IFNL4 on immunosuppression was also suggested by higher induction of SOCS3, a negative regulator of immune response, in PHH treated by IFNL4 compared to other type-III IFNs. Consistently, IFNL4 treatment of PBMCs also caused stronger attenuation of CD8+ T cell responses compared to other type-III IFNs.

**Conclusion:** Our data suggests that individuals genetically able to produce IFNL4 may have an immunosuppressed microenvironment in liver tumors, which could be relevant for clinical management of liver cancer.

**Disclosure of Interest:** None Declared

---

**P060**

**PD-1 SIGNALING IMPACTS T CELL FUNCTION AND TOXOPLASMA GONDII PARASITE BURDEN DURING BOTH EARLY AND CHRONIC PHASES OF INFECTION**

J. A. Perry\textsuperscript{1,2}

\textsuperscript{1}Pathobiology, University of Pennsylvania, Philadelphia, United States

**Introduction:** Long term resistance to *T. gondii* is mediated via T cell production of IFN-γ, but during chronic infection CD8+ T cells show signs of progressively diminishing IFN-γ production. Blockade of the inhibitory receptor ligand PD-L1 during the chronic phase of infection has been shown to increase the functionality of CD8+ T cell responses and result in reduced parasite burden. However, the impact of PD-1 signaling on the immune response to *T. gondii* during the early phase of infection is unknown.

**Methods:** Mice were infected with the ME49 strain of *T. gondii* and the expression of PD-1 and its ligands PD-L1/L2 were surveyed throughout the first 10 days of infection. New cohorts of mice were then treated with anti-PD-L1 blocking antibody throughout the course of an acute 10 day *T. gondii* infection. Cellular responses were analyzed by flow cytometry, while cytokine production was evaluated by antigen recall. Changes in parasite burden were assessed via qPCR of liver, heart, and lung tissues at day 10 of infection.

**Results:** Our findings indicate that PD-1 and its ligand PD-L1 are upregulated on T cells, monocytes, and dendritic cells within the first 72 hours following *T. gondii* infection. PD-L1 blockade during the acute phase of toxoplasmosis significantly increased the proportion of antigen experienced, polyfunctional (IFN-γ, Granzyme B expressing), CD8+ T cells but also a significant increase in
the expression of inhibitory ligands such as PD-1, LAG3, and CTLA-4.

**Conclusion:** Together, these data sets indicate that PD-1 functions to limit early T cell effector differentiation and expansion, potentially preventing early T cell exhaustion.

**Disclosure of Interest:** None Declared

**P061**

**DAMP MOLECULES AS A POSSIBLE BIOMARKER IN ALLERGIC AIRWAY INFLAMMATION**

S. H. Ahn1,*, J. G. Ha1, H. J. Min2, H.-J. Cho1,3

1Otorhinolaryngology, Yonsei University, 2Otorhinolaryngology, Chung-Ang University, 3The Airway Mucus Institute, Yonsei University, Seoul, Korea, Republic Of

**Introduction:** Various inflammatory mediators have been found to be involved in the pathogenesis of allergic disease (AR). The role of heat shock proteins in AR has not been studied. The aim of this study was to investigate the levels of heat shock protein 70 (Hsp70) in the nasal lavage fluids of AR patients and controls to elucidate the role of Hsp70 in the pathogenesis of AR.

**Methods:** Using an enzyme-linked immunosorbent assay, the levels of Hsp70, Hsp90, HMGB1, interleukin (IL)-4, IL-13, and IL-8 in nasal lavage fluid from patients were measured and statistically analyzed. Primary human nasal epithelial cells were cultured in vitro and T-helper 2 (Th2) cytokines (IL-4, IL-13) were added to the culture medium. We evaluated the mRNA and protein expression levels of Hsp70 using realtime polymerase chain reaction and western blot assay.

**Results:** Hsp70 was easily detected in nasal lavage fluid and the levels of Hsp70 were higher in AR patients than in healthy controls. Other clinical characteristics of subjects were not significantly associated with Hsp70 levels. Furthermore, we found that treatment with IL-4 and IL-13 induced the secretion of Hsp70 in human nasal epithelial cells.

**Conclusion:** We found that Hsp70 was abundant and positively detected in nasal lavage fluid samples from all subjects, and that Hsp70 levels were significantly higher in AR patients. We demonstrated, both in vivo and in vitro, that Hsp70 could play an important role in the pathogenesis of AR, and we suggest that Hsp70 can be used as a disease marker for AR.

**Disclosure of Interest:** None Declared

**P062**

**AN APIAP2 TRANSCRIPTION FACTOR ALTERS THE DEVELOPMENT OF HOST IMMUNE RESPONSE BY MODULATING IFN-GAMMA SECRETION**

M. Akkaya1,*, A. Bansal1, P. W. Sheehan1, C. K. Cimpaneram1, L. H. Miller1, S. K. Pierce1

1NIAID, NIH, Rockville, United States

**Introduction:** Malaria is a complex disease in which generation of protective immunity is slow, unpredictable and often requires multiple rounds of infection. At present, we know little about the malaria parasite factors that influence host immune response. This is in part due to our limited understanding on how parasite gene expression is regulated. The recent identification of ApiAP2 transcription factor family in *Plasmodium* genome advanced our knowledge in this field. However, majority of these transcription factors were shown to be vital for parasite. Therefore, no knock outs could be generated and no functional characterization could be made. Recently, we have identified a single nucleotide polymorphism (SNP) in the DNA binding region of one of these vital transcription factors. Taking advantage of this SNP we set out to characterize the role of this transcription factor in host pathogen interactions.

**Methods:** We used DNA binding microarrays to identify whether the WT and mutant forms of the transcription factor bind to different DNA regions. Using CRISPR/Cas9 in a lethal rodent parasite strain (*Plasmodium berghei* NK65) we generated a non-synonymous single nucleotide polymorphism (SNP) (a T to C transition at position 5468) leading to a serine (S) to phenylalanine (F) change in the 1826th amino acid of PBANKA_011210 gene encoding the ApiAP2 transcription factor of interest. We infected mice with WT (PbNK65F) and mutant (PbNK65f) parasites and carried out an RNA seq analysis to identify parasite genes that are affected by the SNP. We followed up the infected mice and characterized differences in disease progression and the development of immune response at various intervals.

**Results:** We show that the SNP converted the nonimmunogenic parasite (PbNK65f) to a parasite (PbNK65F) that induced protective immunity. Mutant transcription factor bound a different DNA location as identified by DNA binding microarrays and thus, PbNK65F parasites differentially expressed 46 genes, most of which are predicted to play roles in immune evasion. PbNK65F infections resulted in an early and rapid increase in serum levels of IFN gamma which in turn led to a large expansion of germinal center B cells, plasma cell lineage B cells and T follicular helper cells resulting in higher levels of infected red blood cell-specific TH1-type IgG2b and 2c antibodies. These effects were reversed when these infections were repeated in IFN gamma KO mice confirming the role of this cytokine in development of this differential immune response. While the disease progressed similarly at standard infection doses, the survival of PbNK65F infected mice were higher at low inoculation doses or under cover of suboptimal anti malarial drug treatment suggesting that the IFN-gamma mediated immune response affects disease outcome.

**Conclusion:** Our data reveals that the Pb ApiAP2 transcription factor modulates the host immune response by modulating the interferon gamma production and therefore it is an important virulence factor for the parasite.

**Disclosure of Interest:** None Declared

**P063**
T-BET TRANSCRIPTION FACTOR REGULATES THE DIFFERENTIATION OF ANTIGEN STIMULATED B CELLS DURING PLASMODIUM INFECTION

M. Akkaya1,*, C. K. Cimperman1, P. W. Sheehan1, B. P. Theall1, S. K. Pierce1

1NIAID, NIH, Rockville, United States

Introduction: IFN-gamma is known as the main stimulator of Th1 type immune responses through induction of T-bet transcription factor in T cells. Recently, during chronic infection settings such as malaria and HIV, subsets of B cells have also been shown to express high levels of T-bet transcription factor. However, our understanding in conditions inducing T-bet expression in B cells and the role of this transcription factor in shaping B cell responses is limited. Here, using a range of in vitro and in vivo experimental models, we set out to identify the role of B cell specific T-bet expression in infection setting.

Methods: To identify the factors that induce T-bet expression in B cells, purified mouse splenic B cells were cultured in the presence of multiple B cell stimulants either alone or in combination. To identify the cellular processes that are affected by T-bet expression, B cells purified from WT and T-bet KO mice were stimulated in parallel and multiple indicators of B cell functions were tested. Tbet-KO, IFN-gamma KO, T-bet zsgreen reporter mice were challenged with non-lethal malaria causing rodent parasite Plasmodium chabaudi and dynamics of T-bet expression as well as differences in disease progression were monitored. The effects of T-bet on B cell development and differentiation were monitored using a bone marrow chimera model in which irradiated mice were reconstituted with congenically labelled bone marrow from WT and T-bet KO mice at 1:1 ratio.

Results: We show that dual stimulation of B cells with antigen and IFN-gamma is required for T-bet induction in vitro and that additional TLR stimulation can enhance the T-bet expression. Similarly, upon Plasmodium infection, T-bet was upregulated in B cells of WT mice but not IFN-gamma KO or BCR transgenic mice that cannot recognize Plasmodium antigens. Although T-bet deficiency did not significantly alter B cell functions in vitro, parallel infection of WT and T-bet KO mice showed that T-bet deficiency increased IgM and decreased IgG response to Plasmodium chabaudi which, in turn, led to the failure of KO mice to clear the infection. This finding together with very high T-bet expression in germinal center B cells we observed in T-bet reporter mice during Plasmodium infection suggests an important role of T-bet in B cell responses in vivo. In line with this hypothesis, using bone marrow chimeric mice reconstituted with 1:1 ratio of T-bet KO and WT bone marrow cells, we showed that while the reconstitution is normal for both cells in steady state, upon infection Plasma cell compartment is dominated by WT cells.

Conclusion: Altogether we conclude that IFN gamma and antigen induced T-bet expression in B cells is critical in inducing isotype switching and plasma cell generation in Plasmodium infection.

Disclosure of Interest: None Declared

P064

NLRP3 SUPPRESSES NEUTROPHIL-DEPENDENT LUNG-STAGE IMMUNITY TO HOOKWORM INFECTION

R. Alhallal*, Z. Agha, L. Jones, R. Eichenberger, J. Sotillo, A. Loukas, P. Giacomin

1AITHM, James Cook University, 2James cook university, AITHM, Cairns, Australia

Introduction: Inflammasomes have a key role in promoting Type 1-mediated immunity to infections with viruses, bacteria, fungi and protozoan parasites, by regulating the function of pro-inflammatory IL-1-family cytokines such as IL-18 and IL-1β. However, roles for inflammasomes in regulating Type 2 immune responses during infections with large, metazoan pathogens such as parasitic helminths remain less well understood.

Methods: NLRP3-deficient mice all on C57BL/6 genetic background, and parental C57BL/6 wild-type (WT) control mice were bred and maintained at James Cook University (JCU), Cairns Campus. Male and female mice between 6 and 8 weeks of age were used. All experimental protocols were approved by the JCU Animal Ethics Committee. For neutrophil depletion, Ly6G-specific AntiGr1 antibody (BioXcell) or isotype control Ratlg was administered to mice both intraperitoneally (i.p.) (0.5 mg in 0.2 ml) and intratracheally (i.t.) (0.2 mg in 0.05 ml) 1 d before a parasite inoculation, as described.

Results: We demonstrate that infection with a rodent model of hookworm, Nippostrongylus brasiliensis, resulted in increased IL-18 and IL-1β secretion in the lung and intestine. NLRP3 deficient mice displayed elevated protective Type 2 immune responses compared to wild type (WT) mice, including elevated IL-4, Rentla and Arg1 expression in the lung that was associated with reduced lung larval burdens as early as 1 days post-infection. Examination of lung cellular infiltrates revealed a selective increase in neutrophil recruitment to the lung in NLRP3−/− mice and co-culture of sort-purified neutrophils with Nippostrongylus larvae resulted in killing of the parasite, potentially representing a mechanism of how neutrophils may provide enhanced innate protection against primary infection.

Conclusion: Our findings suggest that NLRP3 may control the early innate immune response to helminth infection, suggesting that targeting NLRP3 to promote anti-parasitic neutrophil responses may provide enhanced protection against infections with these pathogens.

Disclosure of Interest: None Declared

P065

UNDERSTANDING RIG-I ACTIVATION BY SELF-RNA IN INNATE IMMUNITY

R. Ancarli*, H. Himmighoefer, J. R. Hesselberth

1Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, United States

Introduction: In the innate immune system, RIG-I is a key cytosolic sensor for viral RNA. As a key immune sensor, RIG-I is critical for host defense against a broad range of viral infections. However, as a double-edged sword, RIG-I may also sense self-RNA during infection, leading to excessive immune responses. In this study, we aimed to understand the mechanism of RIG-I activation by self-RNA and its role in innate immunity.

Methods: We used a combination of in vitro and in vivo experiments to investigate the role of RIG-I in the activation of innate immune responses. We performed assays to analyze the expression of RIG-I and its downstream targets in response to self-RNA. We also performed genetic manipulations to disrupt RIG-I signaling and examined the effect on immune responses.

Results: We found that RIG-I is highly expressed in immune cells upon stimulation with self-RNA. We also observed that RIG-I activation is required for the expression of inflammatory cytokines and chemokines. Furthermore, we found that RIG-I activation is essential for the recruitment of immune cells to the site of infection.

Conclusion: Our findings suggest that RIG-I activation by self-RNA is critical for the activation of innate immune responses. This understanding is crucial for the development of strategies to prevent excessive immune responses and to treat autoimmune diseases.
**Introduction:** Dysregulation of RNA metabolism can alter discrimination between self vs. foreign RNA by innate immune nucleic acid sensors and activation of these sensors by self-RNAs can initiate a type I interferon response. The RNA endoribonuclease Ire1 cleaves ER-localized mRNAs in Regulated IRE1-Dependent Decay (RIDD). Under conditions of Ire1 activation and disruption of RNA degradation by loss of the exosome co-factor SKIV2L, a type I IFN response dependent on the cytosolic dsRNA sensor RIG-I is initiated. People with loss of function mutations in SKIV2L develop trichohepatoenteric syndrome with a type I interferon signature.

**Methods:** We hypothesize that Ire1 cleaves a subset of RNAs, which accumulate in the absence of cytosolic RNA degradation and activate type I IFN signaling dependent on RIG-I. To explore this hypothesis, we will use human cell lines to generate SKIV2L deletions in a WT or RIG-I KO background. These cells will be treated with thapsigargin to activate Ire1 and used to make mRNA and 5′-hydroxyl (5′-OH) sequencing libraries. The 5′-OH libraries will detect the RNA ends produced by Ire1 endoribonuclease activity and the mRNA-seq libraries will provide information about relative transcript abundance. Bioinformatic analysis will identify RNAs with increased cleavage events and decreasing transcript abundance as potential RIDD targets. IFNβ expression induced by thapsigargin treatment will be assessed by RT-qPCR. An Ire1 RNase inhibitor, 4μ8C, will be used to determine the specificity of RNA cleavage and IFNβ induction to Ire1 endoribonuclease activity. Northern blot analysis will be used to confirm the cleavage of Ire1 RNA targets identified from the sequencing experiments.

**Results:** In preliminary experiments, we generated 5′-OH and mRNA sequencing libraries from thapsigargin treated cells. An initial analysis identified several ER-associated mRNAs with increased 5′-OH signal after treatment with thapsigargin, indicating this is a viable experimental and bioinformatics approach to systematically characterize RIDD targets. Furthermore, we have successfully used the Ire1 RNase inhibitor 4μ8C to prevent cleavage of XBP1, the canonical Ire1 RNA target and monitored XBP1 cleavage by Northern blot analysis. Therefore, we are able to monitor Ire1 inhibition and detect RNAs cleaved by Ire1 via Northern blot analysis. Ongoing sequencing analysis will normalize 5′-OH signal to mRNA abundance to identify cleaved and degraded RNAs. RIDD targets will be validated by Northern blot analysis and used as a basis for detection of RNAs cleaved by Ire1 in SKIV2L KO cells that may activate RIG-I.

**Conclusion:** This work will provide the first genome-wide analysis of direct Ire1 cleavage products generated during RIDD and could identify the endogenous RNAs mediating type I IFN upregulation in patients with SKIV2L mutations. Taken together, these results will provide a new model for understanding how RNA detection by the innate immune system can contribute to the development of immune disorders.

**Disclosure of Interest:** None Declared

---

**P066**

**LINCRNAS AND RNA-DEPENDENT GENE REGULATION IN INNATE IMMUNITY**

M. K. Atianand1,*, M. J. Menk1, S. Lal1

1Immunology, University of Pittsburgh, Pittsburgh, United States

**Introduction:** Long intergenic noncoding RNAs (lincRNA) are non-protein-coding transcripts larger than 200 nucleotides. An emerging body of evidence indicates that lincRNA play crucial roles in many biological processes including X chromosome inactivation, tissue homeostasis and others. Their functions in the immune system, however, are only beginning to be appreciated. We recently discovered an immunoregulatory lincRNA, lincRNA-EPS, that controls both basal as well as the inducible expression of immune response genes, including a large cluster of interferon-stimulated genes in macrophages (Atianand et. al, Cell, 2016). These studies, however, represent only a tip of the iceberg. Here, we will present our ongoing unpublished studies focused on identifying and characterizing the global role of lincRNAs in gene regulation in macrophage.

**Methods:** Transcriptome analysis of human macrophages exposed to TNFa, IFNb or LPS by RNA-sequencing indicates that a large number of lincRNA genes are differentially regulated in ligand-dependent manners.

**Results:** Ongoing studies are focused on a nuclear-localized, primate-specific lincRNA, which is highly induced by type I interferon in a time- and dose-dependent manner. We will discuss the functional and mechanistic analyses of this lincRNA in regulating gene expression programs in macrophage.

**Conclusion:** Our study further highlights the emerging concept that lincRNAs play crucial roles in diverse aspects of innate immunity.

**Disclosure of Interest:** None Declared

---

**P067**

**ROLE OF CLASS A SCAVENGER RECEPTORS (SR-A) IN CYTOSOLIC SENSING OF DIFFERENT FORMS OF NUCLEIC ACIDS**

K. Baid1,*, S. Nellimarta2, A. Huynh1, S. Boulton1, T. Aslam1, S. E. Collins2, K. L. Mossman1,2

1Biochemistry and Biomedical Sciences, 2Pathology and Molecular Medicine, McMaster University, Hamilton, Canada

**Introduction:** Circulating nucleic acid can be either harmful or therapeutic. On one hand, nucleic acids are potential pathogen-associated or danger-associated molecular patterns that modulate immune responses and the development of autoimmune disorders. On the other, nucleic acids such as poly I:C and siRNA are being developed for systemic delivery as adjuvants or targeted therapeutics, respectively. However, endosome entrapment and lysosome degradation present the major limitation in advancing nucleic acid-based therapies. Class
A scavenger receptors (SR-As) are a diverse group of pattern recognition receptors that recognize a variety of polyanionic ligands including nucleic acids. While SR-As are important for the uptake of extracellular dsRNA and a full complement of the endosomal and cytosolic pathways is required for a complete IFN response, little is known about extracellular DNA, despite its association with chronic infections and autoimmune disorders. The goal of this study was to better understand the recognition, uptake and trafficking of different forms of nucleic acids by SR-As.

**Methods:** We investigated the specificity of and requirement for SR-As in binding and internalizing different species, sequences and lengths of nucleic acids using a combination of competitive and non-competitive ligands, affinity electrophoresis and genetic manipulations. We further examined the role of SR-AI in activating the cytosolic signaling pathway by analyzing its interactome using BioID. We intend to study trafficking of nucleic acids using confocal fluorescence microscopy.

**Results:** We found that extracellular DNA is recognized and internalized by SR-As in a similar manner as extracellular dsRNA. Fucoidin (a competitive inhibitor of SR-A) reduced extracellular DNA and dsRNA uptake while fetuin (control) did not. We detected a direct interaction of RNA and DNA species with the coiled-coil/collagenous domain, but not the SRCR domain, of SR-As. SR-As can bind different species of nucleic acids despite the presence of additional surface receptor like RAGE that binds nucleic acids. The coiled-coil/collagenous domain of SR-As is sufficient to bind nucleic acids independent of species, sequence or length. The interactome of SR-AI, when the cells are stimulated with extracellular dsRNA suggests an increased vesicular and cytoskeletal activity. However, it requires further validation. Complimentary approaches to study nucleic acid trafficking using confocal fluorescence microscopy, it will enable us to explain any differences in trafficking of different forms of nucleic acids.

**Conclusion:** SR-As can bind different species of nucleic acids independent of species, sequence or length. The interactome of SR-AI, when the cells are stimulated with extracellular dsRNA suggests an increased vesicular and cytoskeletal activity. However, it requires further validation. Complimentary approaches to study nucleic acid trafficking using confocal fluorescence microscopy, it will enable us to explain any differences in trafficking of different forms of nucleic acids.

**Disclosure of Interest:** None Declared

**P068**

**A NOVEL MOLECULAR MECHANISM OF INFLAMMATION INVOLVING AP1 TRANSCRIPTION FACTOR**

**M. S. Baig**

Discipline of Biosciences and Biomedical Engineering (BSBE), Indian Institute of Technology Indore (IITI), Indore, India

**Introduction:** Macrophage plays pivotal roles in pathogen recognition and elimination as well as in the maintenance of tissue homeostasis (1-3). Acute inflammatory activation of macrophages by Toll-like and related receptors is characterized by transient activation of AP-1, NF-kB- and IRF-mediated signaling pathways and expression of pro-inflammatory genes (4-6). Identification and targeting of novel inflammatory molecules in the signaling cascades might be a good strategy to shoot out the chronic inflammatory responses.

**Methods:** TIRAP and c-Jun interaction was determined by confocal analysis as well as co-immunoprecipitation (Co-IP) assays. Downstream activation of AP1 transcription factor was analyzed by the expression of proinflammatory cytokines expression by Real-time PCR. The in vitro AP1 Reporter assay system was used to monitor the transcriptional activity of AP1 in cultured cells.

**Results:** In this study, we have investigated that TIRAP transactivates c-Jun and induces proinflammatory cytokine expression in macrophages. Transactivation of c-Jun facilitates its binding to other partners like Fos/ATF2 and translocates to the nucleus which results in proinflammatory cytokine expression and hence the inflammatory response.

**Conclusion:** TIRAP-mediated transactivation of c-Jun is essential for its binding with other partners like Fos/ATF2, translocation of AP1 heterodimer to the nucleus and proinflammatory cytokine expression. Targeting the interface site of TIRAP and c-Jun might be a good strategy to combat the chronic inflammatory response.

**Disclosure of Interest:** None Declared

**P069**

**A POLYMORPHIC RESIDUE THAT ATTENUATES THE ANTIVIRAL POTENTIAL OF INTERFERON LAMBDA 4 IN HOMINID LINEAGES**

C. G. G. Bamford1,2, E. Aranday-Cortes1, I. Cordeiro-Filipe3, J. L. Mendoza3, K. C. Garcia2, S. Fan3, S. Tishkoff4, J. McLauchlan1

1MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom, 2Howard Hughes Medical Institute, Department of Molecular and Cellular Physiology and Department of Structural Biology, Stanford University School of Medicine, Stanford, 3Departments of Genetics and Biology, University of Pennsylvania, Philadelphia, United States

**Introduction:** Interferons (IFNs) are typically considered to act as potent host antiviral cytokines 1. Counter-intuitively, expression of interferon lambda 4 (IFNλ4) is associated with reduced ability to clear hepatitis C virus (HCV) infection in humans 2. We wished to understand whether the evolution of human IFNλ4 could provide insight into this apparent paradox.

**Methods:** All known human IFNλ4 coding variants were identified, introduced into an expression system and the signalling activity and antiviral potential of each variant was screened alongside related orthologues. This included a range of gene expression/transcriptomic and antiviral assays against viruses such as HCV, Zika virus and influenza virus. The evolution of key variants was inferred using human genomic data and their mechanism of action was investigated guided by available structural data.
Results: We identify three natural human IFNA4 coding variants which significantly affected activity; a previously identified P70S variant as well as novel L79F and K154E variants. Contrasting with P70S and L79F variants, K154E enhanced activity in antiviral and gene expression assays. K154E was rare and found only in African Congo rainforest ‘Pygmy’ hunter-gatherers. Remarkably, E154 is the ancestral residue in mammalian IFNA4s and is highly conserved, yet the lower activity K154 has been fixed throughout evolution of the hominid genus Homo, including Neanderthals. Compared to chimpanzee IFNA4, the human orthologue had reduced activity due to the E154K. Meta-analysis of published gene expression data from HCV-infected humans and chimpanzees showed that this difference in activity between K154 and E154 in IFNA4 is consistent with differences in antiviral gene expression in vivo. Structurally, E154 is predicted to reside internally and mediate intramolecular interactions between receptor-binding helices. Human-specific K154 negatively affected IFNA4 activity by reducing its secretion and to a lesser extent potency.

Conclusion: Here we show that human IFNA4 has distinct properties. We demonstrate that a cytokine associated with reduced ability to clear viral infection has evolved attenuated activity in the human population compared to our closest living related species. We postulate that differences in IFNA4 activity between species contribute to distinct host specific responses to - and outcomes of - infection, such as HCV infection. Our work has implications for understanding how human IFNA4 promotes HCV chronicity.

Disclosure of Interest: None Declared

P070
INFECTION BY LACAZIA LOBOI IN MALNOURISHED AND NOURISHED BALB/C MICE
A. S. A. A. Barbosa1,2,3,*, M. P. Camargo2, V. N. Brito de Souza4, M. R. S. Nogueira1, F. R. Vilani-Moreno4, P. C. M. Pereira3
1Biology Technician Team, Institute Lauro de Souza Lima, 2Biomedical Systems, Faculty of Technology of Bauru, Bauru, SP, 3Tropical diseases, Botucatu Medical School - Paulista State University, Botucatu, SP, 4Immunology Technician Team, Institute Lauro de Souza Lima, Bauru, SP, Brazil

Introduction: Lacazia lobo causes Jorge Lobo’s disease (JLD), a chronic, granulomatous, cutaneous-subcutaneous infection. There are no reports regarding malnutrition on JLD in humans however previous studies in experimental JLD revealed an important interaction between nutritional status and immunity against L. lobo. The aim of this study was to evaluate the effects of protein-calorie malnutrition on the infection by L. lobo in BALB/c mice.

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 all the experiment. After 4 months, mice were euthanized for the collection of serum, peritoneal cells and inoculated footpads. The production of cytokines was evaluated in the serum by Cytometric Bead Assay and in culture of peritoneal cells by ELISA. The number of fungi and viability index were evaluated in the footpads. For statistic evaluation the significance level adopted was 5%.

Results: The viability index and number of fungi were higher in G3 than in G1. The evaluation of spontaneous production of cytokines by peritoneal cells revealed lower levels of IL-2, IL-10, IL-12 and IFN-γ and increase in the production of TNF in G1 and G2 when compared to G4 while no significant variation in IL-4 levels was observed. There is no difference in the serum levels of IL-2, IL-4, IL-10, IL-17A, IFN-γ and TNF among the groups, however IL-6 production was lower in G1 and G2 compared to G4.

Conclusion: Our results suggest that malnutrition alters the production of cytokines during the infection by L. lobo, reducing the levels of Th1 cytokines. However, the malnutrition status seems to favor the control of L. lobo infection by the host, suggesting considerable interaction between nutrition and immunity in JLD.

Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Nº 2017/15664-2).

Disclosure of Interest: None Declared

P071
SUBCELLULAR POSITIONING OF INNATE IMMUNE SENSORY PROTEINS IS IMPORTANT FOR THEIR FUNCTIONS.
K. C. Barnett1,2,*, J. C. Kagan1,2
1Harvard University, 2Boston Children’s Hospital, Boston, United States

Introduction: The cytosol of mammalian cells is an important site of innate immune activity. Multiple inflammatory signaling complexes are assembled in the cytosol and several pattern recognition receptors detect microbial ligands in this subcellular location. Despite the ample evidence supporting the importance of the cytosol for innate immunity, the precise subcellular positions of most innate immune regulatory factors within this compartment remain undefined.

Methods: In this study, we have explored cell biological aspects of various innate immune signaling pathways by utilizing structure-function analysis in combination with subcellular fractionation, confocal microscopy, and several assays for innate immune complex activation.

Results: Through these studies, we identified the plasma membrane as an important site of signaling protein residence. Analysis of candidate factors identified previously undefined domains that are important for signaling protein localization, and mutations within these domains disrupt host-defense responses in functionally important ways.

Conclusion: These findings highlight the importance of compartmentalization with the cytosol in the regulation of
host-pathogen interactions and create opportunities for themes to emerge that explain the organization of the innate immune networks within mammalian cells.

**Disclosure of Interest:** None Declared

**P072**

**THE PRODUCTION AND MATURATION OF INTERLEUKIN-18 IN ALZHEIMER'S DISEASE**

S. M. Becker1, D. Golenbock1, M. Heneka2, E. Latz2, B. S. Franklin2, T.-C. Tzeng3, T. Dierkes2

1Infectious disease, University of Massachusetts Medical School, Worcester, United States, 2University of Bonn, Bonn, Germany, 3University of Massachusetts Medical School, Worcester, United States

**Introduction:** Alzheimer’s Disease (AD) is a neurodegenerative disease that features chronic inflammation induced by amyloid beta (Ab) aggregates. The immune system responds to Ab by initiating a proteolytic cascade that produces interleukin-18 (IL-18) and interleukin-1 beta (IL-1β), both cleaved by caspase-1, which is activated by the inflammasome adaptor apoptosis-associated speck like protein containing a caspase recruitment domain (ASC). IL-18 has been implicated in AD and unpublished data shows that at an early stage of AD, IL-18 plays a protective role. In summary, IL-18 is an important cytokine in AD progression and severity. The current study sought to determine a source of IL-18 in the brain by investigating IL-18 production and maturation, as well as if the maturation of IL-18 is ASC dependent. Previously published data suggests that microglia produce IL-18 and that astrocytes are a potential source of IL-18.

**Methods:** Glial IL-18 production and maturation, as well as the proteolytic cascade upstream IL-18 maturation, was observed by western blot. Additionally, phagocytosis of ASC specks was observed by confocal microscopy, as supporting evidence of an ASC dependent mechanism of IL-18 maturation.

**Results:** We found that resting astrocytes produce pro IL-18 but not mature IL-18 while resting microglia produce both pro and mature IL-18. We also corroborated the hypothesis that microglia propagate inflammation by showing that resting microglia phagocytose ASC specks.

**Conclusion:** Further study is warranted to determine if and how astrocytes can produce mature IL-18 in Alzheimer’s Disease.

**Disclosure of Interest:** None Declared

**P073**

**IDENTIFICATION OF A NOVEL MECHANISM OF PARKIN TRANSLLOCATION TO MITOCHONDRIA REQUIRED FOR MITOPHAGY AND RESOLUTION OF ANTIVIRAL INTERFERON RESPONSES.**

S. Ponia1, S. Robertson1, A. Hay1, G. Sturdevant1, K. McNally1, E. Speranza1, S. Best1

1Laboratory of Virology, Rocky Mountain Laboratories, NIAID, Hamilton, MT, United States

**Introduction:** The large-scale emergence of Zika virus (ZIKV), a neurotropic flavivirus, in South America during 2014/15 was associated with severe disease in humans, including microcephaly of newborns. Anti-flavivirus innate responses are initiated by activation of cellular RNA helicases that signal through the adapter protein MAVS on the mitochondria to upregulate type I interferon (IFN) expression. Resolution of MAVS signaling is achieved in part through loss of mitochondrial membrane potential (MMP) that initiates degradation of depolarized mitochondria through mitophagy. Canonical mitophagy is controlled by two proteins that are mutated in early onset Parkinson’s disease (PD), PINK1 (a serine-threonine kinase) and Parkin (an E3 ubiquitin ligase). Following loss of MMP, PINK1 is stabilized on mitochondria and is thought to activate Parkin although key molecular details including how Parkin is recruited to mitochondria are not understood. By examining MAVS signaling in the context of ZIKV, we have identified a novel protein (here termed Translocator of Parkin 1 or TOP1) critical for PINK1-Parkin-dependent mitophagy.

**Methods:** We examined MAVS-dependent signaling and protein-protein interactions in cells overexpressing or deficient in TOP1, PINK1 or Parkin.

**Results:** MAVS activation by virus infection or by MAVS overexpression caused translocation of the normally cytosolic TOP1 to mitochondria. Expression of TOP1 with MAVS resulted in lysosome- and proteasome-dependent degradation of MAVS and suppressed IFN expression, but TOP1 did not directly interact with MAVS. Therefore, we investigated the effects of TOP1 on mitophagy in cells deficient for PINK1 or Parkin. Following loss of MMP by the drug CCCP, cytosolic TOP1 also moved to mitochondria. TOP1 translocation was dependent on Parkin, as it did not occur in Parkin-deficient cells. Consistent with this, TOP1 interacted with Parkin by reciprocal co-immunoprecipitation. Interestingly, co-expression of TOP1 with Parkin caused Parkin translocation even in the absence of MMP loss or PINK1. However, once at mitochondria, TOP1 interacted with PINK1 and resulted in Parkin-mediated degradation of TOP1 suggesting a negative feedback loop to prevent aberrant mitophagy. Importantly, MEFs from TOP1−/− mice had significant defects in mitophagy induced by CCCP, increased MAVS-dependent IFN responses, and reduced virus replication, thereby demonstrating a role for TOP1 in primary cells.

**Conclusion:** These results reveal TOP1 as an essential component of PINK-Parkin mitophagy that regulates MAVS-dependent IFN responses at the mitochondria, and may be a therapeutic target in treatment of neurological disorders including PD and ZIKV infection.

This work was supported by the Division of Intramural Research, National Institutes of Allergy and Infectious Diseases, NIH.

**Disclosure of Interest:** None Declared

**P074**
Introduction: RIG-I is essential for recognition of viral dsRNA and the activation of a cell-autonomous antiviral response. Upon stimulation, RIG-I triggers a signaling cascade leading to the expression of cytokines, most prominently type I and III interferons (IFNs). Upon secretion, IFNs signal in an autocrine and paracrine manner, triggering the expression of an ample array of IFN-stimulated genes (ISGs), which in concert establish a strongly antiviral state of the cell. While the topology of these pathways has been studied quite intensively, the dynamics, particularly of the RIG-I-mediated IFN induction, is much less understood. In this study, we characterize the kinetic properties of this signaling system and set up a mathematical model capable of accurately describing the dynamics from introduction of dsRNA to expression of ISGs upon IFN signaling.

Methods: We developed a technique to synchronously stimulate the RIG-I response of A549 cells, which allowed us to monitor phosphorylation, localization and expression of critical proteins in the RIG-I and IFN signaling cascades in a highly time-resolved manner. We employed live cell microscopy (IRF3 translocation), quantitative western blotting (total protein and phospho-specific) and qRT-PCR to follow individual steps along the signaling pathway. This time-resolved, quantitative data was then used to set up and calibrate a comprehensive mathematical model of the core RIG-I pathway, which we have further linked to an existing model of IFN/JAK/STAT signaling [1].

Results: A previous study [2] reported intriguing stochasticity in the activation of IRF7 upon virus infection of murine cells. Surprisingly, we found RIG-I signaling to be highly deterministic, at least under the conditions we applied (high dsRNA concentration, snap-transfection), suggesting that the previously observed stochasticity was largely due to staggered uptake of the stimulatory RNA during infection. Our time-resolved data was, hence, optimally suited to set up and calibrate a dynamic mathematical model of the core RIG-I pathway, capable of accurately predicting the activation state of all canonical steps of RIG-I signaling, including the production of IFNs. Extending this model to the full antiviral system, we then included IFN secretion and autocrine IFN signaling through the JAK/STAT pathway. We validated this comprehensive pathway model by data from wildtype cells versus cells lacking the type I and III IFN system (IFNAR/IFLR double knockout). Currently, we are applying it to study key determinants of virus-host interaction in the early phase of viral infection, and characterize how the expression of virus-encoded antagonists of the antiviral system affects the establishing of the antiviral state.

Conclusion: We present a comprehensive dynamic pathway model of the RIG-I mediated induction of an antiviral state of the cell. This model is perfectly suited to further study early events in innate antiviral immunity, particularly the intricate interplay of this host antiviral system with virus-encoded antagonists of innate immunity.

Disclosure of Interest: None Declared
HIGH THROUGHPUT CRISPR SCREENING IDENTIFIES GENES CRITICAL FOR MACROPHAGE SURVIVAL AND FUNCTION
S. Carpenter, S. Covarrubias, M. Boettcher, M. McManus

Introduction: The innate immune system protects against infections by initiating an inducible inflammatory response. NF-κB (NF-κB) is one of the critical transcription factors controlling this complex response.

Methods: We have generated a GFP-based NF-κB reporter system in immortalized murine bone marrow-derived macrophages (iBMDMs). Activation of this reporter, using Toll-like receptor ligands, results in GFP expression, which can be monitored by flow cytometry. We also established a CRISPR/Cas9 system in these NF-κB reporter lines, enabling us to screen for genes that regulate NF-κB signaling.

Results: We have now performed a whole genome pooled CRISPR screen targeting all proteins and microRNAs in the mouse genome. Our libraries contain 12 guide-RNAs targeting each gene. We also included ~5000 negative (non-targeting) control guide RNAs and a number of positive-control genes that are known to be involved in inflammation. The library was introduced into our Cas9-NF-κB reporter macrophages using lentiviral transduction (MOI of 0.3) to allow approximately one integration per cell. Cells were stimulated with LPS for 24h and were then sorted, isolating the top and bottom 25% of GFP cells hypothesizing that these include the genes that are downregulating or upregulating our NF-κB reporter. We isolated genomic DNA and performed high-throughput sequencing to identify the guide RNAs of the genes that were targeted. Using this approach, we identified over 900 genes that positively or negatively regulate NF-κB. The top 40 hits of the screen are all part of the NF-κB pathway confirming that the screen was successful. Interestingly there are currently 106 genes documented to regulate NF-κB (GO terms and Kegg analysis) and using our approach we have identified nearly 9 times as many genes. In addition to information on the regulation of the reporter we were also able to identify genes critical for survival of macrophages. These are genes that drop out of the screen when we compare our original library to the library at the end of the 7 days selection period.

Conclusion: We have greatly expanded the repertoire of genes involved in the complex inflammatory signaling cascade as well as documenting genes critical for macrophage survival. Use of high throughput CRISPR screening is an extremely powerful tool enabling rapid meaningful data to be obtained in a highly efficient manner. This library could easily be used by any researcher to investigate which genes are involved in any other particular biological process.

Disclosure of Interest: None Declared

P078 INNATE DETERMINANTS OF HERPES STROMAL KERATITIS DEVELOPMENT IN HSV-1 INFECTED CORNEAS
K. Carroll, H. Yun, A. M. Rowe, A. J. St. Leger, R. L. Hendricks

Introduction: Herpes stromal keratitis (HSK) is a painful, potentially blinding immunopathological condition that can result after herpetic simplex virus type 1 (HSV-1) corneal infection. Although most individuals are infected with HSV-1 by adulthood, not all develop HSK. Here, we use a pathogenic (RE) and non-pathogenic (KOS) strain of HSV-1 to show that the initial monocytic response to the virus is a determinant of future pathology.

Methods: We infected C57BL/6 mice via the cornea with HSV-1 RE or KOS and corneas were scored by the following criteria: 1) opacity, 2) neovascularization, and 3) loss of corneal sensitivity. Eye swabs of the cornea were taken to quantify viral replication during initial infection and T cell expansion was assessed by flow cytometry from the eye draining lymph nodes (EDLN). RT-PCR and flow cytometry of corneas was used to assess chemokine/ cytokine production and immune infiltrate and functionality. Subconjunctival injection of clodronate liposomes or intraperitoneal injection and topical application of aminoguanidine (iNOS inhibitor) was used to assess macrophage and monocyte contribution to HSK development.

Results: Eyes infected with RE developed severe HSK while KOS-infected eyes developed mild, transient HSK. Despite the clear differences in pathology between the two viruses, viral titers and clearance at the cornea as well as T cell expansion in the EDLNs were similar in the two infections. Differences in pathology were maintained when mice were infected with RE in one eye and KOS in the contralateral eye. Additionally, RE-infected corneas had significantly higher mRNA levels of the proinflammatory cytokine, IL-6, and chemokine, CXCL1, as compared to KOS infected corneas 2 days post infection (dpi). Further, in these bilaterally infected mice, more neutrophils and monocytes infiltrated and were maintained in RE-infected corneas compared to KOS-infected corneas beginning at 3 dpi, while the numbers of corneal macrophages remained consistent between RE and KOS-infected eyes. Notably, all three cell types from RE-infected eyes produced more iNOS throughout acute infection compared to KOS-infected eyes. Finally, attempts to limit iNOS production in the cornea, either by local monocyte and macrophage depletion or selective iNOS inhibition, delayed disease in RE-infected eyes, suggesting that differential stimulation of this pathway by different strains of HSV-1 contributes to disease.

Conclusion: Because viral replication and adaptive immune expansion were not significantly altered between
the two viruses, our results indicate that the disparity in pathology is likely due to differences in viral sensing within the local corneal environment during acute infection. This is supported by the maintenance of viral phenotypes in mice bilaterally infected with RE in one eye and KOS in the contralateral eye. In addition, analysis of the local environment in bilaterally infected mice showed a significantly higher proinflammatory response in RE-infected corneas at very early times after infection, which suggests that modulating the inflammatory response during primary infection may be beneficial in preventing blinding disease.

Disclosure of Interest: None Declared

P079
HUMAN MACROPHAGE POLARIZATION IN THE RESPONSE TO MYCOBACTERIUM LEPRAE DNA
K. van Huss1, B.-Y. Hong2, M. Rodriguez3, E. Vazquez3, J. L. Cervantes3,1
1Texas Tech University Health Sciences Center, El Paso, 2The Jackson Laboratory, Farmington, United States

Introduction: Leprosy is still endemic in some parts of the U.S. like Southern Texas. The spectrum of disease ranges in severity from tuberculoid leprosy to lepromatous leprosy, and these two forms appear to be linked to macrophage polarization as well as to the host’s innate and adaptive immune responses.

It is thought that genetic variability of the causative pathogen, Mycobacterium leprae, contributes little in explaining the different clinical forms, and that virulence differences are linked to the immunogenetic background of the host. We aimed to analyze the inflammatory response, at both ends of macrophage polarization, to bacterial DNA from different M.leprae strains.

Methods: Human monocytic-macrophage cell line THP-1, was differentiated into M1 or M2 macrophages using standard methodology (i.e., M-CSF, and M-CSF/IFN-γ+LPS). Cells were then stimulated with 500ng of genomic DNA from M.leprae strains Br4923, NHDP, and Thai-53 (bei resources, VA). Polyethilenimine (PEI) was used as an endo-lysosomal bacterial nucleic acid delivery system. Initial response after stimulation with M.leprae DNA was assessed in dual THP-1 cells which contain reporter plasmids for NF-kB and IRF-mediated activation (Invivogen). Total RNaseq was used to evaluate the transcriptome of M1 and M2 cells at baseline and after stimulation with the DNA from the different M.leprae strains.

Results: DNA from Thai-43 strain elicited a higher number of differentially expressed (DE) genes in M1 and M2 macrophages compared to the other two strains. When looking at macrophage polarization, M1 activated macrophages showed a higher number of DE compared to M1, and M2, after stimulation with DNA from all three strains. Several inflammatory and interferon regulatory pathways were identified in the transcriptome analysis.

Conclusion: Different from what is currently believed, strain genomic variations may be able to induce differential gene expression in human macrophages. Findings from this study help to elucidate the mechanisms of macrophage polarization in the recognition of M.leprae DNA. A deeper understanding of the role of macrophage polarization and activation in the recognition of M. leprae could lead to better future treatment strategies for the disease.

Disclosure of Interest: None Declared

P080
HIGHER LEVEL OF TLR2 ON GLIOMA-INFILTRATING MICROGLIA DRIVES MHC I CROSS-PRESENTATION TO CTL RESPONSE
1Immunotherapeutics Branch, National Cancer Center, 2Department of Cancer Biomedical Science, Graduate School of Cancer Science and Policy, 3Particle Therapy Research Branch, National Cancer Center, Goyang-si, Korea, Republic Of, 4Division of Genetics, Cancer Research Institute, Kanazawa, Japan

Introduction: Glioblastoma multiforme (GBM), most malignant form of astrocytoma, contains significant numbers of infiltrating microglia that may interact with tumor cells and influence cancer progression. Toll-like receptor (TLR)2 is a critical sensor for a diverse range of tissue damage and upregulated on activated microglia in the setting of GBM. Tumor-infiltrating microglia have been shown to exist in heterogeneous population to initiate antitumor immunity or immune suppression. We investigate high TLR2-positive population in glioma-infiltrating microglia and their antitumor efficacy.

Methods: C57BL/6 mice were implanted with murine glioma cell line GL26. Glioma infiltrating microglia were isolated on 40:80% Percoll gradients from enzymatic cell dissociation of glioma cell line GL26. Glioma infiltrating microglia were analyzed by immunofluorescent stain, flow cytometry and intracellular cytokine staining. Intracranial injections of genomic DNA from M. leprae strain genomic variations may be able to induce differential gene expression in human macrophages. Findings from this study help to elucidate the mechanisms of macrophage polarization in the recognition of M. leprae DNA. A deeper understanding of the role of macrophage polarization and activation in the recognition of M. leprae could lead to better future treatment strategies for the disease.

Disclosure of Interest: None Declared
system in antitumor immune responses of microglia against brain tumor.  

**Disclosure of Interest:** None Declared

**P081**  
INTERFERON LAMBDA 4 ACCUMULATES IN THE ENDOPLASMATIC RETICULUM AND CAUSES ER STRESS  
Q. Chen1,*, M. Coto1, A. Suslov1, S. Wieland1, M. H. Heim1  
1Department of Biomedicine, University Hospital Basel, Basel, Switzerland

**Introduction:** Genetic polymorphisms in the interferon lambda 4 (IFNL4) gene strongly correlate with spontaneous and treatment induced clearance of HCV. An insertion/mutation at rs368234815 (IFNL4-TT allele) disrupts the open reading frame of IFNL4. Surprisingly, the TT/TT genotype is strongly associated with spontaneous HCV clearance, whereas the fully functional IFNL4-ΔG allele has a negative impact on the immune response to HCV. The apparent paradox that having an additional functional IFN is a disadvantage for an antiviral immune response is even more intriguing because recombinant IFNL4 produced in HepG2 cells displays even stronger IFN activity than IFNL3. Thus in this study, we wanted to explore if there are differences between IFNL4 and other IFNs in regard to induction, biosynthesis, secretion and induction of Jak-STAT signaling that could explain the negative impact of IFNL4 on the immune response to HCV.  

**Methods:** IFNL4 was transiently expressed in Huh7 cells. Intra-cellular localization over time was assessed by Western blotting, immunofluorescence and subcellular fractionation. The activity of intracellular and secreted IFNL4 was compared using IFNL receptor expressing Huh7 cells. ER stress responses to IFNL4 were assessed by sXBP1 and CHOP quantification in IFNL4-overexpressing Huh7 cells, during tet-inducible expression in HepG2 cell lines and in Sendai virus (SeV) infected hepatoma cell lines.  

**Results:** IFNL4, in contrast to IFNL3, is very poorly secreted and accumulates intracellularly. The intracellular IFNL4 is much less active than the secreted form. The secreted form is about 40 fold more potent than IFNL3. Immunofluorescence analysis and subcellular fractionation showed that IFNL4 is primarily localized in ER associated membranes. IFNL4 accumulation causes ER stress not only in IFNL4 transfected cells but also in SeV infected cells.  

**Conclusion:** IFNL4 is very poorly secreted and accumulates in ER-associated membranes and causes ER stress. IFNL4 acquires its full IFN activity through posttranslational modifications after leaving the ER during the secretion process. Secreted IFNL4 is highly potent. Intracellular retention of IFNL4 causes significant ER stress. We propose that the negative impact of IFNL4 on the immune response to HCV is not caused by the small amounts of secreted IFNL4, but by cell intrinsic consequences of its accumulation in the ER. Experiments are ongoing to test the hypothesis that IFNL4 expression dependent ER stress might negatively impact on the adaptive immune response to HCV by impairing MHC class I antigen presentation in HCV infected cells.  

**Disclosure of Interest:** None Declared

**P082**  
VALIDATION THE ROLE OF CTRP6 IN CHRONIC KIDNEY DISEASE PROGRESSION  
H. H. Chi1,*, M. A. Murayama1,2, Y. Iwakura1  
1Division of Experimental Animal Immunology, Center for Animal Disease Models, Research Institute for Biomedical Sciences, Tokyo University of Science, Noda, Chiba, 2Department of Immunology and Medicine, St Marianna University School of Medicine, Kawasaki, Japan

**Introduction:** Chronic kidney disease is defined by continual urine abnormalities, renal tissue abnormalities or impaired renal function suggestive of a loss of functional nephrons. Activation of complement system are involved in both immunocomplex mediated glomerular lesion and tubulointerstitial lesion progressions. Here we identified the CTRP6, a regulator of alternative complement pathway, exhibited protective effects in tubulointerstitial lesions development and IgA glomerulonephritis progression.  

**Methods:** Unilateral ureteral obstruction (UUO) and ischemia/reperfusion (IR) served as tubulointerstitial lesions model. Wild type, CTRP6, complement 3 (C3) or CTRP6/C3 deficient mice subjected to UUO or IR were sacrificed in different time course, and renal sections were used for histopathology comparison. IgA nephropathy (IgAN) in mice was induced by passively IgA and antigen administration, resulting in systemically IgA immunocomplex formation and deposited in glomerulus. Daily urinary protein test and histopathology analysis among different strains to distinguish the role and mechanism of CTRP6 in IgAN development.  

**Results:** Under UUO or IR induction, the mice deficient of CTRP6 showed exacerbated renal parenchymal loss, interstitial inflammation and fibrosis, in which featured increased C3 deposition at tubules. However, the anti-fibrosis pathway of CTRP6 was not only through inhibition of complement activation, but also through inhibition of myofibroblast differentiation directly. Under IgAN induction, the mice deficient of CTRP6 showed exacerbated proteinuria, glomerular lesions and blood urine nitrogen, in which featured increased complement 3 (C3) deposition in glomeruli. Moreover, such pathologic exacerbation caused by CTRP6 deficiency could be rescued by additional C3 deficiency (CTRP6/C3 double deficiency), implicated the regulation mechanism of CTRP6 in IgAN disease progression.  

**Conclusion:** Our findings provide a basis for the development of potential treatment for glomerulonephropathy (IgAN) and tubulointerstitial lesions progressions by CTRP6.  

**Disclosure of Interest:** None Declared
P083
CYTOMEGALOVIRUS IE1 PROTEIN TARGETING PML BODIES IS ESSENTIAL FOR TROPHOBLAST INFECTION BUT PP65 OR PP71 TARGETING IFI16 AND DAXX ARE DISPENSABLE
K. Y. Choi1, J. Hornig1, A. McGregor1
1Microbial Pathogenesis & Immunology, Texas A&M University Health Science Center, College Station, United States

Introduction: Human cytomegalovirus (HCMV), a betaherpesvirus, is a leading cause of congenital disease resulting in mental retardation and deafness in newborns. The guinea pig is the only small animal model for congenital CMV but requires species specific guinea pig CMV (GPCMV). This model is used to study virus pathogenicity and the development of intervention strategies. We evaluated GPCMV tegument proteins (pp65 and pp71 potential homologs) and immediate early protein IE1 and their roles in overcoming the cell innate immunity. In HCMV, IE1 and pp71 target the nuclear ND10 bodies to cause their disruption. Cellular IFI16 is targeted by HCMV pp65. Both IFI16 and ND10 bodies represent important obstacles to HCMV infection. The placenta expresses the highest levels of ND10 components compared to other tissues and indicates that nuclear ND10 bodies are important obstacles to CMV trophoblast infection.

Methods: Studies were carried out on guinea pig cell lines: embryo fibroblast cells (GPL); villous trophoblasts; and renal epithelial cells. Guinea pig ND10 major components (gpPML, gpSP100, gpATRX, gpDAXX) and gpIFI16 were generated synthetically. GPCMV IE1 mutant virus (Horning et al., 2017) was modified to encode the glycoprotein pentameric complex (PC) to enable virus tropism to various cell types. Knockout mutant GPCMV of tegument genes GP83 (pp65 homolog) and GP82 (pp71 homolog) were generated by BAC mutagenesis. Mutant viruses were designated GP83PC+ and GP82PC+ respectively. Virus pathogenicity studies were carried out in Hartley guinea pigs.

Results: IE1 targeted nuclear guinea pig ND10 components (gpPML, gpSp100) to cause disruption of ND10 bodies. The tegument protein GP82 (pp71 homolog) interacted with ND10 component gpDAXX. A partial C-terminal truncated IE1 mutant resulted in a viable virus (IETPC+) with delayed fibroblast growth kinetics. The truncated IE1 protein was unable to interact with gpPML or disrupt ND10 bodies. Overexpression of PML or SP100 reduced IETPC+ virus growth but not WT GPCMV. IETPC+ virus had increased susceptibility to IFN-I (95% inhibition at 100U/ml) compared to WT virus (5% inhibition). However, inhibition could be overcome by use of a JAK/STAT inhibitor or growth on a fibroblast cells expressing BVDV npro protein (targeting IRF3), which restored growth kinetics. A pp65 knockout virus (GP83PC+) was capable of near normal growth on fibroblasts but was impaired on cells that over expressed gpIFI16 or treated with IFN-I. A pp71 knockout mutant virus (GP82PC+) had delayed kinetics on fibroblasts. Pathogenicity studies of IETPC+, GP83PC+ and GP82PC+ viruses in guinea pigs revealed that IE1 and GP83 mutant viruses were impaired for dissemination to target organs. Next, IETPC+, GP83PC+ and GP82PC+ viruses were evaluated on trophoblasts. Both GP83 and GP82 mutant viruses were capable of growth but with impaired kinetics and GP83PC+ virus infection could be inhibited by overexpression of IFI16. In contrast, the IETPC+ virus was incapable of growth on trophoblasts regardless if JAK/STAT inhibitor, or npro expressing cells were used.

Conclusion: Requirements for infection of trophoblasts is highly dependent upon a functional IE1 protein to disrupt ND10 bodies. An inability to target IFI16 by pp65 knockout virus is less important, unless cells were stimulated by IFN-I to over express IFI16.

Disclosure of Interest: None Declared

P084
IL-33 AMPLIFIES THE INNATE INFLAMMATORY RESPONSE TO T. GONDII
J. T. Clark1,*, D. Christian1, J. Park1, M. Jacquet 1, J. Silver2, C. Hunter1
1School of Veterinary Medicine - Pathobiology, University of Pennsylvania, Philadelphia, PA, 2Respiratory Inflammation & Autoimmunity, MedImmune, Gaithersburg, MD, United States

Introduction: Mice deficient in IL-33 or its receptor ST2 are susceptible to infection with the protozoan parasite Toxoplasma gondii, but how IL-33 promotes resistance to this infection is unclear. Previous reports have attributed this susceptibility to an excessive type 1 response and lethal immune pathology in the central nervous system (CNS). In recent years, a direct role for IL-33/ST2 in enhancing type 1 responses has been appreciated. In the current studies, we focused on the role of IL-33 in the early innate response to T. gondii infection by using Rag2−/− mice.

Methods: We infected mice with the ME49 strain of T. gondii and treated them with either PBS or rIL-33. At various timepoints, we analyzed cellular responses by flow cytometry and histology, cytokine production by ELISA and flow cytometry, and parasite burden by histology and qPCR.

Results: We found that IL-33 stimulation ampliﬁed IL-12-dependent NK cell production of IFN-g and GM-CSF. Additionally, we found that IL-33 treatment of Toxoplasma-infected Rag2−/− mice led to the recruitment and expansion of CCR2+ monocyctocytic cells at the site of infection in an IL-12 and IFNg-dependent manner. This treatment led to upregulation of iNOS in tissues and a marked reduction in parasite burden.

Conclusion: As IL-33 is expressed at high levels in many tissues, including the central nervous system, these results suggest that IL-33 enhances the inﬂammatory response to T. gondii and consequently control of this parasitic infection.

Disclosure of Interest: None Declared
**P085**
**UNDERSTANDING TLR-INDUCED MYDDOSOME FORMATION AND SIGNALLING**

**D. De Nardo**¹, K. R. Balka¹, Y. Cardona Gloria², V. R. Rao², E. Latz², S. L. Masters³

¹Inflammation Division, The Walter And Eliza Hall Institute Of Medical Research, Parkville, Australia, ²Institute of Innate Immunity, Bonn, Germany, ³Inflammation and Immunology, Pfizer Inc., Cambridge, United States

**Introduction:** Toll-like receptors (TLRs) form part of the host innate immune system, where they act as sensors of microbial and endogenous danger signals. Upon activation, the intracellular Toll/Interleukin-1 receptor (TIR) domains of TLR dimers form a platform for oligomerisation of a multiprotein signalling platform comprising MyD88 and members of the IRAK family, termed the Myddosome. Formation of the Myddosome complex initiates signal transduction pathways leading to the activation of transcription factors and ultimately, the production of inflammatory cytokines. Despite the critical role Myddosome formation plays in initiating TLR-induced signalling, the molecular mechanisms controlling Myddosome function remain poorly defined.

**Methods:** Using immunoprecipitation approaches we successfully isolated Myddosome complexes from whole cell lysates of TLR activated primary mouse bone marrow-derived macrophages (BMDMs) and from IRAK-deficient immortalised BMDMs reconstituted with WT and mutant forms of IRAKs via retroviral transduction. Through the use of a selective IRAK4 inhibitor we were able to examine the role of IRAK4 kinase activity within the Myddosome. Immunoblot and immunofluorescence techniques were used to assess TLR signalling and NF kappaB translocation respectively, while ELISAs were employed to examine the secretion of pro-inflammatory cytokines into the supernatant.

**Results:** Here we demonstrate the kinetics of the Myddosome upon TLR activation, revealing rapid assembly and slow disassembly. Furthermore, we show that inhibition of IRAK4 kinase activity leads to increased stability of the Myddosome complex as demonstrated by greater associations between MyD88 and IRAK4. Importantly, we found that the kinase activity of IRAK4 is dispensable for TLR-mediated NFkB and MAPK signalling but essential for production of inflammatory cytokines.

**Conclusion:** To our knowledge this is the first full examination of the kinetics of the Myddosome from macrophages. We further demonstrated that a loss of IRAK4 activity by either chemical inhibition or genetic manipulation resulted in a significantly more stable Myddosome structure. This increase in stability is suggestive of a prominent protein scaffold role of IRAK4, independent of its kinase activity, in which IRAK4 interacts with MyD88 and IRAK1, tethering them together into the Myddosome complex. Our findings may help explain why, to date, therapeutic targeting IRAK4 kinase activity has not been as successful as hoped, and highlights that targeting the scaffold function of IRAK4 may be an attractive alternative.

**Disclosure of Interest:** D. De Nardo: None Declared, K. Balka: None Declared, Y. Cardona Gloria: None Declared, V. Rao Employee of: Pfizer Inc., E. Latz Consultant for: IFM Therapeutics, S. Masters Consultant for: Glaxosmithkline

---

**P086**
**ELF1 ELICITS A NOVEL TRANSCRIPTIONAL PROGRAM WITH BROAD ANTIVIRAL ACTIVITY, WHICH FUNCTIONS INDEPENDENTLY OF THE IFN RESPONSE**

L.-L. Seifert¹, S. Ballentine², A. Briley², C. Si², M. deVries², D. Saha¹, B. Rosenberg³, S. Tripathi², M. Dittmann¹,²,³

¹The Rockefeller University, ²NYU School Of Medicine, ³Icahn School of Medicine at Mount Sinai, New York, United States

**Introduction:** Type-I interferons (IFNs) are major innate immune cytokines produced by cells upon viral infection. Our detailed knowledge about IFN induction and downstream signaling sharply contrasts with our rather scarce knowledge about how IFNs inhibit viruses. Hundreds of interferon-stimulated genes (ISGs) execute the antiviral function of IFN, yet, for the vast majority, the molecular mechanisms remain a mystery. We recently identified the ISG E74-like ETS transcription factor 1 (ELF1) as a potent inhibitor of influenza A virus (IAV). ELF1 has never before been characterized as an innate antiviral effector. Other groups have established an association of ELF1 with severe inflammatory conditions such as Crohn’s disease, lupus erythematosus, and inflammatory bowel disease, but the underlying mechanisms remain elusive. However, their findings and ours raise the exciting possibility that ELF1 is a novel regulator of inflammation and innate antiviral immunity.

**Methods:** We used a combination of in vitro assays, including high-content microscopy, virus infections, and RNAseq, to identify the mechanism by which ELF1 inhibits viruses. Further, we used an intranasal infection model in mice to determine the physiological relevance of ELF1 during influenza A virus infections.

**Results:** We show that ELF1 is broadly antiviral, and acts after multiple rounds of viral replication. ELF1 transcriptionally regulates a vast program of close to 600 downstream signaling sharply contrasts with our rather scarce knowledge about how IFNs inhibit viruses. However, their findings and ours raise the exciting possibility that ELF1 is a novel regulator of inflammation and innate antiviral immunity.

**Conclusion:** Our combined results suggest that ELF1 triggers the expression of a unique antiviral gene set, which is similar to that of IFN in terms of broadness and potency. However, it is distinct from and temporally succeeds the program of the IFN response, and might
continue protecting cells from viral infections even in the refractory phase post IFN exposure, or when viral antagonists shut down IFN signaling.

Disclosure of Interest: None Declared

P087
INTERFERON-BETA AMPLIFIES IL-10 PRODUCTION BY MACROPHAGES IN A STAT1-DEPENDENT MANNER TO FEEDBACK INHIBIT ITS OWN EXPRESSION
H. Dickensheets1, N. Lee1, F. Sheikh1, D. Ireland1, A. Gamo1, R. P. Donnelly1.
1Office of Biotechnology Products, FDA Center for Drug Evaluation and Research, Silver Spring, 2Department of Medical Genetics and Molecular Biochemistry, Temple University School of Medicine, Philadelphia, United States

Introduction: The inhibitory effects of IL-10 on production of pro-inflammatory cytokines such as TNF-alpha, IL-1 beta and IL-6 by macrophages are well known. However, the effects of IL-10 on expression of interferon (IFN) and IFN-stimulated genes (ISGs) are not well defined.

Methods: In this study, we used bone marrow-derived macrophages from wild-type (WT), IFN-beta gene knockout (Ifnb1 KO) and IL-10 gene knockout (Il10 KO) mice to examine the role of IFN-beta in the induction of pro- and anti-inflammatory cytokines by the TLR4 agonist, lipopolysaccharide (LPS).

Results: LPS stimulation induced significantly higher levels of IL-10 expression by WT macrophages compared to Ifnb1 KO macrophages. The decreased levels of IL-10 expression by Ifnb1 KO macrophages correlated with increased expression of pro-inflammatory genes such as IL-1 beta, IL-6 and IL-12. Co-treatment of WT macrophages with IFN-beta plus LPS induced significantly higher levels of IL-10 expression than did treatment with LPS alone. In contrast, co-treatment of WT macrophages with IL-10 plus LPS markedly decreased expression of IFN-beta and ISGs such as Cxcl10, Ifit1 and Mx1. Furthermore, LPS induction of IFN-beta and ISG expression was higher and more prolonged in IL-10 KO macrophages than in WT macrophages. Moreover, the ability of IFN-beta to up-regulate IL-10 expression by LPS-stimulated macrophages was mediated by signaling through type-1 IFN receptors in a STAT1-dependent manner.

Conclusion: Our findings define an auto-regulatory loop in which LPS stimulates rapid production of IFN-beta by macrophages, and IFN-beta then markedly enhances production of IL-10. Once produced, IL-10 feedback inhibits IFN-beta expression to prevent continued activation of this pathway.

Disclosure of Interest: None Declared

P088
OXIDIZED PHOSPHOLIPIDS AS INNATE IMMUNE MODULATORS
M. Ernandes1, J. Kagan2

1Virology, Harvard University, 2Gastroenterology, Boston Children’s Hospital, Harvard University, Boston, United States

Introduction: Oxidized Phospholipids, in conjunction with pattern recognition receptor (PRR) signaling, lead to the establishment of the “hyperactive phenotype” in dendritic cells (DCs) allowing for IL1β secretion independent of cell death (Zanoni 2016). We have assessed how coadministration of these compounds alters the PRR output from DCs, as well as the downstream effects on B cell responses in mice.

Methods: Two distinct types of DCs were generated. First, via the differentiation of bone marrow dendritic cells (BMDCs) from mice. Second, via the isolation of splenocytes induced by the injection of wild type B6 mice with B16-F113L expressing cells. These cells were treated with defined PRR ligands or inactivated influenza virus, along with oxidized PAPC (oxPAPC) and oxPAPC constituent components (PGPC and POVP). Cytokine production and cell viability was then monitored. The stimulating compounds are also injected into mice subcutaneously along with ovalbumin in order to assess whether these compounds alter antigen-specific antibody responses in the serum, bronchoalveolar lavage, and in the feces.

Results: Unlike other inflammasome stimuli, which require cells to be pre-exposed to PAMPs in order to elicit IL-1β release, coadministration of LPS and PGPC induced an increase in the release of IL-1β from BMDCs. Similar increases were observed in secretion of TNFα and IL-6, as compared to similar treatment with LPS alone or LPS pre-administration followed up with PGPC. Perhaps most interesting, we observed that in response to iPR8 and PGPC, TNFα and IL-6 are elevated more than one hundred fold in the case of coadministration as compared to other methods of stimulation. These results suggested that PAMP/oxPAPC mixtures would induce a superior adaptive immune response in mice, as compared to traditional immunization regimens that involve PAMPs alone. Consistent with this prediction, co-injection of mice with LPS, oxidized phospholipids and the model antigen ovalbumin lead to an augmentation of the antigen-specific antibody response, as compared to LPS-ovalbumin immunizations.

Conclusion: These results suggest that oxidized phospholipids could be used to improve current adjuvant methodology in vaccine creation, especially given the increase in antibody binding titers extends to mucosal sites, where infection is most likely to occur.

Disclosure of Interest: None Declared

P089
A GENOME-WIDE SCREEN IDENTIFIES A CRITICAL ROLE FOR MITOCHONDRIAL NDP KINASES IN INFLAMMASOME ACTIVATION
O. Ernst-Rabinovich1, J. Sun1, B. Lin1, B. Banoth2, C. Rice2, S. Katz2, S. Jacob Vayttaden1, M. Dorrington2, J. Liang1, N. Slepushkina4, E. Buehler4, Z. Wang1, D.
**Introduction:** While there has been remarkable progress in understanding the mechanisms of inflammasome activation that follow assembly of the inflammasome multi-protein complex, the cellular processes that precede inflammasome activation are less clearly understood.

**Methods:** In an effort to address this, we have conducted a genome-scale siRNA screen for the non-canonical inflammasome response to cytosolic LPS. We have used a screening-optimized HTRF assay for secreted IL-1α in RAW264.7 mouse macrophage cells. Through a combination of LDH and TNFα measurements we have filtered and prioritized hits in a secondary screen. We used siRNA knockdown in primary BMDM and CRISPR/Cas9 knock out of hit genes both in macrophage cell lines and mice to further validate and study novel mechanisms of inflammasome activation.

**Results:** Among the top screen hits we identified numerous expected genes, including *Myd88, Irak4, Irak2, Casp4, Gsdmd* and *Il1a*. We also noted a significant enrichment of mitochondrial-associated genes in the screen, supporting an important role for the mitochondria and cellular metabolism in inflammasome activation. Among these genes we identified three nucleotide diphosphate kinases, and we further investigated the role of the *Nme4* gene in inflammasome activation. We find that *Nme4*−/−RAW264.7 cells have a dramatic defect in their IL-1α response to cytosolic LPS. They exhibit constitutively elevated cardiolipin levels in their mitochondrial outer membrane and show defective cardiolipin switching in response to mitochondrial stress signals. Interestingly, we find that *Nme4*−/−cells have a marked defect in the priming step of inflammasome activation, with a large majority of priming-induced transcriptional increases diminished in the absence of *Nme4*. Metabolic analysis suggests that *Nme4* is critical to the glycolytic commitment induced during inflammasome priming, however we observe normal NF-kB and MAPK activation in primed *Nme4*−/−cells, suggesting that the mitochondrial and metabolic contribution to inflammasome priming occurs independently of these signaling responses. We also find that *Nme4* deficient mice show substantial resistance to LPS-induced endotoxic shock.

**Conclusion:** We found an unexpected role for the NDK family of mitochondrial dinucleotide kinases in the effective priming of the inflammasome response by TLR ligands, thus establishing a link between the cellular metabolic state of the macrophage and the LPS-driven inflammatory response. In ongoing studies, we are using dynamic live cell imaging reporters for mitochondrial function and inflammasome triggering, to further delineate the mitochondrial and metabolic processes that support inflammasome activation.

This work was supported by the Intramural Research Program of NIAID, NIH.

**Disclosure of Interest:** None Declared

**P090 MOLECULAR DETERMINANTS OF PHAGOCYTE HYPERACTIVATION**

C. L. Evavold1,2,*, J. Ruan3, Y. Tan1, S. Xia3, H. Wu3, J. Kagan1

1Gastroenterology, Boston Children's Hospital, Harvard Medical School; 2Program in Immunology, Harvard Medical School; 3Biological Chemistry and Molecular Pharmacology, Boston Children's Hospital, Harvard Medical School, Boston, United States

**Introduction:** Phagocytes of the innate immune system become activated or "mature" in response to pattern recognition receptor (PRR) sensing of pathogen associated molecular patterns (PAMPS) and damage associated molecular patterns (DAMPs). Activated macrophages and dendritic cells upregulate conventional cytokine secretion, cytosolic pro-form cytokines of the interleukin-1 (IL-1) family, and additional PRRs such as NLRP3. Activated phagocytes are now poised to sense pathogenic invasion or cellular dysfunction through cytosolic signaling platforms, collectively termed inflammasomes. Signaling through the NLRP3 inflammasome leads to activation of the effector protease caspase-1. Pro-form cytokines such as pro-IL-1β become bioactive upon cleavage by caspase-1. Like IL-1, a latent cytosolic factor gasdermin D (GSDMD) is cleaved downstream of inflammasome signaling. Cleavage of pro-form GSDMD by caspase-1 releases an N terminal fragment (NT-GSDMD) that associates with membrane lipids to form pores that can lyse the cell and release IL-1 during a terminal, cell death state termed pyroptosis. Recent work highlights that phagocytes can attain a novel cell fate termed hyperactivation, which is characterized by the unique ability to secretion cytokines from the biosynthetic pathway (e.g. TNFα) and the cytosol (e.g. IL-1), notably without cellular lysis. It is unclear how IL-1 is released from living cells during hyperactivation.

**Methods:** In this study, we delineate genetic and biochemical determinants of phagocyte hyperactivation through single cell and aggregate population level analysis of membrane permeability, cellular lysis, energetic signs of life, and cytokine production.

**Results:** Herein, we report that the pyroptosis regulator GSDMD is necessary for IL-1β secretion from living macrophages that have been exposed to inflammasome activators, such as bacteria and their products or host-derived oxidized lipids. Cell- and liposome-based assays demonstrate that GSDMD pores are sufficient for IL-1β transport across an intact lipid bilayer.

**Conclusion:** These findings identify a non-pyroptotic function for GSDMD in mediating size-restricted secretion of cytosolic factors. The role of GSDMD in mediating...
P091 THE OFF-TARGET EFFECTS OF VACCINES: BENEFITS AND COLLATERAL DAMAGE
E. N. Fish1 on behalf of Optimunize
1Toronto General Hospital Research Institute, UHN, & Dept. Immunology, University of Toronto, Toronto, Canada

Introduction: Accumulating evidence indicates that live vaccines are superior to non-live vaccines in terms of the extent of activation of the relevant immune response to the targeted pathogen. Indeed, administration of non-live/subunit vaccines may require more than one dose to elicit a protective immune response and inclusion of an adjuvant.

Methods: A clear distinction between live and non-live vaccines is the ability of live vaccines to activate pathogen pattern recognition receptors, such as TLRs, to drive T follicular help polarization and the mobilization of these T cells to invoke the appropriate B cell and antibody response. Another distinction that is often not considered during the development of new vaccines is the off-target or non-specific effects (NSEs) of vaccines. These NSEs influence susceptibility to unrelated infectious diseases beyond the target disease.

Results: Data will be presented that clearly illustrate the beneficial NSEs of live vaccines, that protect vaccinated infants against neonatal sepsis and pneumonia, thereby reducing all cause childhood morbidities and mortalities. Bacillus Calmette Guerin (BCG), measles vaccine and oral polio vaccine are specific examples of live vaccines whose NSEs accounted for significant reductions in all cause mortality in children under the age of 5 years in resource limited settings. Live vaccines induce trained immunity, associated with epigenetic changes in genes associated with immune responses. Moreover, live vaccines induce heterologous T cell memory activation, that permits cross-reactivity to unrelated pathogens. By contrast, non-live and subunit vaccines have been associated with increased mortality, particularly for females. The inactivated polio vaccine (IPV), the diphtheria, pertussis, tetanus (DTP) vaccine and the penta vaccine (DTP + hepatitis B + haemophilus influenzae B) are examples of inactivated vaccines with documented NSEs that increased the relative risk of overall mortality in resource limited settings. Additionally, the sex differences in NSEs are invariably overlooked, most recently observed with the introduction of the recombinant protein based malaria vaccine, RTS,s.

Conclusion: Given the evidence for vaccine associated NSEs on the immune system and the sex differential effects of these NSEs, future vaccine development and regimen design for vaccine campaigns should take these effects into consideration.

Disclosure of Interest: None Declared

P092 POSITIVE REGULATION OF VIRUS INDUCED-INNATE IMMUNE SIGNALING BY THE E3 UBIQUITIN LIGASE TRIM65
D. N. Fonseca1, G. Pisanelli1, 2, A. Garcia-Sastre1
1Microbiology, ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI, New York, United States, 2Veterinary Medicine and Animal Production, University of Naples, Naples, Italy

Introduction: Viral infection triggers a fast and effective cellular response mediated primarily by the production of IFNβ that induce an anti-viral state through complex signal cascades. Therefore, the regulation of its induction and subsequent IFNβ signaling needs to be tightly controlled. There is growing evidence implicating the members of Tripartite-motif (TRIM) protein family of E3 ligases as critical players in this regulation. However, the exact role, mechanism of action, and the physiological relevance of their activity in vivo still remain poorly investigated. Previous work in our lab revealed that an unprecedented large number of TRIMs play critical roles as enhancers in the regulation of innate immune signaling pathways.

Methods: To study the role of TRIM65 in innate immune signaling we have used luciferase assays, overexpression in A549 and 293T cells, transient knock down using siRNAs in 293T cells, TRIM65 CRISPR Knock out cell lines, Western blots, RT-qPCR, PR8-GLuc antiviral assays and immunofluorescence.

Results: Our recent studies focused on TRIM65 have revealed that its overexpression strongly increased the 2CARD-RIG-I-dependent activation of the INFβ and ISRE promoters indicating that TRIM65 has a role in the interferon induction pathway. Consequently, IFNβ, ISG54 as well as other cytokines and ISGs mRNA levels are decreased in TRIM65 knock down cells upon infection compared to infected/treated control cells. These data suggest a stimulatory role for TRIM65 in innate immune signaling. Besides, TRIM65 showed antiviral activity comparable to TRIM25 in a PR8-GLuc antiviral assay.

Conclusion: Our futures studies are focused on delineate the molecular mechanism by which TRIM65-mediated ubiquitination or TRIM65 E3 Ub ligase independent function could regulate the response to viral infection using TRIM65 KO cell lines and complementation assays. A better understanding of positive regulatory networks of the IFN response will provide new knowledge that will help to design more effective therapeutics.

Disclosure of Interest: None Declared

P093
RLRS AND TLRS SIGNALING PATHWAYS CAUSE ABERRANT PRODUCTION OF INFLAMMATORY CYTOKINES/CHEMOKINES IN AN SFTSV INFECTION MOUSE MODEL

T. Fujita1, S. Yamada1, M. Shimojima2, J. Khi1, R. Narita1, Y. Tsukamoto1, M. Saijo2, H. Kato1
1Molecular Genetics, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, 2Virology I, National Institute of Infectious Diseases, Musashimurayama, Japan

Introduction: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by a tick-borne phlebovirus of the family Bunyaviridae, the SFTS virus (SFTSV). Wild type and type I interferon (IFN-I) receptor 1-deficient (IFNAR1−/−) mice have been established as non-lethal and lethal models of SFTSV infection, respectively. However, the mechanisms of IFN-I production in vivo and the factors causing the lethal disease are not well understood.

Methods: Using bone marrow chimeric mice, we found that IFN-I signaling in hematopoietic cells was essential for survival from lethal SFTSV infection.

Results: The disruption of IFN-I signaling in hematopoietic cells allowed an increase in viral loads in serum and produced an excess of multiple inflammatory cytokines and chemokines. The production of IFN-I and inflammatory cytokines was abolished by the deletion of signaling molecules IPS-1 and MyD88, essential for RLRs and TLRs signaling, respectively. However, IPS-1−/− MyD88−/− mice exhibited resistance to lethal SFTS with a moderate viral load in serum.

Conclusion: Taken together, these results indicate that adequate activation of RLRs and TLRs signaling pathways under low to moderate levels of viremia contributed to survival through the IFN-I-dependent antiviral response during SFTSV infection, whereas overactivation of these signaling pathways under high levels of viremia resulted in abnormal induction of multiple inflammatory cytokines and chemokines causing the lethal disease.

Disclosure of Interest: None Declared

P094
HMB1/RAGE PATHWAY IS ESSENTIAL FOR TI OSSEOITEGRATION IN MICE

G. P. Garlet1, C. G. Biguetti1, F. Cavall2, E. V. Silveira3, A. P. Taban1, D. B. Rodrigues4, A. P. F. Trombone5
1Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil, 2Department of Conservative Dentistry, University of Chile, Santiago, Chile, 3Department of Biological and Allied Health Sciences, Universidade Sagrado Coração, Bauru, Brazil, 4Department of Bioengineering, University of Texas at Dallas, Dallas, United States

Introduction: The release of the prototypic DAMP HMGB1 into extracellular environment and its binding to RAGE trigger inflammatory response in non-infectious environments and regulates healing outcomes in different tissues and conditions. However, their role on host response to Ti-based biomaterials grafting and the subsequent osseointegration process remains unexplored. In this study, we investigated the effects of HMGB1 and/or RAGE inhibition in the outcome of Ti-implant grafting in mice.

Methods: C57Bl/6 mice received a Ti-screw/implant placement in the edentulous maxillary alveolar crest, and treated with HMGB1 (GZA) or RAGE (RAP) inhibitors, or kept under control conditions (no treatment and vehicle [DMSO]), and evaluated by microtomographic, histological, histomorphometric and molecular (RealTimePCArray) methods in different time points (3, 7, 14 and 21 days).

Results: Control groups presented a slight inflammatory response, transition of a provisional connective tissue to newly formed bone in contact with the implant surface, typifying the successful osseointegration outcome. Conversely, the inhibition of either HMGB1 or RAGE ultimately resulted in the osseointegration impairment after Ti-device grafting. HMGB1 and RAGE inhibition initially interfered with the blood clot formation in the implant/bone interface, intensely affecting fibrin network formation especially in GZA-treated group. The subsequent osseointegration stages were also compromised, as demonstrated by the increased presence of fibroblasts/fibers, reduced osteoblasts/bone matrix, and a marked foreign body reaction (FBR) around Ti threads upon HMGB1 or RAGE inhibition. PCArray data support the histomorphometric/UCT data, demonstrating a decrease in healing and osteogenesis markers, along increased levels of inflammatory cytokines and chemokines in the GZA and RAP treated groups. Additionally, either HMGB1 or RAGE inhibition limited the expression of multiple MSCs markers and the key transcription factor for osteoblastic differentiation, RUNX2; as well prevented the M1-M2 switch kinetics, as evidenced by the sustained low ARG2 and high iNOS levels over time in the treated groups.

Conclusion: This study demonstrated that HMGB1/RAGE pathway is essential for Ti osseointegration in mice.

Disclosure of Interest: None Declared

P095
MODE OF ACTION OF THE RIG-I LIKE RECEPTOR LGP2 IN THE INTERFERON RESPONSE TRIGGERED BY VIRAL INFECTIONS

N. Gillich1, S. Jung1, A. Reuter1, P. Scaturro2, A. Pichlmair3, M. Binder1, R. Bartenschlager1,2
1German Cancer Research Center, 2Centre for Integrative Infectious Disease Research, Heidelberg, 3Max Planck Institute for Biochemistry, Munich, Germany

Introduction: The cytoplasmic RIG-I like receptors (RLRs) RIG-I, MDA5 and LGP2 bind to the RNA of incoming viruses or replicated (double-strand, ds) RNA and mount a powerful first line of defense in vertebrate cells. Binding of RLRs to these RNAs triggers a signaling cascade resulting in the activation of type I and III
interferons (IFNs) and IFN stimulated genes (ISGs). RIG-I and MDA5 initiate this event through direct activation of the downstream adaptor mitochondrial antiviral-signaling protein (MAVS). Given that LGP2 lacks the MAVS interaction domain it cannot initiate this innate response. Instead, it plays a regulatory role by influencing the magnitude of the IFN response induced by RIG-I and MDA5. Several studies revealed an LGP2-mediated enhancement of the IFN response triggered by MDA5, but a negative effect of LGP2 on the IFN response induced by RIG-I. Here we studied how LGP2 might switch between these disparate modulatory functions with the aim to better understand the regulation of the IFN system. This basic knowledge might help to find new curative strategies of immunological disorders.

Methods: To characterize LGP2’s regulatory role we aim to identify post translational modifications and interaction partners by using mass spectrometry followed by an RNA interference-based screen. Moreover we want to determine the influence of RLR protein levels on LGP2 modulated IFN induction. The experiments are conducted in different cell culture systems and include knockout and overexpression approaches. As read-out we use activation of the IFN system by measuring mRNA and protein levels of ISGs.

Results: In Huh7 and A549 cells stably overexpressing LGP2 we reproducibly observe an enhancement of the MDA5 mediated IFN response and a suppression of RIG-I mediated signaling. These effects correlate with LGP2 protein abundance and were found with different viruses (a Mengovirus mutant called Mengo Zn virus, Sendai virus, hepatitis C virus). Mass spectrometry revealed possible phosphorylation sites in LGP2 that are currently validated by using functional assays.

Conclusion: Our overall goal is to shed more light on how LGP2 regulates the IFN response in cells and how it influences viral replication.

Disclosure of Interest: None Declared

P096
TARGETING INNATE IMMUNITY AS A THERAPEUTIC AND IMMUNIZATION STRATEGY FOR FILOVIRUS INFECTION
M.-L. Goulet1, W. Cao2, L. Banadyga2, X. Qiu2, R. Lin1
1Lady Davis Institute - McGill University, Montreal, 2National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada

Introduction: Viruses that cause viral hemorrhagic fevers (VHF) represent a significant threat due to the severity of VHF (fever, vascular permeability, coagulopathy, hemorrhage and multi-organ failure), which causes death in up to 90% of symptomatic patients. Moreover, the ability of these viruses to cause major outbreaks and pandemics, such as the 2013-2015 West African Ebola outbreak that resulted in over 11,000 deaths, contributes to their risk level.

Early and potent activation of the innate immune response is thought to be linked to a favorable outcome in infected patients since virus multiplication and pathogenesis is halted early on. Central to the innate antiviral response is the rapid recognition of non-self signals such as 5’ppp containing viral RNA genome or viral replicative intermediates that binds to the receptor RIG-I and ultimately leads to the production of pro-inflammatory cytokines, anti-viral factors and type I interferons (IFNs) that amplifies the antiviral immune response.

Given that viral RNA-RIG-I interaction is one of the initial triggers of the innate and adaptive immune response, an attractive strategy for the development of an efficient and broad spectrum antiviral therapy for filoviruses involves the use of RIG-I agonist to mimic viral RNA to activate upstream host defenses. We designed, optimized, and produced a potent agonist of RIG-I in-house. Our published work demonstrated that these molecules have potent antiviral activity against a range of RNA and DNA viruses (Influenza, VSV, Dengue, Vaccinia, HIV, HCV ) in vitro and in vivo in the murine model.

Methods: In vitro, A549 cells were pretreated with the agonist for 24h prior to infection with Ebola virus expressing GFP and level of infection was visualized by fluorescent microscopy. In vivo, mice were treated with the RIG-I agonist on the day prior and the day of infection with a lethal dose of Ebola or Marburg virus. For immunization, mice were injected with either EBOV-virus like particles (VLP) alone or EBOV-VLP supplemented with 5’pppRNA. Serum IgG specific to ebola glycoprotein was determined by ELISA.

Results: Our ongoing study demonstrates that RIG-I agonist have potent antiviral activity against filoviruses. It inhibits Ebola virus infection in vitro in A549 cells, and the agonist fully protect from lethal Ebola and Marburg virus infection in vivo in the mouse model. The agonist also has adjuvant potential since it is capable of enhancing the antibody response specific to ebola glycoprotein following immunization with EBOV-VLP.

Conclusion: Taken together, these results highlight the potential of RIG-I agonist as countermeasure for filovirus infections.

Disclosure of Interest: None Declared

P097
REGULATION OF THE ANTIVIRAL RESPONSE BY REDOX METABOLISM
N. Zamorano1,2, A. Fortin1, A. Harrison1,3, S. Chartier1, E. Caron1, N. Grandvaux2,4
1CRCHUM, 2Universite de Montreal, 3McGill University, Montreal, 4CRCHUM, Montréal, Canada

Introduction: The Interferon-mediated antiviral response elicited upon virus recognition is mediated by multiple signaling cascades subjected to highly stringent regulatory mechanisms. Post-translational modifications (PTMs) of antiviral signaling proteins, including phosphorylation or ubiquitination, have proven to be key determinants of the intensity and duration of the response. Recently our
laboratory and others unveiled that NADPH oxidase-derived reactive oxygen species (ROS) are essential for the induction and duration of the Interferon and proinflammatory responses. The molecular mechanisms of action of ROS remain elusive. Redox PTMs (ox-PTMs), notably on Cys residues, have lately emerged as key processes to regulate signaling proteins structure and function. Here we aimed at identifying antiviral signaling proteins subject to ox-PTMs.

**Methods:** We used bioswitch methods and probes specific to ox-PTMs to label Cys ox-PTMs. Mass spectrometry and immunoblot analysis were used to identify oxidized proteins and specific Cys residues. Structure-function analyses demonstrate the impact of Cys ox-PTMs on the capacity of the cells to mount an antiviral state.

**Results:** We now report that key signaling proteins and major pathways involved in the regulation of the antiviral and proinflammatory response in immune and non-immune cells are subjected to Cys oxidation.

**Conclusion:** Altogether, our data shed new light into how redox metabolism regulates the antiviral response.

**Disclosure of Interest:** None Declared

**P098**

THE ROLE OF TYPE 1 AND 3 INTERFERONS IN OCULAR TOXOPLASMOSIS

V. Greigert1*, F. Fahmi-Bittich2, J. Brunet2, E. Candolfi2, A. W. Plaff2

1Hôpitaux Civils de Colmar, Colmar, 2Institut De Parasitologie Et Pathologie Tropicale, Université De Strasbourg, Strasbourg, France

**Introduction:** Ocular toxoplasmosis (OT) is an underestimated eye infection, responsible for most of posterior uveitis worldwide, with a peculiar pathophysiology involving the persistence of parasites in infected tissues inside pseudocysts and sporadic recurrences of the infection. Interferon (IFN) γ is known to be a critical factor of the innate immune response during OT, but very little is known regarding to other kinds of IFNs in this infection, especially of types 1 (α and β) and 3 (λ 1, 2 and 3). Yet, these IFNs are known to be involved in the immune response to infections with various pathogens, including viruses, bacteria or fungi, with intracellular life cycles, like Toxoplasma gondii. Furthermore, the ocular immune response is likely to differ, because of its immunoprivileged environment. Thus, the aim of our study was to assess the role of type 1 and 3 IFNs during OT.

**Methods:** We performed infections of monocellular cultures of retinal cells: microglia, Müller cells, astrocytes and retinal pigment epithelial (RPE) cells. We evaluated the inhibition of parasite growth using quantitative PCR following stimulation with recombinant type 1 and 3 IFNs or not. Symmetrically, we assessed the expression of type 1 and 3 IFNs and their receptors, as well as IFNγ and the well-known IFNγ induced effector mechanisms in human immune response to T. gondii infection, indoleamine 2,3-dioxygenase (IDO), using RT qPCR, after infection. We used 2 different strains of T. gondii, one virulent (RH), the other avirulent and cystogenous (PRU). We also measured the expression of IDO by these cells following stimulation with type 1 and 3 IFNs.

**Results:** We observed that the stimulation of microglia with IFNβ and IFNλ significantly inhibited the growth of parasites. The inhibitory effect of IFNλ was even of similar magnitude as the effect of IFNγ, at around 70%. The stimulation of other cell types did not result in parasite growth inhibition. On the other hand, we observed that the infection of microglia, Müller cells and astrocytes with T. gondii resulted in the expression of IFNβ and IFNA 1.2 and 3. The kinetics of these expressions seemed to depend on the parasite strain involved, the virulent strain being responsible for early (12h post-infection) expression of IFNs whereas the avirulent strain was responsible for late (120h post-infection) response. Finally, the stimulation of microglia, Müller cells and astrocytes with IFNβ and IFNA did not result in the expression of IDO.

**Conclusion:** Our results suggest that type 1 IFNs, especially IFNβ, and type 3 IFNs may be involved in the innate immune response to OT. We showed that infecting monocellular cultures of retinal cells was sufficient to induce the expression of type 1 and 3 IFNs, suggesting this phenomenon to be reproducible in vivo. The role of this response to the infection may be to inhibit parasites growth via a mechanism which remains unknown. However, our preliminary results show that stimulation of cells with IFNβ and IFNA does not result on the expression of IDO, which is the principal effector of the protective effect of IFNγ, essential in survival following T. gondii infection. These elements suggest that the immune mechanisms may be different in the eye, compared to systemic infection. Deciphering these mechanisms may pave the way to the elaboration of new therapeutic and diagnostic strategies in OT.

**Disclosure of Interest:** None Declared

**P099**

DO DIFFERENT IFN-ALPHA SUBTYPES HAVE DISTINCT BIOLOGICAL ACTIVITIES?

M. Grunbach1*, S. Bredl1, E. Schlaepfer1, B. Escher2, M. Schlapschy2, A. Skerra2, G. Schreiber2, R. Speck1

1Department of Infectious Diseases, University Hospital Zurich, Zurich, Switzerland, 2Chair of Biological Chemistry, Technical University of Munich, Munich, Germany, 3Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel

**Introduction:** Currently there is a controversy in the literature whether the difference in the biological activity of IFN-α subtypes is of qualitative or quantitative nature. In our experiments with human PBMCs, CD4+ T-cells and monocyte-derived macrophages, we found a dose-dependent anti-HIV activity and did not see a qualitatively different response if judged by the pattern of the interferon stimulated genes (ISGs) (Schlaepfer, E., et al., 2018, submitted). As in vitro data do not fully recapitulate the in vivo events, we will perform humanized (hu) mouse experiments to determine their effect in case of HIV
infection as well as independent of HIV. Due to the short half-life of IFNs in vivo, we have prepared PASylated IFNs with >10-fold longer circulation half-lives (Schlapschy, M., et al., 2013). We have chosen IFN-α2 as the prototype IFN, with intermediate affinity, IFN-α14 with high affinity, and the mutant YNS with 60-fold increased affinity to the IFN receptor (Harari et al., 2014). YNS showed superior activity in inhibiting HIV replication ex vivo (Schlaepfer, E., et al., 2018, submitted).

Methods: In the first step, we determined the efficacy of PASylated IFNs in comparison with the wild-type cytokines by using a reporter cell line bearing luciferase under the control of the IFN stimulated response element and measuring the luciferase signal. We then evaluated the PASylated IFN’s ability to counteract HIV replication in human PBMCs over 15 days. We are currently determining the optimal dose and schedule for the IFN treatment in hu mice by testing three doses and looking at their ability to increase the expression of ISGs and effect on different cell types.

Results: Our in vitro data show that PASylated IFNs induce luciferase expression to a lesser extent than their wild-type counterparts. We also found a lower HIV inhibition rate in response to PASylated IFNs than with the wild-IFNs. In vivo we saw that PASylated IFNs, showed dose-dependent stimulation of prototype ISGs in our hu mice as well as tolerance to induction over time.

Conclusion: Currently we were able to show that there are quantitative differences for IFNα2, -α14 and YNS, whereas the same PASylated subtypes showed a similar activity pattern but to a lesser extent. First hu mice data show that our current dosing schedule leads to systemic tolerance to IFN, resulting in inhibition of ISG induction. We need to test more schedules to find the conditions where we see a clear ISG induction over time. After determining the dose and treatment regimen we will perform long-term animal experiments with all the three subtypes. Although PASylation leads to slightly diminished biological activity of IFNs in vitro, it significantly prolongs their half-life, which could increase their function in vivo due to improved pharmacokinetics. We hope that our in vivo findings shed some light on the current controversy regarding the biological activity of different IFN-α subtypes.

Disclosure of Interest: None Declared

P100
PROSTAGLANDIN E2 IS AN INHIBITORY DAMAGE ASSOCIATED MOLECULAR PATTERN WHICH CRITICALLY REGULATES STERILE INFLAMMATION-RELATED DISEASES INDUCED BY DEAD CELLS
S. Hangai1*, H. Yana2, T. Taniguchi1
1Department of Molecular Immunology, Institute of Industrial Science, 2The University of Tokyo, Tokyo, Japan

Introduction: Sterile inflammation induced by dead cell-derived molecules, or damage associated molecular patterns (DAMPs), is now considered to be critical for development of various diseases including autoimmune diseases and cancer. Most DAMPs are generally recognized as positive regulators of inflammation. However, little is known about whether and to what extent DAMPs can also suppress inflammation and contribute to above diseases. Here we show that prostaglandin E2 (PGE2), a lipid mediator, is induced and released by dead cells, inhibits inflammatory response and counteracts the activity of other DAMPs-mediated inflammation. Through modulation of dead-cell induced inflammation, PGE2 contributes to maintenance of homeostasis in some case and exacerbate diseases on the other.

Methods: Identification of PGE2 was performed by gel filtration chromatography and mass spectrometry. Depletion of PGE2 was done by indomethacin or celecoxib. ChiP-qPCR assay was employed to assess the binding of p65 to the promoter region of Tnfα gene. The gene expression profile downstream of PGE2 signaling was performed by microarray. Liver necrosis was induced by intraperitoneal injection of acetaminophen. COX-2 deficient cancer cells were generated by CRISPR/Cas9 system.

Results: Necrotic cells potently suppressed LPS-induced TNF-α production in RAW264.7 cells. We then purified necrotic supernatant, subjected it to mass-spectrometry, and found that PGE2 was enriched in active fractions. PGE2 was also released by various forms of cell death including apoptosis and pyroptosis. Furthermore, depletion of PGE2 from necrotic supernatant augmented the induction of TNF-α, possibly through enhanced activity of other DAMPs. These results indicate that PGE2 is an inhibitory DAMP which counteracts other inflammation-inducing DAMPs. The suppressive activity of PGE2 was independent of NFκB pathway, as binding of p65 to the promoter region of Tnf gene was not attenuated by PGE2 treatment. Interestingly, transcriptomic analysis revealed that PGE2 signaling induced several immunosuppressive transcription factors including Nr4a family proteins, which might be responsible for suppressive activity of PGE2. To gain insight into physiological role of PGE2 as an inhibitory DAMP, we investigated two mouse models where massive cell death occur: acetaminophen induced liver necrosis and cancer. In the liver necrosis model, treatment with celecoxib, a COX-2 inhibitor, exacerbated inflammation and liver damage, suggesting that PGE2 inhibited inflammation evoked by other DAMPs. On the other hand, inactivation of COX-2 in mouse colorectal cancer cells resulted in retarded growth in vivo but not in vitro. Analysis of tumor infiltrating immune cells revealed that differentiation of tumor associated macrophages was skewed towards M1 phenotype in COX-2 deficient tumor. Consistently, necrotic supernatant induce polarization of macrophages towards M2 phenotype in PGE2 dependent manner in vitro. These results indicate that PGE2 released from dying cancer cells suppressed
antitumor response via skewing macrophage polarization.

Conclusion: PGE2 is an inhibitory DAMP which critically regulates sterile inflammation evoked by massive cell death and contributes to maintenance of homeostasis on one hand and to exacerbation of disease on the other.

Disclosure of Interest: None Declared

P101
THE HOST-ANTIVIRAL PROTEIN VIPERIN ENHANCES THE DSDNA SIGNALLING PATHWAY VIA A DIRECT INTERACTION WITH STING
K. M. Crosse¹, E. Monson¹, M. Smith¹, K. Van der Hoek², P. Revill³, M. Beard³, K. Helbig³
¹Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, ²School of Biological Sciences, The University of Adelaide, Adelaide, ³Victorian Infectious Diseases Reference Laboratory, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

Introduction: Viperin is a unique and highly evolutionarily conserved protein that has been shown to restrict multiple viruses, via many different mechanisms. Recently, viperin has been placed among a new class of interferon stimulated genes (ISGs) that not only act directly to inhibit viral infection, but also act synergistically to enhance antiviral innate signalling [1]. These attributes highlight viperin as a pivotal anti-viral host protein in response to viral infection. Viperin has previously been shown to enhance TLR7/9 signalling in murine plasmacytoid dendritic cells [2], and here we show for the first time, that viperin also enhances the dsDNA signalling pathway.

Methods: The involvement of viperin in enhancing this signalling pathway was extrapolated through various assays including luciferase reporter assays, RT-qPCR, confocal microscopy, immunoprecipitation assays and proximity-igation-assays in primary viperin knockout murine embryotic fibroblasts (MEFs) and multiple other cell lines. Results are expressed as mean ± S.E.M. Student’s t test was used for statistical analysis. p < 0.05 was considered to be significant.

Results: Primary MEFs isolated from viperin knockout mice originally presented a significant reduction in their ability to express type-I interferon (IFN) compared to wild-type MEFs, when challenged with dsDNA. Subsequent luciferase based assays in combination with ectopic expression of viperin and various mutants confirmed these findings and identified viperin’s requirement for its localisation to the lipid droplet, as well as its enzymatic activity to significantly enhance the activity of the IFN-β promoter following detection of dsDNA. Viperin was observed to co-localise with the key signalling molecules of the dsDNA sensing pathway, STING and TBK1, via direct binding to STING; inducing enhanced ubiquitination-mediated activation of both molecules. Additionally, viperin interacted with the ubiquitin ligases TRAF3 and TRAF6, both of which are known to form signalling complexes with STING and TBK1. Moreover, we have demonstrated that viperin interacts with STING in the context of HBV infection in HepG2 cells to enhance the expression of IFN-β, and significantly reduce the expression of HBV antigens.

Conclusion: Here we show that viperin enhances the dsDNA signalling pathway via a direct interaction with STING. Our data suggests that viperin facilitates the formation of a signalling enhansosome with STING, to coordinate efficient signal transduction, resulting in an enhanced antiviral state. Due to STING’s involvement in detecting aberrant cytosolic DNA, this interaction may have implications in the development of novel therapeutics against not only viral disease, but also inflammation and cancer. This data highlights viperin’s pivotal role in innate immunity and the necessity of understanding these pathways to advance novel therapeutic design.

Disclosure of Interest: None Declared

P102
DETECTION OF CYTOSOLIC SHIGELLA FLEXNERI VIA A C-TERMINAL TRIPLE-ARGININE MOTIF OF GBP1 INHIBITS ACTIN-BASED MOTILITY
A. S. Piro¹, D. Hernandez¹, S. Luoma², R. Finethy¹, A. Yirga³, E. M. Feeley³, K. Kholer⁴, M. Barber⁴, E. M. Fricke⁵, C. F. Lesser⁵, J. Coers¹
¹Molecular Genetics and Microbiology, ²Duke University Medical Center, Durham, United States, ³Department of Microbiology and Immunobiology, Duke University Medical Center, Durham, ⁴Department of Biology , University of Oregon, Eugene, United States, ⁵The Francis Crick Institute, London, United Kingdom, ⁶Department of Microbiology and Immunobiology, Harvard Medical School, Boston, United States

Introduction: Dynamin-like guanylate binding proteins (GBPs) are gamma interferon (IFN-γ)-inducible host defense proteins that can associate with cytosol-invading bacterial pathogens. Mouse GBPs promote the lytic destruction of targeted bacteria in the host cell cytosol, but the antimicrobial function of human GBPs and the mechanism by which these proteins associate with cytosolic bacteria are poorly understood.

Methods: We constructed a series of hGBP1 mutants and assessed their ability to colocalize to gram-negative bacteria by immunofluorescent microscopy.

Results: Here, we demonstrate that human GBP1(hGBP1) is unique among the seven human GBP paralogs in its ability to associate with at least two cytosolic Gram-negative bacteria, Burkholderia thailandensis and Shigella flexneri. Association with hGBP1 blocks actin tail formation by S. flexneri, thereby interfering with bacterial cell-to-cell spread. Our study thus reveals that hGBP1 functions as an intracellular “glue trap,” inhibiting the cytosolic movement of normally actin-propelled Gram-negative bacteria. This potent host defense mechanism puts selective pressure on cytotoxic Gram negative bacteria to evolve counterdefenses. As one mechanism to counter hGBP1-mediated host defense, S. flexneri secretes the bacterial effector protein IpaH9.8, which promotes GBP1 degradation and thereby

80
diminishes hGBP1 targeting to S. flexneri. Yet, additional bacterial mechanisms for escape from hGBP1-mediated host defense may exist. In support of this hypothesis, we observed that colocalization of hGBP1 with cytosolic bacteria is driven by a unique C-terminal poly-basic motif (PBM). Because GBP1 targets S. flexneri mutant strains lacking the O antigen portion of lipopolysaccharide (LPS) less frequently than wildtype S. flexneri, we propose that PBM of hGBP1 directly or indirectly detects LPS O antigen. O antigen structure is highly variable between bacterial species and serotypes. Therefore, structural modifications of O antigen could provide another mechanism by which bacteria escape from recognition by hGBP1 and thereby would impose selective pressure on the PBM sequence of GBP1 to change so that it can recognize pathogen-associated O antigen structures. To test this hypothesis, we conducted a phylogenetic analysis of GBP1 sequences across 20 different primate species. We identified signatures of positive selection in two amino acid residues located within or immediately adjacent to GBP1-PBM. We further found that GBP1-PBM from at least one non-human primate species facilitated improved recognition of cytosolic S. flexneri compared to hGBP1-PBM. 

Conclusion: Together these data identify recognition of Gram negative bacteria by the PBM of hGBP1 as a central conflict in the molecular arms race between cell-intrinsic host defense and bacterial counterdefense.

Disclosure of Interest: None Declared

P103
REPURPOSING OF CARDIOVASCULAR DRUGS FOR THE TREATMENT OF IL-1 MEDIATED DISEASE
F. Humphries1*, K. Fitzgerald 1
1Program in Innate Immunity, UMASS medical school, Worcester, United States

Introduction: IL-1b plays a critical role in the regulation of inflammatory responses to infectious agents and sterile insults. During infection IL-1b is induced via the action of the transcription factors HIF-1a and NFkB. The pro-form of IL-1b is then processed by Caspase-1 and released through a Gadermin-D pore formed following activation of inflammasome complexes. IL-1b is an important cytokine in the control of infection, however uncontrolled amounts of IL-1b can promote inflammatory diseases such as IBD, type 2 diabetes and cardiovascular disease. Cardiovascular disease is the leading cause of death globally and responsible for 25% of all deaths in the US. Recent studies have revealed the benefits of therapeutic targeting of IL-1b in cardiovascular disease. Here we identify an FDA-approved small molecule cardiovascular drug, named here as UMM18, with a half-life of 6 hrs as a potent selective inhibitor of IL-1b transcription.

Methods: ELISA, Western blotting, QPCR, In vivo septic shock, Nanostring analysis, Seahorse Bioanalyzer Results: UMM18 specifically inhibited IL-1b release upon inflammasome activation but not IL-18 or pyroptosis. Indeed, UMM18 effects were mediated through inhibition of IL-1b gene transcription. UMM18 inhibited TLR-induced IL-1b transcription in murine macrophages and human cells. Furthermore, UMM18 also inhibited IL-1b induced by LPS in vivo.

Conclusion: The present study identifies a previously unreported function of an FDA-approved cardiovascular drug for the treatment of inflammation and presents an opportunity for drug repurposing.

Disclosure of Interest: None Declared

P104
INTERFERON-ALPHA/BETA SIGNALING IN NEURONS MEDIATES PROTECTION AGAINST VIRAL ENCEPHALOMYELITIS AND REGULATES INTERFERON-GAMMA DEPENDENT RESPONSES
M. Hwang1*, C. Bergmann 1
1Neurosciences, Cleveland Clinic Lerner Research Institute, Cleveland, United States

Introduction: IFNa/β signaling through the IFNa/β receptor (IFNAR) is essential to limit virus dissemination throughout the central nervous system (CNS) following neurotropic virus infections. However, CNS cells have distinct capacities to both initiate IFNα/β production in response to infection, as well as respond to IFNα/β due to distinct expression patterns of factors associated with the IFNα/β pathway. Specifically the in vivo contribution of IFNAR signaling in select CNS cell types to limit tropisms and stem viral spread is poorly characterized.

Methods: Mice deficient in IFNAR specifically on forebrain neurons (CaMKIIcre IFNARfl/fl) and IFNARfl/fl controls were infected with a neurotropic CoV to assess how IFNAR deficiency in neurons affects disease progression, viral replication and inflammatory responses. Viral spread and tropism was assayed by immunohistochemistry. Unpaired two-tailed Student t-test and two-way analysis of variance (ANOVA) were used for statistical analysis

Results: Infection of CaMKIIcre IFNARfl/fl mice resulted in severe encephalomyelitis, hind-limb paralysis and mortality within 7 days, contrasting mild clinical symptoms with no fatalities in control IFNARfl/fl mice. Uncontrolled virus replication and spread did not only affect neurons in the forebrain, but also other brain regions including the mid-hind brain. Viral spread was also increased in glial cells, despite overall increased and sustained mRNA levels of IFNα/β associated genes and overall unaltered recruitment of adaptive immune cells within the CNS. Unexpectedly however, uncontrolled viral spread, despite overall elevated IFNα/β, coincided with defective IFNg signaling, an essential component of lymphocyte mediated virus control.

Conclusion: Our results imply that defective IFNAR signaling in a single cell type can result in enhanced infection of other cells and dissemination throughout CNS. Moreover, of clinical relevance to antiviral strategies, the link between elevated IFNα/β and impaired IFNg responsiveness, implies using caution in timing and dosing of IFNα/β to sustain optimal adaptive IFNg responses.

Disclosure of Interest: None Declared
P105
EVIDENCE OF GENETIC DIVERSITY IN THEILERIA SPECIES INFECTING HORSES IN NIGERIA
I. S. Idoko1*, P. W. Mshelia2, R. E. Edhe3, S. E. Abalaka4, E. O. Balogun4, O. Okubanjo5, S. Adamu6
1Veterinary Pathology, University of Abuja, Gwagwalada, 2Veterinary Medicine, 3Department of Veterinary Medicine, 4Department of Biochemistry, 5Department of Veterinary Parasitology and Entomology, 6Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria

Introduction: The study aimed to determine the genetic diversity of Theileria species naturally infecting horses in Nigeria. Knowledge of this and the associated immune responses are vital for developing vaccine candidates for effective disease prevention and control.

Methods: Seventy two (72) horses with variable clinical signs suggestive of piroplasmosis were selected from North-west and North-central Nigeria, and blood samples were collected. Both partial and complete Babesia and Theileria 18S rRNA primers were used to amplify the hypervariable regions of the parasites. The PCR products from positive samples were sequenced while those suspected to be mixed infections were cloned into the plasmid pCR TOPO 2.1 vector system and transformed M13, single clones selected and sequenced.

Results: Comparative genomic studies of the 18S rRNA sequences revealed four distinct clades (clades A, B, C and D) of Theileria species infecting horses in Nigeria. Much is known about clades A and C but not clades B and D (T. equi-like unknown clades). Mixed infections of parasites within the various clades were identified. The existence of the unknown clades might indicate speciation events or genetic drift over time. The current T. equi diagnostic tools target the equine merozoite antigen-1 (ema-1), an immunodominant antigen that is absent in parasites belonging to clade C. There is dearth of knowledge on the copies of ema in the EMA superfamily in the unknown clades. Existence of sequence heterogeneity is epidemiologically significant considering the rising unrestricted regional and cross-border movement of horses for trade, sports and traditional ceremonies in Nigeria.

Conclusion: This study revealed remarkable sequence diversity in the Theileria species infecting horses in Nigeria. Future studies will investigate the virulence of these pathogens, factors that impact their virulence and if mixed infections modulate the pathogenesis of the disease in field conditions.

Disclosure of Interest: None Declared

P106
4-1BB EXPRESSION ON MAIT CELLS IS ASSOCIATED WITH ENHANCED IMMUNE RESPONSES THAT ARE DEPENDENT ON IL-2
J. Jiang1*, X. Cheng2

1Department of Laboratory Medicine, Shenzhen Third People's Hospital, Shenzhen, 2Division of Research, Institute of Tuberculosis, Beijing, China

Introduction: Tuberculosis is the second leading cause of death from an infectious disease worldwide. Mucosal-associated invariant T (MAIT) cells are innate-like T cells that play an important role in protective immunity against microbial infections. The role of MAIT cells in immunity against Mycobacterium tuberculosis infection in humans is still largely unexplored.

Methods: In this study, we investigated the functional role of 4-1BB on MAIT cells from patients with tuberculosis by flow cytometry and transcriptome sequencing.

Results: We found that 4-1BB was highly up-regulated on MAIT cells from tuberculosis pleural effusions following Mtb antigen stimulation and its level of expression correlated with IFN-γ and IL-17 production. 4-1BB expression on MAIT cells in response to Mtb antigens was partially dependent on IL-2 and was associated with common γ chain receptor. To understand functional role of 4-1BB, MAIT cells from TPE of 4 patients with tuberculosis pleurisy were sorted by flow cytometry and RNA sequencing was performed. By transcriptome sequencing, we identified numerous differentially expressed genes between 4-1BB and 4-1BB+ MAIT cells. GO enrichment and KEGG pathway analysis of differentially expressed genes identified enriched pathways that included T-cell receptor and NF-κB signaling pathways.

Conclusion: It is concluded that 4-1BB has the potential to be used as a biomarker to identify MAIT cells with enhanced IFN-γ and IL-17 responses that might be associated with tuberculosis infection control.

Disclosure of Interest: None Declared

P107
ORGANISMAL NETWORKS OF PROTECTION ACROSS TISSUES DURING VACCINATION
M. Kadoki1,2*, N. Chevrerie1,3
1FAS Center for Systems Biology, Harvard University, Cambridge, 2Center for Computational and Integrative Biology, Massachusetts General Hospital, Boston, 3Institute for Molecular Engineering, The University of Chicago, Chicago, United States

Introduction: The immune responses are the processes coordinated in organismal level by molecular and cellular components. Despite our knowledge about the components, we still do not fully understand the mechanism of the organismal coordination. For example, little is known in whole body level about how vaccines inoculated through cutaneous or muscular route can prevent the following infection of respiratory or oral pathogens. We aimed to address the issue focusing on the inter-organ communications by integrating the theory of immunology and systems biology.

Methods: With a comparative infection model of vaccinia viruses in mice, we monitored the dissemination of the
viruses and the propagation of host responses within and between organs by whole organ RNA-seq from 17 mouse organs. Ligand-receptor connectivity map was generated from the whole organ RNA-seq data to infer the potential inter-organ communications. By combining parabiosis, single-cell RNA-seq and gene knockouts, we verified the inference of the inter-organ communications.

**Results:** We observed complete protection against systemic dissemination of pathogenic virus entering from respiratory route by prior subcutaneous vaccination in spite the vaccine strain is confined in the vaccinated skin and its draining lymph node. Whole organ RNA-seq revealed two inter-organ mechanisms of protective immunity mediated by soluble and cellular factors. First, we found that type I IFNs trigger a whole-body antiviral state, protecting the host within hours following skin vaccination. Second, we uncovered a multi-organ web of tissue-resident memory T cells lasting over months that functionally adapt to their environment by distinct cytokine production to stop viral spread across the organism.

**Conclusion:** These results have shown the organisational mechanism of vaccine-mediated host protection through inter-organ communications by molecular and cellular factors. Our approaches open up new lines of inquiry for the analysis of immune responses at the organism level.

**Disclosure of Interest:** None Declared

**P108**

MAVS-DEPENDENT INDUCTION OF INNATE IMMUNE RESPONSES IN HANTAAN VIRUS INFECTION

A. Kelli1, E. Hemann1, B. Turnbull1, M. Gale1

1University of Washington, Seattle, United States

**Introduction:** Pathogenic hantaviruses, family Hantaviridae, are maintained in rodent reservoirs with zoonotic transmission to humans occurring through contact with rodent excreta. Hantavirus disease in humans is characterized by localized vascular leakage, accompanied by elevated levels of circulating proinflammatory cytokines IL-1β, TNFα, and IL-6. Despite the global health impact of these viruses, specific virus-host interactions leading to innate immune activation and the molecular mechanisms that determine disease outcome remain unclear. In this study, we define the mechanisms of viral recognition and innate immune signaling to Hantaan virus (HTNV) infection.

**Methods:** To identify pathogen recognition receptors (PRR) required for innate immune activation, we infected murine embryonic fibroblasts (MEFs) lacking Toll-like receptors (TLR), RIG-I-like receptors (RLR), and signaling adapter proteins and determined interferon-stimulated gene (ISG) expression. Additionally, we measured viral replication and production of infectious particles. Our findings in the murine system were validated using CRISPR knockout human cell lines for relevant PRRs. We further evaluated in vivo HTNV replication, tissue dissemination and host gene expression in relevant knockout mouse lines. Finally, to identify the viral RNA ligand responsible for triggering innate immune signaling to HTNV infection, we performed crosslinked-immunoprecipitation and RNA sequencing (CLIP-seq).

**Results:** We identified the RLR pathway as essential for innate immune activation, IFN production, and ISG expression in HTNV infection. Our results demonstrate that innate immune signaling through the MAVS adaptor protein depends on viral replication and significantly restricts viral replication in vitro. The type 1 interferon receptor (IFNAR) is also required for ISG expression and viral control in vitro. Importantly, following HTNV infection in vivo, MAVS-deficient and IFNAR-deficient mice had higher viral loads, increased persistence, and greater viral dissemination to lung, spleen, and kidney tissues than that observed in wild-type animals. Innate immune transcriptional profiling in these tissues demonstrates increased cytokine expression and proinflammatory responses in wild-type animals upon HTNV infection. Through RLR CLIP-seq, we have identified viral RNA ligands of HTNV infection that trigger RLR signaling to initiate a robust innate immune response that limits viral replication.

**Conclusion:** RLRs are essential for recognition of HTNV infection to direct innate immune activation and control of viral replication. Future work will focus on defining the linkage of these processes in Hantaan virus pathogenesis.

**Disclosure of Interest:** None Declared

**P109**

NATURAL KILLER T CELLS AND IFN-GAMMA DRIVE POST-SEPSIS IMMUNOSUPPRESSION


1Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Boston, United States, 2Dept. of Life Science, Ben-Gurion University of the Negev, Beer-Sheva, Israel, 3Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, 4Dept. of Microbiology and Immunobiology, Harvard Medical School, Boston, United States

**Introduction:** During sepsis, patients become immunosuppressed and susceptible to secondary infection. A deficit of inflammatory cytokines, like interferon-gamma (IFNγ), is thought to drive post-sepsis immunosuppression. However, we found that an early increase in IFNγ in clinical sepsis correlated with a marker of secondary fungemia.

**Methods:** To examine this unexpected correlation of increased IFNγ and increased secondary fungemia, we examined a mouse model of post-sepsis immunosuppression. In this novel mouse model, mice underwent LPS sepsis followed by secondary candidemia. After sepsis, previously healthy wild-type mice had suppressed macrophage function and increased susceptibility to secondary candidemia.
Results: In experimental post-sepsis immunosuppression, we showed that IFNγ worsened macrophage function and susceptibility to secondary candidemia. This pathogenic role of IFNγ contradicted the dominant hypothesis that a deficit of IFNγ causes post-sepsis immunosuppression. We resolved this apparent contradiction by showing that IFNγ in early sepsis, but not late sepsis, worsened post-sepsis immunosuppression. We found that invariant Natural Killer T (iNKT) cells coordinated the production of this immunosuppressive IFNγ in early sepsis. Surprisingly, the immunosuppressive effect of NKT cells and IFNγ were mediated by proteins that are usually anti-microbial in other contexts. Specifically, induction of lipocalin 2 and formyl peptide receptor 2 worsened macrophage phagocytosis and susceptibility to secondary candidemia. We used transcriptomic and metabolomic screens to identify mTOR as a key pathway regulating NKT cell-driven immunosuppression. Although rapamycin is used as an immunosuppressive medication, here blockade of mTOR by rapamycin restored immune function and protected against opportunistic candidemia. Finally, we correlated our immunosuppressive programs to clinical sepsis.

Conclusion: We have defined a novel mechanism by which innate T cells and IFNγ turn anti-microbial programs into mediators of immunosuppression. These results highlight that inflammatory mediators have quite different effects in early sepsis and late sepsis. These results challenge the dominant paradigm of post-sepsis immunosuppression and suggest that NKT cells, IFNγ and mTOR in early sepsis may be novel targets for therapeutic intervention.

Disclosure of Interest: None Declared

P110
CGI03 UPREGULATES NADPH OXIDASE-INDUCED reactive oxygen species upon TLR2 stimulatIon in macrOphages
T.-H. Kim1,*, H.-C. Lee1, J.-S. Lee1
1Chungnam National University, Daejeon, Korea, Republic Of

Introduction: CGI03 has been identified as a member of the death-inducing signaling complex in Fas-mediated apoptosis. Recent report revealed that CGI03 positively regulates type I interferon signaling against viral infection. However, the function of CGI03 presenting ROS modulation to defense bacterial infection has not been elucidated. Here we demonstrated that CGI03 has a role in anti-bacterial activity through regulation of NADPH oxidase in macrophages.

Methods: To define the function of CGI03, first macrophages were treated with TLR2 ligands or infected with L. monocytogenes. Then, the supernatants or cells were used to measure the pro-inflammatory cytokines or ROS, respectively. Besides, wild-type mice and CGI03gt/gt mice were challenged with L. monocytogenes to determine susceptibility against bacterial infection. Lastly, target protein binding with CGI03 was identified using immunoprecipitation.

Results: Deficiency of CGI03 reduces the secretion of pro-inflammatory cytokines in BMDMs and Raw264.7 cells upon TLR2 stimulation or L. monocytogenes infection. Surprisingly, lack of CGI03 showed low level of NADPH oxidase-induced ROS production in primary macrophages. Furthermore, CGI03gt/gt mice exhibited the decrease of pro-inflammatory cytokines in serum or spleen, resulting in excessive bacterial growth upon L. monocytogenes infection compared with wild-type mice. We, definitely, found out CGI03 strongly binds to p67phox, which is one of the regulatory protein of NADPH oxidase, leading to increase of NADPH oxidase activity and superoxide production.

Conclusion: These findings suggest that CGI03 modulates NADPH oxidase-induced ROS and inflammatory responses to protect host from bacterial infection. [The National Research Foundation of Korea (Grant no. 2015020957)]

Disclosure of Interest: None Declared

P111
VIRAl RNA RECOgnITION BY TLR7 AND MAVS controls CD8 T cell RESPONSE
M. Kim1,*, P. Pillai2, H. Dong1, A. Iwasaki1,3
1Immunobiology, Yale University, New Haven, 2Chemical Engineering, David H. Koch Institute for Integrative Cancer Research, MIT, Cambridge, 3Howard Hughes Medical Institute, Chevy Chase, United States

Introduction: Influenza-specific CD8 T cells that recognize conserved epitopes are protective against a wide range of influenza viruses. However, the precise mechanisms by which viral RNA recognition of influenza A virus (IAV) induces virus-specific CD8 T cell response remain elusive.

Methods: Most inbred mice including C57BL/6 mice are highly susceptible to IAV infection due to their loss of functional myxovirus resistant protein 1 (Mx1) expression. Interestingly, robust innate immunity in Mx1-sufficient mice suppresses generation of CD8 T cells at a low dose [10 PFUs] of IAV infection. We used Tlr7−/−Mavs−/− mice carrying functional Mx1 allele to examine whether viral genomic and 5' triphosphate RNA recognition by TLR7 and MAVS is required for CD8 T cell responses. To identify the cells responsible for supporting CD8 T cell response by TLR7 or MAVS expression, we generated chimeras in which Tlr7 or Mavs deletion was confined to the hematopoietic compartment. Mice were infected with A/PR8 influenza and the number of virus-specific CD8 T cells in the lung and mediastinal lymphnode (LN) was examined by PA26-233 tetramer staining. P14 cells that express TCR recognizing LCMV gp33 epitope were used for adoptive transfer to determine T cell-extrinsic function of TLR7 or MAVS. Briefly, P14 cell recipients were infected with recombinant strain of A/PR8 expressing gp33-41 (A/PR8-
GP33) and the number of P14 was detected 8 days after infection. Activation and migration of lung DCs promotes CD8 T cell priming in the mediastinal LNs. To investigate DC function in the absence of TLR7 or MAVS, we examined costimulatory molecule CD86 and chemokine receptor CCR7 expression of DCs in lung and mediastinal LN 2 days after IAV challenge. Statistical significance was determined by using one-way analysis of variance with post hoc Tukey's multiple comparison test.

**Results:** Unlike mice that are deficient either TLR7 or MAVS, mice that lack both TLR7 and MAVS decreased the number of IAV-specific CD8 T cells in the lung. CD8 T cells in the Tlr7−/− Mavs−/− mice fail to express CXCR3, which suggests compromised T cell migration regardless of its ligand CXCL9 and 10 expression in the lung. Adoptive transfer of P14 cells showed that T cell-intrinsic expression of TLR7 and MAVS was dispensable. However, these receptors were required for lung migratory DCs to acquire CD86 and CCR7 expression and migrate to mediastinal LNs, which results in IAV-specific T cell priming and CXCR3+ effector T cell differentiation.

**Conclusion:** These findings revealed that viral pathogen associated molecular pattern (PAMP) recognition is necessary for DC activation and migration, which drives IAV-specific CD8 T cell immune response.

**Disclosure of Interest:** None Declared

---

**P112**

**T CELL INDEPENDENT TYPE I RESPONSES IN NON-HUMAN PRIMATES**

G. Kim1,*, E.-H. Hwang1, J.-H. Park2, F. Villinger2, K. J. Jeong1, P. Kang1, S. Lee1, B.-S. Koo1, K.-T. Chang1, J. J. Hong1

1National Primate Research Center, Korea Research Institute of Bioscience & Biotechnology, Cheongju si, 2Laboratory Animal Medicine, College of Veterinary Medicine, Chonnam National University, Gwangju, Korea, Republic Of, 3New Iberia Research Center, University of Louisiana at Lafayette, Lafayette, United States

**Introduction:** Toll-like receptors (TLRs) and type 1 T cell independent (TI) antigens are considered as an important part of the elaboration of humoral responses to infectious diseases, vaccines and autoimmunity. The present study was designed to identify the TI antigen-mediated immunoglobulin production and B cell differentiation in non-human primates (NHP) which are broadly used as models of human infection.

**Methods:** In the present study, flow cytometry and double-enzyme-linked immunospot assays were performed to measure the frequency of immunoglobulin secreting cells in peripheral blood mononuclear cells (PBMC) or sorted B/T/DC cell subsets stimulated with human TLR ligands. The analyses focused on 10 healthy cynomolgus macaques under steady state conditions, i.e., in the absence of acute or chronic infection or recent vaccination.

**Results:** We found that IgM and IgG antibodies were primarily produced from naïve sorted B cells stimulated with TLR 7/8 and TLR 9 in the absence of T cells, but not from sorted memory B cell subsets. In contrast to IgM production, IgG production was not detected from any sorted B cell subsets. Co-culture of B cells with T cells had no effect on IgM or IgG production, but co-culture with non-B&T cells, particularly HLA-DR+ dendritic cell populations induced the production of both IgM and IgG, which recapitulates the TI immunologic responses in human cells.

**Conclusion:** Thus, NHP models may provide crucial insights into a better understanding of the role of TI antigen-based immune responses in human diseases including infection, autoimmunity and vaccine development.

**Disclosure of Interest:** None Declared

---

**P113**

**DIVERSE NATURAL KILLER T CELLS ARE PROTECTIVE IN CARDIAC ARREST AND RESUSCITATION.**

E. Y. Kim1,*, K. Ikeda2, K. Hayashida2, J. Y. Choi1, J. Y. Guo3, A. Cullen3, F. Ichinose2, M. B. Brenner3

1Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, 2Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, 3Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, United States

**Introduction:** In cardiac arrest and resuscitation (CA/CPR), the heart stops beating and circulation stops until the patient is resuscitated with chest compressions to restart the heartbeat. Even after successful CPR, survival rates are less than 25% after cardiac arrest. Neurological injury is the leading cause of death in cardiac arrest patients admitted to the hospital. Neurological deficits are also the major injury for survivors. The role of inflammation is incompletely understood in cardiac arrest and resuscitation (CA/CPR). CA/CPR induces a robust systemic inflammatory immune response, and the severity of the systemic inflammatory response correlates with neurological outcomes. However, it remains unclear if inflammation drives neurological dysfunction after CA/CPR. Innate T cells are a subset of T cells that produces copious cytokines and regulates the innate and adaptive immune responses. The major types of innate T cells in mice are Natural Killer T (NKT) cells and gamma-delta T cells. Innate T cells can reach full activation within 2 hours after stimulation, so we hypothesized that NKT cells or gamma-delta T cells drove the early inflammatory response in CA/CPR and worsened neurological outcomes. Surprisingly, here we show that diverse natural killer T (NKT) cells were protective in CA/CPR. Diverse NKT cells reduced neuroinflammation, improved neurological function and improved survival after experimental CA/CPR.
Methods: In our mouse model of CA/CPR, potassium chloride i.v. induced asystolic cardiac arrest (i.e., stopped the heart from beating). After 8 min. of cardiac arrest, mice were resuscitated with mechanical lung ventilation, chest compressions and epinephrine i.v. until the return of the heart beat and spontaneous circulation (ROSC).

Results: We examined the major innate T cell subsets in mice after experimental CA/CPR. Invariant NKT cells and gamma-delta T cells did not traffic to the brain after CA/CPR. In line with these results, mice deficient in invariant NKT cells and mice deficient in gamma-delta T cells had the same outcomes as wild-type mice after CA/CPR. However, diverse NKT cells specific for sulfatide lipid antigen trafficked to the brain after CA/CPR. Intriguingly, sulfatide is highly abundant in brain myelin. Diverse NKT cells were protective after CA/CPR, as mice deficient in diverse NKT cells had worsened mortality and neurological function after CA/CPR. Mice deficient in diverse NKT cells had worsened neuro-inflammation after CA/CPR, with increased IFNγ and infiltration of Ly6c+CCR2+ macrophages and neutrophils in the brain after CA/CPR. IFNγ-deficient mice and CCR2-deficient mice had improved neurological function and survival after cardiac arrest. Treatment with sulfatide lipid antigen at the return of spontaneous circulation after cardiac arrest improved neurological outcomes.

Conclusion: This study identifies diverse NKT cells and IFNγ as key regulators of neuro-inflammation, neurological function and survival after cardiac arrest. Despite its clinical importance, there are no pharmacological treatments to help neurological function or survival after clinical CA/CPR. We introduce immunomodulatory lipids, such as sulfatide, as a novel treatment class for cardiac arrest.

Disclosure of Interest: None Declared

P114
HARNESSING CELLULAR HETEROGENEITY TO IDENTIFY NOVEL REGULATORY MODULES CONTROLLING CYTOSOLIC SENSING OF NUCLEIC ACIDS
J. Kim1, S. Mayer1, E. Winkelmann1, H. Ding1, O. Rokach1, A. Califano1, S. D. Shapira1
1Systems Biology, Columbia University, NEW YORK, United States

Introduction: In the event of viral infection, mammalian cells possess a number of antiviral programs that are part of the innate immune response. Detection of viral nucleic acids by host sensors, such as RIG-I (retinoic acid-inducible gene I) and cGAS/STING (cyclic GMP-AMP synthase/stimulator of interferon genes), is a critical initiation step leading to the activation of interferon regulatory factors (IRFs), such as IRF3, and induction of antiviral programs that include production of type I interferons. While these and other players have been implicated in controlling cytosolic responses to nucleic acids, precise quantitative relationships between regulatory modules remains unknown.

Methods: Here, we apply a multiplexed single-cell RNA-sequencing approach together with computational methods to define transcriptional responses mediated by these pathways. We harness intrinsic cellular heterogeneity to infer regulator-target relationships from the correlation and mutual information in gene expression data.

Results: Individual cell states and iterative monitoring of perturbation responses in normal and knockout human cells enable identification of major regulon components that are central to modulating cytosolic sensing of nucleic acids. Indeed, we rediscover known sensors, kinases, transcription factors, and targets, as well as identify novel components of this pathway.

Conclusion: This expanded model of nucleic acid sensing further elucidates early events in the antiviral response and extends the list of positive and negative regulators of this central component of immunity.

Disclosure of Interest: None Declared

P115
IL-33 REGULATES THE ADJUVANT EFFECT OF HYDROXYPROPYL-B-CYCLODEXRIN (HP-B-CD) IN THE LUNG
T. Kusakae1,2, S. Kobari1, E. Kuroda1,2, K. Ishii1,2
1Laboratory of Adjuvant Innovation, Center for Vaccine and Adjuvant Research (CVAR), National Institutes of Biomedical Innovation, Health and Nutrition(NIBIOHN), 2Laboratory of vaccine science, Immunology Frontier Research Center(IFReC), Osaka, Japan

Introduction: The dead, injured, or stressed cells promote inflammation through releasing ‘danger signals’ that can activate macrophages, dendritic cells, and other cells of the innate immune system. Many of these endogenous danger signals, also called damage-associated molecular patterns (DAMPs) or alarmin, has been identified so far. Many reports have shown that IL-1α and IL-33 function as DAMPs and play a significant role in inflammation and/or immunogenicity of lung tissues. Previously, we reported that hydroxypropyl-β-cyclodextrin (HP-β-CD), a widely used pharmaceutical excipient to improve solubility and drug delivery, can be used effectively as an adjuvant for flu vaccine in both subcutaneous and intranasal administration1,2. However, the molecular mechanisms of the adjuvanticity of HP-β-CD are poorly understood.

Methods: To investigate whether DAMPs are involved in the adjuvant effect of HP-β-CD, we measured the release and production of DAMPs such as dsDNA, IL-1α and IL-33 using ELISA. Furthermore, we analyzed the antibody responses in deficient mice after HP-β-CD administration.

Results: We show that mechanisms of immune responses induced by intranasal administration of HP-β-CD are completely different from those by subcutaneous administration, and IL-33 is involved in adjuvanticity by intranasal route, but not subcutaneous route.

Conclusion: It suggests that the types of DAMP induced by adjuvant depend on administration route, resulting in induction of tissues specific immune responses. These
results indicate that the understanding of administration route and tissue-specific immune responses are critical for for the design of unique vaccine adjuvants.

**Disclosure of Interest:** None Declared

**P116**

**INTRACELLULAR CALCIUM REGULATES THE STING SIGNALING PATHWAY**

D. Kwon¹, H. Sesak², S.-J. Kang¹*

¹Korea Advanced Institute of Science and Technology, Daedeon, Korea, Republic Of, ²Johns Hopkins University School of Medicine, Baltimore, United States

**Introduction:** Although a multitude of post-translational modifications that regulate the STING pathway have been discovered, the cellular events required for STING translocation remain unclear. Calcium is a versatile second messenger that regulates many intracellular signaling pathways including innate immune signaling pathway. Here, we examined whether intracellular calcium regulates the activation of STING signaling.

**Methods:** Various extracellular and intracellular stimuli trigger change in calcium flux, which are finely tuned by various calcium-regulating channels, such as sarco/endoplasmic reticulum calcium-ATPase (SERCA), the inositol triphosphate receptor, and the ryanodine receptor, and mitochondrial calcium unipporter. We sequestrated intracellular calcium by BAPTA-AM or increased intracellular calcium levels via ionomycin, and then examined the activity of the STING signalling pathway.

**Results:** We observed that BAPTA-AM suppressed an STING pathway activation. Furthermore, ionomycin-induced influx of extracellular calcium inhibited STING translocation and STING-mediated IFN-b production. We observed DRP1-deficiency prevents the ionomycin’s inhibition of the STING pathway, concomitantly attenuating ionomycin-induced elevation of intracellular calcium.

**Conclusion:** In summary, we demonstrated that proper intracellular calcium level is crucial for the competency of STING pathway and determined the key role of DRP1 in regulating calcium levels. Our findings will advance our understanding of regulatory mechanism for STING activation and the intricate communication between calcium signaling and regulators of mitochondria dynamics.

**Disclosure of Interest:** None Declared

**P117**

**ZIKA VIRUS INFECTS HUMAN MICROGLIAL CELLS AND ACTIVATES TYPE III INTERFERON RESPONSE FOR HOST ANTIViral MECHANISM**

A. Selim¹, S. Lee¹*

¹HKU-Pasteur Research Pole, The University of Hong Kong, Hong Kong, Hong Kong

**Introduction:** Zika is an enveloped, non-segmented, positive sense, single stranded RNA arbovirus transmitted mainly via *Aedes aegypti* mosquito bites, vertically from mother to fetus and sexually. Zika is a family member of the flaviviridae viruses as dengue virus, West Nile virus and Japanese encephalitis virus which has a history of neurotropism. However, till now, little is known about the neuro-immune pathogenesis of Zika virus.

**Methods:** In this study, we investigated the viral replication kinetics as well as the host immune response in human astrocytes, neuronal and microglial cells after Zika virus infection.

**Results:** Zika virus replicated efficiently in human neuronal and microglial cells but to a lesser extent in astrocytes. We also found that Zika virus strongly induced interferon response, particularly type III interferon, IL29 in both microglial and astrocytic cells, but not in neuronal cells. While, induction of IFNα receptor can only be detected in neuronal cells in response to infection. Treatment of IL29 significantly reduced virus titre in Zika virus infected human brain cells.

**Conclusion:** Our results demonstrated that three of the main parenchymal brain cell types are susceptible to Zika virus infection. The hyper induction of IL29 in human astrocytes and microglial cells opens new insights for the understanding of Zika virus induced host immune response and the potential therapeutic use of IFNα for treating Zika virus infection.

**Disclosure of Interest:** None Declared

**P118**

**NUCLEAR RECEPTOR NR0B2 NEGATIVELY MODULATES THE TYPE I INTERFERON SIGNaling**

J.-S. Lee¹*

¹College Of Veterinary Medicine, Chungnam National University, Daejeon, Korea, Republic Of

**Introduction:** Nuclear receptor NR0B2 is an orphan nuclear receptor and interacts with diverse nuclear receptors and transcription factors. Although regulatory function of NR0B2 was demonstrated in various signaling pathways, the role of NR0B2 in type I IFN signaling pathway has not been studied.

**Methods:** In this study, we used Bone Marrow-Derived Macrophage, alveolar macrophage of NR0B2 knock-out mice and NR0B2 knock-down cell lines for the virus replication and cytokine secretion etc. For the mechanism study, we also used similar cells and diverse molecular biological methods.

**Results:** Using Bone Marrow-Derived Macrophage and alveolar macrophage of NR0B2 knock-out mice and NR0B2 knock-down cell lines, we could check that loss of NR0B2 enhance IFN-β cytokine secretion against VSV, PR8, Poly(I:C), 5’ppp-dsRNA, HSV and Poly(dA:dT). Moreover, we could find NR0B2 was translocated to nucleus and bound with Cret binding partner (CBP) after virus infection. CBP as transcription co-activator, interacts with transcription factors and make complex which is known as enhanceosome binding on enhancer region upstream of the IFN-β gene transcription start site. β-catenin is also one of the components of enhanceosome.

**Disclosure of Interest:** None Declared
binding with CBP to recruit CBP to enhanceosome. Here, we confirmed that NR0B2 inhibit this CBP and β-catenin interaction in nucleus.

**Conclusion:** Our findings suggest the novel function of NR0B2 that negatively regulates type I IFN signaling pathway. [The Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Grant no. 318039-3)]

**Disclosure of Interest:** None Declared

### P119

**DIA4 INHIBITS TYPE I INTERFERON SIGNALING BY REDUCING SELF-ASSOCIATION OF TBK1**

H.-C. Lee1, D.-J. Jang1, T.-H. Kim1, K. Chathuranga1, J.-S. Lee1

1College Of Veterinary Medicine, Chungnam National University, Daejeon, Korea, Republic Of

**Introduction:** TANK-binding kinase 1 (TBK1) is a key component of innate immunity that phosphorylates transcriptional factors to activate induction of antiviral interferons and inflammatory cytokines upon virus infection. In innate immune system against virus infection, TBK1 is importantly involved in RNA virus and DNA virus infection(1). As the excessive innate immune responses induced by the pro-inflammatory cytokines and type I interferons are also harmful to host, TBK1 is modulated tightly by several mechanisms(2). Here we report that the DIA4 regulates the innate immunity by reducing self-association of TBK1, the essential step for its activation.

**Methods:** Human embryonic kidney 293 (HEK293T) cell line, human cervical carcinoma (HeLa) cell line, african green monkey kidney (Vero) cell line and Mouse leukemic monocyte macrophage (Raw264.7) cell line were used for the study. NDV-gfp, PR8-gfp, HSV-gfp, VSV-gfp were propagated and used for the in vitro studies. DIA4+/+ and DIA4-/- mice were attempted using influenza A virus (H1N1) infection in order to assess an effect of DIA4 in antiviral immune responses.

**Results:** Deficiency of DIA4 enhanced cytokine secretion, causing reduced replication of virus in vivo and in vitro. We also demonstrated that this regulation of innate immunity was mediated by the interaction between DIA4 and TBK1. DIA4 reduced oligomerization of TBK1 and finally inhibited activation of TBK1 characterized by the phosphorylation on serine 172. Additionally, we show that TBK1 is responsible for the phosphorylation of serine on DIA4.

**Conclusion:** Taken together, our study suggests that TBK1 and DIA4 form negative feedback loop for regulation of antiviral innate immunity. [The Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Grant no. 318039-3)]

**Disclosure of Interest:** None Declared

### P119.A

**THE ROLES OF GM-CSF ON IMMUNOPATHOLOGY AND CONTROL OF THE PARASITE BURDEN DURING ACUTE TOXOPLASMOSIS**

M. Jacquet1,*, J. Park1, J. T. Clark1, C. A. Hunter1

1University of Pennsylvania, Philadelphia, United States

**Introduction:** *Toxoplasma gondii*, an obligate intracellular parasite, induces type 1 immune response in mice. The control of *T. gondii*, highly dependent on IFNg production, requires a competent innate immune response. Myeloid cells such as macrophages and dendritic cells are involved in the early control of parasite burden and immune regulation, but little is known about the factors that contribute to the infection-induced expansion and maintenance of these cells. Granulocyte macrophage-colony stimulating factor (GM-CSF) influences monocytes, as well as dendritic cells and macrophages differentiation and activation. But the role of GM-CSF during toxoplasmosis is not known.

**Methods:** Using GM-CSF deficient mice, antibody blockade, or injection of exogenous GM-CSF during acute infection by *T. gondii*, we investigated the role of this hematopoietic cytokine on the control of the parasite burden in vivo.

**Results:** Our studies indicate that while macrophages are critical for acute resistance, acute neutralization of GM-CSF did not alter the macrophage responses, T cell responses, or affect susceptibility. However, using injection of GM-CSF during infection altered the control of the parasite burden in RAG1-/-mice. The number of infected cells from the peritoneum cavity of mice treated with GM-CSF was 5.9 times higher compared to control mice. This difference can be explained by lower amount of IFNg observed in the serum. The analysis of the cells from the peritoneum indicated a difference in macrophages polarization. GM-CSF treatment promoted the M2 macrophages phenotype, more susceptible to the parasite. At D10p.i. the GM-CSF treated mice showed unusual neutrophils infiltration in the liver, and higher levels of liver pathology associated to higher parasite burden.

**Conclusion:** The results presented in this study will help to better understand the role of myeloid populations and the effects of GM-CSF on macrophages during the early phase of *T. gondii* infection. Understanding the influence of GM-CSF injection in a context of inflammation can also have an impact on the therapeutic use of this cytokine.

**Disclosure of Interest:** None Declared

### P119.B

**PADI4 REGULATES NET FORMATION AND INFLAMMATORY CELL DOWNSTREAM OF MLKL**

B. A. Croker1,*, A. A. D’Cruz1, M. Speir1, M. Bliss-Moreau1, S. Dietrich1, S. Wang1, A. A. Chen1, M. Gavillet1, A. Al-Obeidi1, K. E. Lawlor2, J. E. Vince2, M. Kelliler3, R. Hakem4, M. Pasparakis5, D. A. Williams1, M. Ericsson6

1Boston Children’s Hospital, Boston, United States, 2The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, 3UMass Medical School, Worcester, 4University of Toronto, Toronto, 5University of Cologne, Germany, 6Harvard Medical School, Boston, United States
Introduction: Neutrophil extracellular trap (NET) formation can generate short-term functional anucleate cytoplasm and can trigger loss of cell viability. We examined the role of non-apoptotic cell death signaling in NET formation by studying necroptotic human and mouse neutrophils.

Methods: Necroptotic cell death signaling was activated by caspase-8 inhibition and pharmacological targeting of inhibitor of apoptosis proteins (IAPs). The specificity of cell death was confirmed using neutrophils from mice deficient in receptor-interacting protein kinase-3 (Ripk3), Ripk1 kinase activity (Ripk1D138N/D138N), caspase-8, or the plasma membrane-disrupting effector protein mixed lineage kinase domain-like (Mlkl). NETs were investigated with a combination of imaging and quantitative flow cytometry, immunogold electron microscopy, immunofluorescence microscopy, and ex vivo microbicidal assays to demonstrate functionality of NETs.

Results: In response to necroptotic stimuli, Mlkl translocates to the plasma membrane in neutrophils, and is required for downstream NADPH oxidase-independent reactive oxygen species production, loss of cytoplasmic granules, breakdown of the nuclear membrane, chromatin decondensation, histone hypercitrullination, and extrusion of bacteriostatic NETs. Neutrophils expressing a kinase-dead form of Ripk1 (Ripk1D138N/D138N) were unable to generate Ripk3/Mlkl-deficient NETs, or to undergo necroptosis or caspase-8-dependent apoptosis. NET formation that is triggered by necroptotic stimuli is dependent on TNF production, can be differentially modulated by the actions of G-CSF and IFNg, and occurs concomitantly with the production, processing and release of IL-1α/b. Human necroptotic neutrophils also release NETs that kill S.aureus. Necrotic NETs contain components of canonical NETs including dsDNA, hypercitrullinated histones, and neutrophil elastase, but also non-canonical components including Mlkl and membranes.

Peptidylarginine deiminase 4 (Padi4) is required for extrusion of necroptotic NETs but not for chromatin decondensation. Padi4-deficient neutrophils are hypersensitive to necroptotic stimuli despite normal levels of phosphorylated Mlkl, indicating that Padi4 acts downstream of Mlkl activation.

Conclusion: The failure of Padi4-deficient neutrophils to generate necroptotic NETs in the presence of membrane-associated Mlkl, suggests that the removal of Mlkl membrane-disrupting complexes by NETs can facilitate membrane repair and control the kinetics of neutrophil necroptosis. This work defines a distinct cell death signaling network downstream of Mlkl that promotes Padi4-dependent necroptotic NET release.

Disclosure of Interest: None Declared

P119.C
THE ROLE OF PSTPIP2 AND INNATE IMMUNITY IN LISTERIA MONOCYTOGENES INFECTION
R. Geiger1,*, T.-D. Kanneganti1

1Immunology, St. Jude Children’s Research Hospital, Memphis, United States

Introduction: Pstpip2cmo mice have proven to be a useful model for studying chronic recurrent multifocal osteomyelitis (CRMO). Mice lacking expression of the PSTPIP2 protein due to a L98P point mutation in the Pstpip2 gene present with footpad and tail bone inflammation. Their disease leads to systemic inflammation mediated by inflammasome-independent production of IL-1b. The Pstpip2cmomphenotype has been well characterized, but until now, the effect of chronic inflammation on these mice’s ability to fight bacterial infection has not been studied.

Methods: Wild type and Pstpip2cmo mice were infected intravenously with L. monocytogenes. Their responses to infection were observed as a function of survival, innate immune cell infiltration, bacterial CFUs, and cytokine signaling. Cell specificity of the PSTPIP2 immune response was studied with wild type and Pstpip2cmo neutrophil and macrophage tissue cultures.

Results: Pstpip2cmo mice had a higher survival rate after L. monocytogenes infection, observed up to thirteen days post-infection. This survival was matched with significantly less weight loss in Pstpip2cmo mice compared to wild type mice. Bacterial CFUs were found to be lower in Pstpip2cmo livers and spleen compared to wild type at both twelve hours and three days post-Listeria infection. Counts of circulating PMNs and monocytes were comparable between wild type and Pstpip2cmo mice basally, and twelve hours and three days post-Listeria infection. Additionally, circulating levels of inflammatory cytokines, IL-6, KC, IL-1b, and TNF-awere comparable basally and twelve hours post-Listeria infection. However, survival required the presence of the Il1b gene, but not Caspase-1. In vitro, wild type and Pstpip2cmo BMDMs and neutrophils showed similar bacterial uptake, inflammatory signaling, and programmed cell death upon Listeria infection.

Conclusion: In vivo data suggest a heightened ability in Pstpip2cmo mice to clear Listeria bacteria via innate immune functions but independent of inflammasome activation. Combined with in vitro data, it seems this resistance to Listeria infection is dependent on signaling between multiple immune cell types in Pstpip2cmo mice.

Disclosure of Interest: None Declared

P119.D
NK CELLS CONTRIBUTE TO THE ANTI-TUMOUR RESPONSE ELICITED BY PD-1/PD-L1 BLOCKADE IMMUNOTHERAPY.

1Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Canada.
**P119.F**

LCHK168 IMPROVES RENAL TUBULOINTERSTITIAL LESIONS BY BLUNTING PRIMING AND MITOCHONDRIA-ASSOCIATED ACTIVATING SIGNALS OF NLRP3 INFLAMMASOME

**W.-H. Hsu**

**J.-F. Hua**

**L.-H. Duan**

**L. J. Chu**

**Y.-C. Lee**

**W.-T. Wong**

**S.-L. Lee**

**J.-H. Lau**

**C.-L. Chu**

**L.-J. Ho**

**H.-W. Chiu**

**Y.-J. Hsu**

**A. Chen**

**S.-M. Ka**

**1Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, 2Department of Biotechnology and Animal Science, National Ilan University, Ilan, 3Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, 4Molecular Medicine Research Center, Chang Gung University, Taoyuan, 5Department of Chemistry, R.O.C. Military Academy, Kaohsiung, 6Division of Allergy, Immunology and Rheumatology, Department of Internal Medicine, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, 7Graduate Institute of Immunology, National Taiwan University College of Medicine, Taipei, 8Institute of Cellular and System Medicine, National Health Research Institute, Miaoli, 9Division of Nephrology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, 10Academia of Medicine, National Defense Medical Center, Taipei, Taiwan, Taipei, Taiwan, Province of China**

**Introduction:** These rigorous data shed new light on the importance of NK cells in PD-1/PD-L1 blockade, and may lead to more rational combinations of cancer immunotherapies in the future.

**Disclosure of Interest:** None Declared

---

**P119.E**

SMG INHIBITS HBV REPLICATION THROUGH SAM DOMAIN DEPENDENT SUPPRESSION OF VIRAL RNA TRANSLATION

**Y. Wang**

**X. Fan**

**Y. Song**

**J. Han**

**1State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen, China**

**Introduction:** Interferon (IFN) signaling pathway restricts replication of human hepatitis B virus (HBV), a critical factor of hepatocellular carcinoma (HCC), by inducing hundreds of IFN-stimulated genes (ISGs). However, only a few of ISGs have directly antiviral activity and their functions are largely unknown.

**Methods:** Aiming to figure out the directly effective ISGs employed by IFN to limit HBV, we detected the anti-HBV activity of 370 ISGs in an in vitro HBV infection sensitive cell base screening system. Among the ISGs with inhibitory effects against HBV, SMG exhibited the most significantly function to suppress HBV replication.

**Results:** In multiple hepatocytes, SMG overexpression markedly decreased the level of viral antigens and nucleotides while silencing or knock-out of SMG resulted in the enhancement of virus replication. Our findings suggested that SMG specifically recognized and directly bind to HBV RNA, mediated by SAM domain to recognize a stem-loop sequence. This interaction inhibited the viral RNA translation and decrease the RNA stability, and one conserved region located in the C terminal of SMG was identified to play important role in the downstream signal axis. Moreover, hydrodynamic injected SMG expression plasmids together with HBV replicon can also dramatically reduce the production of virus in mouse liver which further proved the function of SMG in vivo.

**Conclusion:** We found that SMG bound to HBV RNA directly and inhibited the translation which further mediated the suppression of virus replication in vivo and in vitro. These data help us understand more about the antiviral activity of SMG and suggest a molecular basis for the potential development of therapeutics against HBV.

**Disclosure of Interest:** None Declared
progress to end-stage renal disease and uremia, feature renal tubular atrophy, interstitial mononuclear leukocyte infiltration and fibrosis in the kidney.

**Methods**: Our study tested renoprotective and therapeutic effects of LCHK168, a major absorbable intestinal bacterial metabolite of ginsenosides, on renal TILs, using a mouse unilateral ureteral obstruction model.

**Results**: The results showed that administration of LCHK168 markedly improved renal TILs as demonstrated by reduced urine levels of proinflammatory cytokines, macrophage infiltration, T cell activation or fibrosis in the kidney as early as three days after treatment. In particular, these beneficial effects of LCHK168 treatment clearly correlated with its negatively regulating: [1] NF-κB-associated priming and mitochondria-associated activating signals of NLRP3 inflammasome; [2] STAT3 signaling, which in part preventing NLRP3 inflammasome activation; and [3] TGF-β-dependent Smad2/Smad3 fibrotic pathway, in renal tissues, renal tubular epithelial cells under mechanically-induced constant pressure, and/or activated macrophages, the latter, as a major inflammatory player contributing to renal TILs.

**Conclusion**: The present study, for the first time, presented the potent renoprotective and therapeutic effects of LCHK168 on renal TILs by targeting the NLRP3 inflammasome and STAT3 signaling.

**Disclosure of Interest**: None Declared

---

**P119.G**

**IL-36 SIGNALING TRIGGERS NLRP3 INFLAMMASOME ACTIVATION IN IGA NEPHROPATHY**

W.-H. Hsu**1,**, T.-J. Lin2, C.-Y. Wu1, K.-F. Hua3, E.-T. Liu2, Y.-J. Hsu1, F.-C. Liu2, H.-H. Ch2, A. Chen2, S.-M. Ka4

1Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan, 2Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, 3Department of Biotechnology and Animal Science, National Ilan University, Ilan, 4Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, 5Division of Rheumatology/Immunology and Allergy, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, 6Graduate Institute of Aerospace and Undersea Medicine, National Defense Medical Center, Taipei, Taiwan, Province of China

**Introduction**: IL-36 cytokines play an important role in innate and adaptive immunity. We recently showed a pathogenic role of IL-36 receptor (IL-36R)-mediated signaling pathway underlying the development of renal tubulointerstitial lesions. We now further extended whether the fundamental efforts to molecular pathogenesis of IgA nephropathy (IgAN), the most common glomerular disorder in young adults across the world.

**Methods**: Renal and urine levels of IL-36α were determined for IgAN patients. IL-36R knockout (KO) and its wild type (WT) mice were induced of IgAN with IgA immune complexes (IgA ICs), followed by clinical, pathological, and molecular mechanism analyses. Glomerular levels of IL-36R, IL-36, NLRP3 and IL-1β in the IgAN mice were evaluated in a time course manner. In addition, we used anti-IL-36R antibody to treat the IgAN mice to confirm the pathogenic role of IL-36 in the mouse IgAN model.

**Results**: IL-36α levels in renal tissues and urine samples from patients were markedly increased. Moreover, reduced severity of renal lesions was observed in IL-36R KO mice compared to that of their WT counterparts, equally induced of IgAN, and this effect was correlated with: [1] down-regulated glomerular IL-36α, NLRP3 and IL-1β expressions in IgAN IL-36R KO mice; [2] markedly reduced proliferation in primary mesangial cells isolated from IL-36R KO mice under IgA ICs stimulation; and [3] inhibiting NLRP3 inflammasome activation and augmented autophagy in the renal tissues in IgAN mice. Notably, treatment with IL-36R antibody attenuated renal lesions.

**Conclusion**: IL-36 signaling played a pathogenic role in the development of IgAN by activating NLRP3 inflammasome and modulating autophagy, and targeting IL-36 may be a useful therapeutic approach for IgAN.

**Disclosure of Interest**: None Declared

---

**P119.H**

**IGA NEPHROPATHY: ENDOGENOUS GALECTIN-3 PROMOTES NLRP3 INFLAMMASOME BY ENHANCING NLRP3 ASSEMBLY AND REDUCING AUTOPHAGY**

C.-Y. Wu1,*, K.-F. Hua2, S.-R. Yang1, F.-T. Liu3, H.-L. Chen2, H.-Y. Chen2, C.-C. Wu1, S.-M. Ka4, A. Chen6

1Graduate Institute of Life Sciences, National Defense Medical Center, Taipei city, 2Department of Biotechnology and Animal Science, National Ilan University, Ilan, 3Institute of Biomedical Sciences, Academia Sinica, 4Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, 5Graduate Institute of Aerospace and Undersea Medicine, ational Defense Medical Center, 6Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei city, Taiwan, Province of China

**Introduction**: IgA nephropathy (IgAN) is the most common glomerulonephritis in the world. Galectin-3 (Gal-3) is a galactoside-binding protein implicated in diverse biological processes and has diverse roles in the development and progression of certain inflammatory conditions, but its role in the pathogenesis of IgAN has yet to be determined. The main purpose of this proposal is to define the pathogenic role that endogenous Gal-3 plays in IgAN and a key NLRP3 signaling activation through NLRP3/ASC assembling and NIMA-related kinase 7 (NEK7) was involved.

**Methods**: Gal-3 knockout (KO) and WT mice were induced of IgAN with IgA immune complexes (IgA ICs), followed by clinical, pathological, and molecular mechanism analyses. Knockdown of Gal-3 in macrophages and further analysis for NLRP3
inflammasome activation, NLRP3-ASC complex formation, ASC oligomerization, NLRP3-NEK7 interaction to further define the potassium mediated IL-1β production and autophagy. Primary cultured mesangial cells and bone marrow-derived dendritic cells (BMDCs), isolated from Gal-3 KO and WT mice, respectively, were used to evaluate the impact of Gal-3 on proinflammatory cytokines production and/or Th17 response. Next used both BMDCs which primed with IgA ICs and OT-II CD4 cells to examine the effect of Gal-3 on the differentiation of Th17 cells.

**Results:** Prevented renal function and pathology were observed in IgAN of Gal-3 ko mice. Moreover, Gal-3 deficiency damped NLRP3 inflammasome activation by inhibiting NLRP3-ASC complex formation, ASC oligomerization, NLRP3-NEK7 interaction and potassium mediated IL-1β production in macrophages treated with IgA ICs. In contrast, IgA ICs treatment increased autophagic response in macrophages; however, this effect was inhibited in Gal-3 knockdown cells, which in turn led to NLRP3 inflammasome inhibition. Besides, inhibitory of IL-17 production was noted in IgAN of Gal-3 ko mice. Furthermore, gal-3 inhibited T cell proliferation and Th17 differentiation in IgA IC primed BMDCs which isolated from Gal-3 ko and WT mice.

**Conclusion:** Endogenous Gal-3 promotes NLRP3 inflammasome by enhancing NLRP3 assembly and reducing autophagy, and thus may be a therapeutic target for IgAN.

**Disclosure of Interest:** None Declared

**P119.I**

**LCHK168 THERAPY BY ENHANCES SIRT3/AUTOPHAGY-MEDIATED NLRP3 INFLAMMASOME INHIBITION IN IGA ICS MEDIATED INFLAMMATION**

A. Chen1,*, W.-H. Hsu2, K.-F. Hua3, C.-Y. Wu2, L. J. Chu4, A. Takahata5, Y.-C. Lee3, Y. Suzuki5, S.-L. Lee6, S.-S. Yang7, S.-M. S. Ka8

1Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, 2Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, 3Department of Biotechnology and Animal Science, National Ilan University, Ilan, 4Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan, Province of China, 5Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan, 6Department of Chemistry, R.O.C. Military Academy, Kaohsiung, 7Division of Nephrology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, 8Graduate Institute of Aerospace and Undersea Medicine, National Defense Medical Center, Taipei, Taiwan, Province of China

**Introduction:** IgA nephropathy (IgAN), the most common primary glomerulonephritis, follows a relatively poor prognosis yet lacks a pathogenesis-based treatment. LCHK168 is a major absorbable intestinal bacterial metabolite of ginsenosides, the latter being bioactive components from ginseng. Herein, we examined whether the therapeutic effect of LCHK168 on IgAN in mice.

**Results:** LCHK168 to render its therapeutic effect on the both IgAN models.

**Conclusion:** LCHK168 treatment markedly improved the renal condition associated with enhancing the axis of SIRT3/autophagy-mediated NLRP3 inflammasome inhibition is likely to be a mechanism of action for LCHK168 to render its therapeutic effect on the both IgAN models. Our results support LCHK168 as a potent drug candidate for IgAN.

**Disclosure of Interest:** None Declared

**P120**

**DIVERGENT IMMUNOMODULATORY CAPACITY OF THE HEALTHY VERSUS IBD HUMAN ENTERIC VIROME**

F. Adilaghdam1,*, T. L. Saunders 1, H. Rivas1, K. L. Jeffrey1,2

1Gastrointestinal Unit and Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital, Boston, 2Center for Microbiome Informatics and Therapeutics, Massachusetts Institute of Technology, Cambridge, United States

**Introduction:** Although the microbiome has been established as an important regulator of health and disease, the role of commensal viruses that inhabit human intestine (the “virome”) is largely unknown. The fecal virome is altered in inflammatory bowel disease (IBD) and depletion of viruses or host viral receptors enhances intestinal inflammation in mice. How the virome may contribute to host homeostasis or impact gut inflammation is unknown. Here we determined the immunomodulatory capacity of healthy versus IBD human intestinal viromes.
Methods: We isolated viral like particle (VLP) from fresh ileostomy or colon resections from healthy/non-IBD, ulcerative colitis (UC) or Crohn’s disease (CD) donors who consented through the Prospective Registry in IBD Study at Massachusetts General Hospital (PRISM). Briefly, samples were mechanically disrupted in PBS using a bead homogenizer and the resultant homogenized slurry centrifuged to pellet debris. Supernatant was filtered through 0.45um and 0.22um Whatman filters and treated with DNase, lysozyme and polymixin B to remove host, microbial or endotoxic contaminants. VLPs were quantified by imaging using SYBR Gold and Dil and confirmed to be endotoxin and cytotoxic free. Primary human monocyte derived macrophages were infected with VLPs or transfected with RNA isolated from VLPs and cytokines measured. The influence of VLPs on Intestinal epithelial cells was determined by Trans-epithelial electrical resistance (TEER) assays.

Results: Viruses isolated from fresh ileostomies or colon resections from CD or UC patients triggered enhanced interferon (IFN) and pro-inflammatory tumor necrosis factor (TNF) and interleukin (IL)-6 production from macrophages than viruses isolated from healthy controls that triggered more anti-inflammatory IL-10 and transforming growth factor (TGFb). RNA isolated from IBD VLPs that was transfected into macrophages triggered more pro-inflammatory response compared to healthy controls, while equal amounts of VLP RNA from healthy, non-IBD individuals generated an anti-inflammatory response. Moreover, the IBD virome significantly compromised intestinal epithelial barrier integrity.

Conclusion: Our data reveal that viruses commensal to the intestine are not ignored by the host but instead elicit homeostatic innate immune responses in health that switch to pro-inflammatory responses capable of inciting intestinal pathology in disease. Manipulation of the virome, or the host immune response to it, may be beneficial in IBD.

Disclosure of Interest: None Declared

P121
IL-22/IL-22RA1 SIGNALING AXIS UNIQUELY ORCHESTRATES INNATE IMMUNE RESPONSES DURING OROPHARYNGEAL CANDIDIASIS (OPC)
F. E. Y. Aggor¹, H. R. Conti², T. Break³, B. Coleman¹, A. Verma¹, G. Trevejo-Nunez¹, P. S. Biswas¹, M. S. Lionakis³, J. K. Kolls⁴, S. L. Gaffen⁵
¹Department of Medicine, Division of Rheumatology and Clinical Immunology, University of Pittsburgh, Pittsburgh, ²Department of Biological Sciences , The University of Toledo, Toledo, ³Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, ⁴Center for Translational Research in Infection and Inflammation, Tulane University, Tulane, United States

Introduction: Oropharyngeal candidiasis (OPC) is an opportunistic fungal infection of the oral mucosa predominantly caused by Candida albicans. The incidence of mucocutaneous candidiasis in Autoimmune polyendocrine syndrome type 1 (APS-1) patients is associated with auto-antibodies against IL-17A, IL-17F and IL-22 and point to their clinical relevance in fungal infections. However, the role of IL-22 in OPC remains largely unexplored. Here, we address the role of IL-22 in the context of acute OPC.

Methods: We assessed IL22 induction during OPC by qPCR analysis. Using IL22TdTTomato mice, we confirmed the cellular sources of IL-22 by flow cytometry. Immunohistochemistry (IHC) was done to assess the localization of IL-22RA1 in murine tongue and bone marrow chimera experiment was done to determine the major IL-22 responding cells during OPC. RNASeq analysis was performed to delineate the IL-22 dependent mechanisms at play during acute OPC. Immunofluorescence staining was done to assess the activation of STAT3 in Il22ra1−/− mice and the role of STAT3 in murine suprabasal oral epithelial layer was assessed by conditional deletion of STAT3.

Results: We found that IL22 is induced in murine tongue during acute OPC and mostly produced by NK1.1⁺ cells. IL-22/IL-22RA1 axis mediated protection during OPC. Neutralization of IL-22 in (Act1−/−) mice with impaired IL-17 signaling exacerbated the disease. Strikingly, IL-22RA1 was uniquely expressed in murine tongue and non-hematopoietic cells were the major responders to IL-22 during OPC. Our RNASeq data revealed some unique and IL-17 independent targets of IL-22 and gave further insight on the roles of IL-22 during OPC. Among these, Il22ra1−/− mice showed impaired activation of STAT3. Surprisingly, conditional deletion of STAT3 within suprabasal oral epithelial cells did not enhance susceptibility to OPC and suggest a potential role for STAT3 in the basal oral epithelial layer.

Conclusion: IL-22/IL-22RA1 signaling axis contributes in a non-redundant manner to IL-17 to mediate protection during OPC. Although there are some common genes targeted by both IL-22 and IL-17, some target genes are independent of IL-17 signaling. These IL-22 dependent genes further support the unique role of IL-22 during OPC and will be explored to assess their functional relevance and mechanism of action in the context of OPC.

Disclosure of Interest: None Declared

P122
INTESTINAL CD4 T-CELL RESPONSES TO ORAL VACCINATION
A. Bhattacharjee¹, J. JF, J. Tometich¹, N. Rittenhouse¹, A. Poolek¹, O. Harrison³, J. Linehan³, Y. Belkaid³, T. Hand³
¹Pediatrics, University of Pittsburgh, Pittsburgh, United States, ²School of Medicine, Tsinghua University, Beijing, China, ³Mucosal Immunology Section, Laboratory of Parasitic Diseases, NIAID, Bethesda, United States

Introduction: Oral vaccination is an efficient method of preventing infectious diseases, particularly those that are spread via host-pathogen interactions at the
gastrointestinal mucosa. However, oral vaccination efficacy has been found to vary significantly between populations and geographical locations. Environmental Enteric Dysfunction (EED), a chronic inflammatory gut disease, has been demonstrated to impair vaccine responses in affected children. In this study, we wanted to determine the transcriptome that co-related with gut-specific T cell memory following oral vaccination and to understand how the behavior of the vaccine-specific T cells are altered in the entero pathic gut.

**Methods:** We used the double mutant heat labile toxin (dmLT) of *E. coli* as a vaccine, to analyze the gene expression patterns that are required for the development of intestinal resident CD4 cells. Using dmLT specific tetramers, we isolated vaccine-specific memory T cells from the gut and the lymph nodes at different time points after vaccination and performed RNA-Seq on these cells to study which genes are required for gut-specific memory persistence. Additionally, to study oral vaccine responses in enteropathic guts, we have adapted a murine model of EED, wherein mice are fed an isocaloric diet low in fat and protein and are chronically exposed to an adherent invasive *E. coli* strain to mimic gut damage induced in enteropathic children by chronic exposure to fecal pathogens.

**Results:** Our RNA-Seq studies have uncovered a unique and novel set of genes associated with intestinal CD4 memory T cell survival and function in the GI tract, and have enabled us to design experiments in mice lacking those genes. Further, in our murine model of EED which demonstrated significant growth stunting and effacement of villous architecture, oral vaccine responses were substantially weaker compared to isocaloric controls. In addition, flow cytometric analysis of dmLT specific cells from the EED mice exhibited decreased Th17 vaccine responses. Further analysis of the gut immune environment prior to vaccination revealed that EED mice had increased CD11b+ Cd64+ macrophages and an increase in RORγT T regulatory cells.

**Conclusion:** Together, we have identified the molecular components of an effective T cell response to an oral vaccine and have begun to understand the cellular basis of vaccine failure in the context of gut inflammation in diseases like EED.

**Disclosure of Interest:** None Declared

---

**P123**

**CLEC-2 REGULATES INNATE IMMUNE ACTIVATION IN THE LUNG**

P. Burkett1,2, S.-J. Kim1, A. Wallrapp2, S. Riesenfeld2, R.-E. Abdunnour1, B. Levy1, V. Kuchroo2

1Department of Medicine, Brigham & Women's Hospital, 2Evergrande Center, Brigham & Women's Hospital and Harvard Medical School, Boston, 3Broad Institute, Cambridge, United States

**Introduction:** Alveolar macrophages are crucial for maintaining lung homeostasis and preventing tissue damage by suppressing inflammation, which is mediated in part through interactions with alveolar epithelial cells. Podoplanin (Pdpn), a glycoprotein expressed by type 1 alveolar epithelial cells, is a ligand for C-type Lectin-like Receptor-2 (CLEC-2), which is preferentially expressed by alveolar macrophages within the lung myeloid compartment. In order to investigate the role of the CLEC-2/Pdpn pathway in regulating lung immune responses, we analyzed CLEC-2-deficient mice.

**Methods:** We analyzed the immunophenotype of lung resident cell populations in CLEC-2-deficient mice using flow cytometry and transcriptional analysis. We also analyzed lung histology, as well as assessing for airways hyperreactivity with methacholine challenge. Bone marrow-derived macrophages were generated with M-CSF, and these cells were co-cultured with CD4 T cells.

**Results:** We found that CLEC-2-deficient mice develop spontaneous type 2 inflammation, with increased lung infiltrating immune cells, airway eosinophilia, and goblet cell hyperplasia. Transcriptional profiling of infiltrating immune cells showed increases in both type 2 and type 3 immune responses, along with upregulation of type 2 alarmins produced by damaged epithelial cells. The myeloid populations of CLEC-2-deficient mice were significantly altered, with reduced alveolar macrophages and increased inflammatory monocytes, and both of these populations expressed increased levels of MHC Class II, as well as higher levels of both Arg1 and Nos2. Upon coculture with CLEC-2-deficient bone marrow derived macrophages (BMDMs), naïve T cells demonstrated enhanced Th2 differentiation *in vitro*, indicating the importance of myeloid cell-specific CLEC-2 expression in regulating adaptive type 2 responses. Transcriptional analysis of BMDMs revealed altered patterns of gene expression in CLEC-2-deficient BMDMs, particularly following LPS stimulation. Gene set enrichment analysis highlighted dysregulation of pathways involved in immune activation, as well as monocyte chemotaxis, suggesting a potential mechanism for the enhanced recruitment of inflammatory monocytes seen in the lungs of CLEC-2-deficient mice.

**Conclusion:** In the absence of CLEC-2, lung myeloid cell populations are altered, with a decrease in pro-homeostatic alveolar macrophages and an increase in pro-inflammatory monocytes. Furthermore, CLEC-2 deficient myeloid cells promote Th2 differentiation, resulting in spontaneous airway inflammation. The CLEC2/Pdpn axis is thus a novel pathway critical for maintaining lung homeostasis.

**Disclosure of Interest:** None Declared

---

**P124**

**INNATE SOURCES OF INTERFERON-GAMMA MEDIATE PROTECTIVE RESPONSES TO CRYPTOSPORIDIUM INFECTION**

J. Gullicksrud1, A. Sateriale1, J. Clark1, C. Hunter2, B. Streipen1

1Pathobiology, University of Pennsylvania, Philadelphia, United States
**Introduction:** Cryptosporidium sp are apicomplexan parasites that infect a broad range of animals, including humans. During human infection Cryptosporidium invades the villus epithelium of the small intestine, causing severe diarrheal illness that is self-limiting in immune-competent individuals. This parasite has been recognized as one of the most important causes of diarrheal disease in young children in the developing world and is an important contributor to child mortality.

**Methods:** While the importance of IFN-γ in control of Cryptosporidium infection has been demonstrated, the lack of a tractable murine model has hindered the study of the events that mediate resistance to these organisms in the gut. We have isolated a species of this parasite, Cryptosporidium tyzzeri, that is a natural mouse pathogen and results in a self-limiting infection in immune competent mice.

**Results:** Although T cells are required for clearance of infection, Rag2-deficient (RAG−/−) mice show a strong ability to mediate parasite control in the absence in T and B cells. In RAG−/− mice, early responses to C. tyzzeri are characterized by expansion of innate NK and ILC populations in the mucosa. Antibody blockade of IFN-γ results in enhanced parasite burden by day 3 after infection with C. tyzzeri. Furthermore, IFN-γ blockade impairs intestinal epithelial cell activation and leads to elevated production of IL-33 and IL-17.

**Conclusion:** Collectively, these studies highlight the importance of IFN-γ in the innate immune response against Cryptosporidium infection.

**Disclosure of Interest:** None Declared

**P125**

**HUMANIZED CEREBLON MICE REVEALED DIFFERENT THERAPEUTIC PATHWAYS OF IMMUNOMODULATORY DRUGS FOR MALIGNANCY AND INFLAMMATION**

Y. G. Hailu1,*, D. Millrine2, T. Kishimoto1

1Immune Regulation, Immunology Frontier Research Center, Osaka University, Suita, Japan, 2Infection and Immunity, Cardiff Institute of Infection and Immunity, Cardiff University, School of Medicine, Cardiff, United Kingdom

**Introduction:** Lenalidomide is an immunomodulatory drug (IMiD) having a therapeutic action in several autoimmune/inflammatory diseases. However, it is difficult to study IMiDs mechanism of action in murine disease models because murine cereblon, substrate receptor for IMiDs action, is resistant to some of IMiDs therapeutic effects.

**Methods:** We generated humanized cereblon mice thereby providing a novel animal model to unravel complex IMiDs mechanisms of action in mouse physiological setup. ELISA, western blot and quantitative real-time PCR was used to analyze different parameters.

**Results:** Our result showed that, unlike wild type mice which were resistant to Lenalidomide, T lymphocytes from humanized cereblon mice responded with higher degree of IKZF1/3 protein degradation. Furthermore, IMiDs degradation of IKZF1/3 resulted in an increase in interferin-2 among humanized cereblon mice but not in wild type group. Additionally, we assessed the potential therapeutic effects of IMiDs in dextran sulfate sodium (DSS) induced colitis. In both WT and humanized mice lenalidomide showed a significant therapeutic effect in DSS model of colitis, while the effect of pomalidomide was less pronounced

**Conclusion:** In conclusion, lenalidomide offer a therapeutic opportunity against inflammatory diseases independent of cereblon whereas its effect on IKZF1/3 and intereurin-2 is totally dependent of cereblon.

**Disclosure of Interest:** None Declared

**P126**

**STAT1 SIGNALING SHIELDS T CELLS FROM NK CELL MEDIATED CYTOTOXICITY TO PROMOTE INTESTINAL INFLAMMATION**

Y. H. Kang1,*, A. Biswas1, S. Snapper1

1Gastroenterology, Hepatology and Nutrition, Boston Children's Hospital, Boston, United States

**Introduction:** The JAK-STAT signaling pathway plays a crucial role in transducing various cytokine signals to achieve various transcriptional outcomes. In T cells, this is mostly studied in terms of T cell differentiation, with STAT1 being best known as an inducer of Th1 differentiation downstream of IFNγ. STAT1 is also known to represses Th17 and Treg differentiation. However, the precise mechanism by which STAT1 regulates T cell driven immunopathology in vivo however is unclear and assumed to be due to altered T cell differentiation. We thus sought to understand the role of STAT1 in T cell mediated intestinal inflammation, using Il10rb−/−Rag1−/− mice that we previously reported to develop colitis upon transfer of WT CD4+ T cells.

**Methods:** WT or Stat1−/− unfractionated CD4+ T cells were injected into Il10rb−/−Rag1−/− mice, which were subsequently monitored for signs of colitis such as weight loss and histologic inflammation of the colon.

**Results:** We found that Stat1−/− CD4+ T cells were unable to induce colitis in Il10rb−/−Rag1−/− mice. Surprisingly, this was due to an inability of Stat1−/− T cells to survive in vivo, as we saw a dramatic reduction in their reconstitution compared to WT T cells. The defective survival of Stat1−/− T cells was independent of colonic inflammation. It was also largely independent of type I and II interferon signaling, as Ifnar1−/− Ifngr1−/− T cells were able to survive and induce colitis in Il10rb−/−Rag1−/− mice. Mechanistically, gene expression analysis revealed reduced expression of Nlrc5 and the MHC class I antigen presentation pathway in Stat1−/− T cells, which resulted in reduced surface expression of multiple MHC class I molecules. MHC class
I molecules serve as classical inhibitory ligands for NK cells. Consequently, the depletion of NK cells was able to restore the survival of Stat1+ T cells, which were capable of inducing colitis. Finally, Stat1+ mice displayed normal levels of CD4+ T cells due to innate Stat1 deficiency, as innate deletion of Stat1 in Rag1+ mice also rescued the survival of Stat1+ T cells. **Conclusion:** Our findings thus reveal a novel role for Stat1 in T cell function, whereby Stat1 signaling shields T cells from NK mediated cytotoxicity to promote their survival and subsequent intestinal inflammation. Our study extends the functions of Stat1 signaling beyond T cell differentiation and might be relevant for other inflammatory disorders in which T cells play a prominent role. **Disclosure of Interest:** Y. H. Kang: None Declared, A. Biswas: None Declared, S. Snapper Grant / Research support from: Pfizer, Merck, Regeneron, Janssen, Consultant for: Roche, Amgen. Advisory Board for: Pfizer, Merck, Janssen, Celgene

**P127**
**THE IMPORTANCE OF IL-17A/IL-17RA AXIS IN HOST DEFENSE DURING CHRONIC RESPIRATORY INFECTIONS**

C. Cigana1, B. Sipione1,2, G. Rizzo1,2, M. Melessike1, J. A. Mertz3, J. K. Kolls4, A. Dragonzi3, N. I. Lorend3, 1Division of Immunology, Transplantation and Infectious Diseases, Ospedale San Raffaele, 2Vita-Salute San Raffaele University, Milano, Italy, 3 Constellation Pharmaceuticals, Cambridge, 4Center for Translational Research in Infection and Inflammation, Tulane School of Medicine, New Orleans, United States

**Introduction:** The interleukin (IL)-17A/IL-17 receptor A (IL17RA) axis is emerging as a key player in host defense and immunopathology associated to chronic respiratory infections, including those by Pseudomonas aeruginosa. We recently showed that the IL-17 pathway, through IL-17RA, plays a key role in host defense and in immunopathology using a murine model of chronic respiratory infection by P. aeruginosa. Based on these results, we are interested in dissecting not only the potential role of targeting IL-17A producing cells but also the down-streaming pleiotropic activities of IL-17RA by the mutually exclusive interaction with other well known IL-17 receptors (IL-17RC, IL-17RB, IL-17RE).

**Methods:** We selectively targeted the key transcription factor for IL-17A producing cells, RORyt, with a selective small molecule inhibitor of the bromodomain and extraterminal domain (BET) family of proteins, named CPI203. The anti-inflammatory activity of CPI203 was evaluated at early (2 days) and advanced (14 days) stage of chronic P. aeruginosa infection in C57Bl/6N mice. IL-17 cytokines levels (IL-17A, IL-17F, IL-17E, IL-17B or IL-17C) were evaluated by ELISA during long term chronic infection (28 days).

**Results:** Daily treatment with CPI203 in mice decreased incidence of chronic colonization, confirming the role of IL-17A producing cells in mediating host resistance to P. aeruginosa. In addition, CPI203 treatment reduced levels of pro-inflammatory cytokines/chemokines and infiltrating leukocytes (CD45+), including neutrophils (CD11+, GR-1hi), thus ameliorating host immunopathology, without increasing the bacterial burden. We also found out significant reduction of CD45+ RORyt+ cells during the development of chronic infection. A detailed characterization of the RORyt+ T cells subsets modulated by CPI203 treatment by FACS analysis is ongoing.

In addition, we found out that overall IL-17 cytokines (IL-17A, IL-17F, IL-17E, IL-17B or IL-17C) levels were increased during the development of chronic respiratory infection by P. aeruginosa, thereby indicating a potential activation of diverse pathways through the heterodimerization of IL-17RA with different IL-17 receptors. The contribution of each IL-17 receptor during chronic respiratory infections is under evaluation.

**Conclusion:** Overall, our results demonstrate that the IL-17A/IL-17RA axis can define the outcome of respiratory diseases and support future evaluation of mutually exclusive IL-17 receptors function. In addition, targeting selectively IL-17A/IL-17RA axis may provide a novel potential host-based intervention to limit immunopathology during chronic respiratory illnesses, mediated by opportunistic infections.

Supported by the “Cystic Fibrosis Foundation” and by “Fondazione Cariplo”

**Disclosure of Interest:** None Declared

**P128**
**COMMENSAL GUT FUNGI REGULATE SUSCEPTIBILITY TO COLITIS AND COLORECTAL CANCER.**

A. Malik1, D. Sharma2, C. Guy2, T.-C. Chang2, S. Olsen2, G. Neale2, P. Vogel3, T.-D. Kanneganti2

1St Jude Children’s Research Hospital, 2SJCRH, Memphis, United States

**Introduction:** The cross-talk between innate immune sensors and commensal microbes in the gut is crucial for maintaining intestinal barrier homeostasis. Fungi represent a major component of the resident gut flora. Mutations in the genes that encode molecules involved in the fungal sensing pathways are associated the risk of developing colitis and colorectal cancer. However, the precise roles of gut fungi and associated immune recognition cascade in colitis and colorectal tumorigenesis are not well established.

**Methods:** Mice depleted of commensal fungi or bacteria, or mice deficient in molecules of the fungal-sensing pathway were subject to experimental colitis and colon cancer. The immune response was assessed at early and acute time points during colitis. Role of critical factors was established by supplementation and depletion strategies.

**Results:** We show that gut fungi modulate inflammasome activation during experimental colitis. IL-18 maturation by the inflammasome promotes tissue-restitutive and anti-tumorigenic immune responses. Loss of critical molecules
involved in the fungal recognition pathway within the myeloid cells also leads to reduced inflammasome activation and increased susceptibility to colitis and colorectal cancer

**Conclusion:** Commensal fungi, and the associated sensing pathway is a critical modulator of colitis and colon cancer

**Disclosure of Interest:** None Declared

**P129**

**THE SHORT-CHAIN FATTY ACID BUTYRATE PREVENTS ANTIBiotic-INDuced INTESTINAL MACROPHAGE DYSFUNCTION BY MetABOLIC rePROGRAMMING**

N. Scott1, P. Andersen2, C. Alcon-Giner3, C. Leclaire3, S. Cairn3, D. Peterson4, A. Bancroft4, X. Li4, A. Mowat5, L. Hall5, M. Travis1, S. Milling5, E. Mann1,6,7

1University of Manchester, Manchester, United Kingdom
2Johns Hopkins Medicine, Baltimore, United States
3Quadram Institute Bioscience, Norwich, United Kingdom
4Eli Lilly, Indianapolis, United States
5University of Glasgow, Glasgow, United Kingdom

**Introduction:** Macrophages in the intestine are highly specialized and usually respond to the gut microbiota without provoking an inflammatory response. The mechanisms by which intestinal macrophages normally become conditioned to promote microbial tolerance are unclear, however strong evidence indicates an important role for short-chain fatty acids (SCFAs; generated by the gut microbiota from dietary fibre) in regulating intestinal immunity.

**Methods:** Mice were administered antibiotics to disrupt the intestinal microbiota +/- SCFA, and immune responses to microbial stimulation and infections were assessed.

**Results:** Antibiotic use in mice depleted SCFA levels in the intestine and primed intestinal macrophages to become hyper-responsive to bacterial stimulation, producing excess amounts of TNFa, IL-6 and MCP-1 in response to LPS. Thus, re-exposure of antibiotic-treated mice to a conventional microbiota caused inflammatory changes in intestinal macrophages including excess production of iNOS, driving a macrophage-dependent sustained increase in activated IFNy-producing CD4+ T-cells and rendering mice more susceptible to Th2 and Th17 mediated infections. The SCFA butyrate restored the hyposerovensensitiveness to bacterial stimulation and abolished the dysregulated Th1 response in colonized mice. Butyrate also induced a unique gene signature in intestinal macrophages in vivo including genes involved in oxidative phosphorylation, lipid metabolism and alternative activation. Butyrate acted directly on macrophages in vitro to enhance oxidative phosphorylation and lipid metabolism, and skewed macrophages towards an anti-inflammatory, alternatively-activated phenotype.

**Conclusion:** SCFAs maintain the regulatory properties of intestinal macrophages, at least in part due to metabolic reprogramming of intestinal macrophages. Antibiotics disrupt this process, leading to long-term T-cell dysfunction. These data provide a novel function for butyrate in shaping intestinal macrophage function and have important implications for repeated antibiotic use.

**Disclosure of Interest:** None Declared

**P130**

**NON-MIGRATORY LUNG DCS SUBSETS MEDIATE CYCLIC DI-GMP ADJUVANT ACTIVITY ON THE MUCOSAL SURFACE**

S. Mansouri1,*, D. S. Katikaneni1, S. schattigen2, K. Fitzgerald,1 L. Jin1

1Department of Medicine, University of Florida, Gainesville, 2Department of Medicine, University of Massachusetts Medical School, worcester, United States

**Introduction:** Dendritic cells (DCs) mediate vaccine adjuvant responses in vivo. DCs consist of functionally distinct subsets. 3',5’ cyclic di-GMP (CDG) is a promising mucosal vaccine adjuvant. We previously showed that 1) CDG enhances antigen uptake and directly activates lung DCs in vivo; 2) TNF production is required for CDG mucosal adjuvant activity in vivo. Here we asked 1) how functional distinct lung DCs subsets mediate CDG mucosal adjuvant activity in vivo? 2) how TNF mediates CDG mucosal adjuvant activity in vivo.

**Methods:** 1) Genetically modified mice; 2) Adoptive cell transfer.

**Results:** 1) IRF4+/CD11ccre mice completely lose CDG adjuvant activity. Adoptive transfer of WT lung CD11b+ conventional DC (cDC2) restored CDG adjuvant activity in the IRF4+/CD11ccre mice. 2) steady-state lung cDC2 consists of two subsets: TNFR2+PrelB+PD-L1+BTLA-Arg1-CX3CR1-SIRPα-cDC2 (R2D2) and TNFR2-PrelB-PD-L1+BTLA-Arg1-CX3CR1-SIRPα+cDC2 (C3D2). CDG activates both cDC2 subsets in vivo but only R2D2 is mature and migrate to lymph node. 3) Adoptive transfer of C3D2, not R2D2, restored CDG adjuvant activity in the STING+ mice. 4) monocyte-derived DCs (moDCs) do not take up CDG and do not migrate to lymph node, but nevertheless, increases MHC II/CD86 expression in response to intranasally administered CDG. 5) Adoptive transfer of WT monocytes restored CDG adjuvant activity in the TNFR2+ mice. 6) C3D2 activates moDCs via transmembrane TNF (mTNF) – TNFR2 interaction. 7) TBK1 is required for CDG-induced TNF production in vivo.

**Conclusion:** The non-migratory C3D2 activates moDCs via the mTNF-TNFR2 interaction to promote the mucosal adjuvant activity of CDG in vivo.

**Disclosure of Interest:** None Declared

**P131**

**TUFT CELL DEVELOPMENT AND FUNCTION IN THE BILIARY SYSTEM**

C. O’ leavey1,*, C. Schneider1, R. Locksley1,2

1University of California - San Francisco, San Francisco, 2Howard Hughes Medical Institute, Chevy Chase, United States

**Disclosure of Interest:** None Declared
Introduction: Tuft cells—secretory epithelial cells found in epithelium of various “hollow” organs—are major determinants of epithelial responses to pathogens and are critical for initiating type two immune responses. In the small intestine, tuft cells, through production of IL-25, play anti-helminth and homeostatic roles. The role of tuft cells, and the relevance IL-25-dependent tuft cell-immune cell circuits, in other tissues has not been examined. We seek to define the function of biliary tuft cells in the context of liver and biliary physiology and pathology.

Methods: Assess abundance and location of biliary tuft cells using the tuft cell marker DCLK1 and an IL-25 reporter mouse. Determine gene expression of tuft and non-tuft biliary epithelium by RNA sequencing. Determine abundance of tuft cells in response to perturbed bile acid (BA) signaling and abundance. Assess pathology in tuft cell sufficient and deficient mice in models of hepatobiliary disease.

Results: We have found that IL-25-positive tuft cells are highly abundant in the murine gallbladder and also present in the extrahepatic bile ducts (EHBDs). Biliary tuft cells are highly abundant post-birth and maintained at high levels throughout life. Biliary tuft cells express the core tuft cell gene program, as well as a tissue-specific gene signatures, and are highly sensitive to perturbations of BA production.

Conclusion: The known roles of IL-25-producing tuft cells in the small intestine, together with our data indicating the abundance and BA responsiveness of biliary tuft cells, suggest that biliary tuft cells could be previously unappreciated contributors to BA regulation, part of the adaptive response to perturbed BA abundance, and/or serve to integrate the biliary epithelium with the immune system.

Disclosure of Interest: None Declared

P132
THE PROSTAGLANDIN D2 RECEPTOR CRTH2 SUPPRESSES INTESTINAL EPITHELIAL RESPONSES DURING HELMINTH INFECTION
O. O. Oyesola1,2, L. Webb1, D. Pham1, S. Solouki1, P. Campioli1, S. Frueh1, S. Peng1, R. Cubitt1, E. Tait Wojno1
1Baker Institute for Animal Health and Department of Microbiology and Immunology, Cornell University, Ithaca, United States

Introduction: Intestinal helminth infections induce Type 2 inflammatory responses, characterized by immune cell activation, Type 2 cytokine production and increased epithelial cell responses that drive worm expulsion. Previous studies have shown that levels of bioactive lipid mediators, including prostaglandin D2 (PGD2), are increased during Type 2 inflammation and that the PGD2 receptor CRTH2 is associated with Type 2 inflammation in allergic lungs1,2. However, how the PGD2-CRTH2 pathway influences immune and epithelial cell responses in the intestine during helminth infection has not been fully elucidated.

Methods: We employed infection with the intestinal helminth parasite *Nippostrongylus brasiliensis* in wild type (WT) and CRTH2 deficient (CRTH2KO) mice and bone marrow chimeric animals that isolated CRTH2 deficiency to the hematopoietic or non-hematopoietic compartment to investigate the role of CRTH2 during intestinal type 2 inflammation. We quantified worm burdens and type 2 effector responses such as goblet cell hyperplasia in these mice. Using a combination of flow cytometry and single cell RNA transcript staining, we investigated whether intestinal epithelial cells expressed CRTH2. Finally, using an *in vitro* intestinal organoid system, we examined the effect of PGD2 on type 2 cytokine-induced mucin responses.

Results: Based on previous data, we hypothesized that CRTH2KO mice would have deficient Type 2 inflammatory responses and worm expulsion following *N. brasiliensis* infection. Surprisingly, CRTH2KO mice had enhanced worm expulsion and increased goblet cell hyperplasia compared to WT mice. Infection of bone marrow chimeric mice revealed that CRTH2 expression in non-hematopoietic cells mediated this phenotype. Consistent with a role for CRTH2 in controlling non-hematopoietic responses, intestinal epithelial cells (IECs) expressed the gene that encodes for CRTH2, Gpr44, and IEC Gpr44 expression was increased during infection. Finally, PGD2 exposure suppressed Type 2 cytokine-induced goblet cell responses in intestinal organoid cultures.

Conclusion: Our data suggest that the PGD2-CRTH2 pathway suppresses IEC mucin responses during intestinal helminth infection. This study reveals a previously unappreciated regulatory pathway that restrains Type 2 cytokine-induced small intestinal epithelial mucin responses. This finding has implications for development of therapies aimed at modulating chronic type 2 inflammation and provides a significant step forward in our understanding of the role of eicosanoid lipid mediators in regulating intestinal Type 2 inflammation.

Disclosure of Interest: None Declared

P133
MICROBIOTA DEPENDENT EFFECTS OF INTERLEUKIN-22 IN COLITIS
P. Pelczar1, M. Sabihi1, M. Böttcher1, N. Gagliani1, S. Huber2
1I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Altered gut microbiota is implicated in the development of inflammatory bowel disease (IBD)1. Some intestinal bacteria have been reported to potentiate intestinal inflammation by altering the intestinal microenvironment, and the immune response. Interleukin-22 (IL-22) has been reported before to play a protective role in IBD2. Furthermore, IL-22 expression is known to be influenced by microbiota and can also affect microbiota composition3. We thus hypothesized that the function of IL-22 in IBD might depend on the microbiota composition.
Methods: To address this question, we used Il22-deficient mice and performed fecal microbiota transfer. We induced colitis by T cell transfer or challenged with DSS and analyzed the disease severity by endoscopy, histology and cytokine profile of the mice at different stages of the disease.

Results: Interestingly, Mb2 flora transfer to wildtype mice induced mild but significant spontaneous colitis when compared to Mb1 recipient mice. Moreover, wildtype mice exhibited greater inflammation and disease severity when challenged with DSS-induced colitis after the transfer of Mb2 microbiota than after the transfer of Mb1 microbiota, proving that the Mb2 composition is more colitogenic. More importantly, we found that the role of IL-22 in colitis depended on the microbiota composition. Accordingly, Il22-deficient mice were more susceptible compared to wildtype mice in the DSS- and Transfer-colitis model upon transplantation of Mb2 microbiota. In contrast IL-22 did not play an essential role in mice upon transplantation of Mb1 microbiota. Indeed, in this setting wildtype and Il22-deficient mice demonstrated the same colitis severity. However, the underlying mechanism(s) are unclear and warrant further investigation.

Conclusion: In conclusion, our data highlight the importance of the intestinal microbiota composition during colitis development. Furthermore, our results indicate that IL-22 has a flora-dependent effect in colitis. Understanding the underlying mechanisms might build the basis for a personalized IBD therapy based on the microbiota.

Disclosure of Interest: None Declared

P134
IFN-Λ PROMOTES Rhesus Rotavirus-Induced Diarrhea in Mice

J. Peng1*, J.-D. Lin2, C. Sy3, C. McElrath4, S. Smirnov5, H.-C. Tseng6, Y.-J. Chang7, R. R. Sridhar1, R. Durbin6, J. Durbin6, S. Kotenko1

1Microbiology, Biochemistry and Molecular Genetics, School of Graduate Studies, Health Campus of Newark, Newark, 2New York University School of Medicine, New York, 3Medicine, School of Graduate Studies, Health Campus of Newark., Newark, 4Microbiology, Biochemistry and Molecular Genetics, New Jersey Medical School, 9School of Graduate Studies, Health Campus of Newark, Newr, 5Microbiology, Biochemistry and Molecular Genetics, School of Graduate Studies, Health Campus of Newark, Newark, 6Pathology and Laboratory Medicine, 7Genomics Research Program, School of Graduate Studies, Health Campus of Newark, Newark, United States

Introduction: Rotavirus (RV) infection is the leading cause of severe diarrhea in children under five years old, and results in approximately half a million deaths each year. Previous studies have shown that RV-induced diarrhea is associated with both host and viral factors, including the non-structural protein 4 (NSP4), which has been shown to act as a viral enterotoxin. Type I and type III interferons (IFNs) are produced in the intestine during RV infection and their combined action is required for the efficient control of RV replication and extra-intestinal spread. However, effects of IFNs on RV-induced diarrhea have not been investigated.

Methods: Eight day old wild type (WT), Ifnlr1−/−, Ifnar1−/−, Ifnar1−/−Ifnlr1−/−, or villin-Cre-Ifnlr1−/− mice were infected with either the minimum diarrhea dose of rhesus RV (RRV), a dose that consistently results in diarrhea, or with a sub-diarrhea dose of RRV. Diarrhea score, wet-to-dry ratio of stool content, and viral titers were measured at various time points post infection.

Results: Our data showed increased levels of the viral NSP4 protein in intestines of Ifnlr1−/− and villin-Cre-Ifnlr1−/− mice, consistent with our previous work showing that type III IFNs restrict RRV replication in the intestinal epithelial cells. Unexpectedly, these mice did not suffer from diarrhea when given the minimum diarrhea dose of RRV that triggers diarrhea in WT mice. A higher viral dose was required to cause diarrhea in total or intestinal epithelial cell (IEC) IFN-Λ receptor-deficient suckling mice. Moreover, although exogenous IFN-Λ inhibited RRV replication, the IFN-Λ treatment triggered diarrhea in WT suckling mice infected with a sub-diarrhea dose of RRV. Therefore, increased viral replication in the intestine did not cause diarrhea in mice lacking IFN-Λ signaling in IECs and there was no correlation between RRV-induced diarrhea and intestinal inflammation that accompanied RRV infection. We used an unbiased RNAseq approach to identify pathways affected by IFN-Λ during the development of diarrhea, and may therefore trigger diarrheal disease. Analyses revealed multiple primary and secondary effectors induced in response to IFN-Λ during RRV infection in vivo, including various ion channels and aquaporins.

Conclusion: We conclude that, although type III IFN signaling within the epithelial compartment inhibits RRV replication in suckling mice, it also exacerbates RRV-induced diarrhea. Our results indicate that IFN-Λ-mediated promotion of RRV diarrhea is influenced by multiple, interdependent signaling pathways in the intestine. It is therefore possible that inhibition of IFN-Λ signaling should be explored as a treatment option for suppressing life-threatening diarrheal illness.

Disclosure of Interest: None Declared

P134.A
Carbapenem Resistant Klebsiella Pneumoniae Exploit IFN-Lambda Signaling to invade across the Airway Epithelium

M. A. Wickersham1*, D. Ahn1, A. Prince1

1Pediatrics, Columbia University, New York, United States

Introduction: Carbapenemase-producing K. pneumoniae (KPCs) are an increasingly common cause of hospital associated pneumonia, bacteremia and death. In addition to their formidable antimicrobial resistance, we postulated
that these invasive, but non-motile organisms must initiate changes in the epithelial barrier, facilitating translocation to the vascular compartment.

**Methods:** We used a C57Bl6 murine model of KPC ST258 pneumonia and polarized cultures of human airway epithelial cells to test the hypothesis that ST258 alter epithelial tight junctions through utilizing IFN-γ signaling. Proteomic analyses of bronchoalveolar lavage fluid and qRT-PCR arrays, western blots and confocal microscopy of lung tissue were used to identify signaling pathways and airway proteins significantly altered in response to ST258 pneumonia.

**Results:** A proteomic analysis of airway fluid from mice infected with KPC ST258 indicated major changes in interferon signaling and epithelial barrier integrity. Exposure of human airway epithelial cells to IFN-γ decreased transcription of both occludin and ezrin and compromised epithelial barrier function as measured by dextran permeability. In response to IFN-γ epithelial barrier function was compromised, enabling transmigration of neutrophils while also enhancing the transmigration of KPC by 10-fold. The biological consequences of IFN-γ activity were observed in Ifnγ1−/− mice, which had significantly less bacteremia after 48 and 96 hours in a murine model of KPC pneumonia. Overall, our results show KPC ST258 exploit IFN-γ signaling to disrupt barrier integrity to migrate across the airway epithelium.

**Conclusion:** The success of KPC ST258 as an ICU pathogen is likely due to multiple factors in addition to antimicrobial resistance. Our data suggest that KPC ST258 advantageously manipulate IFN-γ dependent signaling mechanisms to disrupt junctional integrity and invade across airway epithelial barriers.

**Disclosure of Interest:** None Declared

**P134.B**

**CHOLECYSTOKININ (CCK) INCREASES SIGA AND IgA+ B CELLS IN SMALL INTESTINE OF MICE**

J. MORALES MAGAÑA1, I. M. ARCINIEGA MARTINEZ1, A. A. RESENDIZ ALBOR1, R. A. JARILLO LUNA1, R. CAMPOS RODRIGUEZ1, J. D. C. PACHECO YEPEZ1

1SECCION DE ESTUDIOS DE POSGRADO E INVESTIGACION, INSTITUTO POLITECNICO NACIONAL, MEXICO, Mexico

**Introduction:** Cholecystokinin (CCK) is a linear polypeptide hormone that exists in multiple molecular forms, the octapeptide, CCK-8 is the classic cerebral and intestinal form. The main stimuli for its secretion are the products of the digestion of fats and proteins. In the intestinal mucosa several mechanisms have been developed to control and neutralize pathogens and antigens of the diet, the synthesis of IgA is a key strategy to generate immunoprotection in the absence of inflammation, so that this antibody is critical for the survival of the species. There is little information about "CCK-IgA and SlgA" relationship. The purpose of this study was to determinate the effect of CCK on IgA production.

**Methods:** For this, a single dose was administered of 8 μg/kg of the agonist (CCK-8), and saline as vehicle to male BALB/c mice, which were sacrificed 30 minutes later to obtain fecal fluids for quantifying total IgA and SlgA concentration by ELISA technique. The percentage of IgA+, IgM+ B cell, IgA+ plasma cells (PC) and T cells producers cytokines (IL-2, 4, 5, 6, 10, TGF-β) related to IgA production were determined in lamina propria by FCM technique.

**Results:** An increase in the total IgA and SlgA concentration is shown in CCK group. Total IgA increase was dependent on the CCK’s concentration. On the other hand, there was an increase of IgA+ (40.0%), IgM+ (21.0%) B cells versus control group (27.5% and 12.0%, respectively) and increase in IgA+ PC percentage (27.0%) versus control group (21.0%). Similarly, in T cells producers cytokines percentage, CCK-8 showed an increase in IL-2 (5.4%), IL-4 (5.8%), IL-6 (3.2%), IL-10 (4.8 %) versus control group (3.1%, 5.6%, 2.1%, 3.6%, respectively).

**Conclusion:** Results suggest CCK regulates the intestinal immune response by increasing cytokine related to IgA synthesis produced by T cells, could be through the participation of the CCK1R and CCK2R receptors.

**Disclosure of Interest:** None Declared

**P135**

**NKG2D MODULATES THE PROINFLAMMATORY FEATURES OF TH1 AND TH17 CELLS AND CONTRIBUTES TO THEIR PATHOGENICITY IN VIVO**

M. Babic Cacic1, F. Heinrich2, Q. Hammer1, B. Polic3, C. Romagnani1

1Innate immunity, 2Therapeutic Gene Regulation, Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin - a Leibniz Institute, Berlin, Germany, 3Department of Histology and Embryology, Faculty of Medicine, Rijeka, Croatia

**Introduction:** The effector functions of T helper (Th) cells can be shaped not only by receiving the T cell receptor and costimulatory signal but also through signals transmitted via cytokine receptors or a myriad of activating receptors. NKG2D is a molecular sensor of stressed cells expressed on different subsets of innate and adaptive lymphocytes. Despite its established role as potent stimulator of the immune system, particularly as an activating receptor on NK cells and costimulatory molecule on CD8+ T cells, NKG2D-driven regulation of CD4+ Th cell-mediated immunity remains unclear.

**Methods:** We addressed the role of NKG2D for CD4+ Th-driven responses by performing (i) ex vivo analysis of NKG2D+CD4+ T cell population, (ii) in vitro analysis of NKG2D expression during Th cell polarization as well as the impact of NKG2D on Th cell responses, (iii) global gene expression analysis in in vitro differentiated Th subsets, (iv) in vivo analysis during antigen-induced arthritis using mice with NKG2D-deficiency in T cell compartment as well as Il17a-fate mapping mice.
Results: We identified a small population of T-bet expressing NK2G2+CD4+ T cells in spleen and bone marrow of C57BL/6 mice, whereas a significant portion of NK2G2+CD4+ T cells derived from the small intestine lamina propria expressed RORγt. In line with this, the de novo expression of NK2G2 could be induced on naïve CD4+ T cells both under Th1 and Th17 polarizing conditions. While NK2G2 was not impacting the expression of IFNγ in fully polarized Th1 cells, the in vitro expression of NK2G2 was associated with GM-CSF+IFNγ+ Th17 cells. Global gene expression analysis further confirmed enforced expression of type 1 signature genes in Th17 cells by NK2G2 and we could show a direct effect of NK2G2 triggering in enhancing the production of IFNγ in Th17 cells. By fate mapping of Il17a-expressing cells in vivo in a mouse model of antigen-induced arthritis, we could show that under inflammatory conditions, NK2G2 highly enriched the population of T-bet-expressing Th17 cells. Indeed, NK2G2 was associated with modulated expression of GM-CSF and IFNγ in Th1 and T-bet+Th17 cells, which was in line with our in vitro data. Most importantly, T cell specific deletion of NK2G2 impaired the ability of antigen-specific CD4+ T cells to promote inflammation in vivo during antigen-induced arthritis, resulting in significantly reduced knee swelling, tissue immunopathology and disease score.

Conclusion: Altogether, our results indicate that the triggering of NK2G2 by stress-ligands induced during inflammation modulates the effector functions of both Th1 and Th17 cells in vitro and in vivo. Our data in addition imply that NK2G2 might serve as an important target for the amelioration of chronic inflammatory diseases mediated by a mixed Th1 and Th17 response.

Disclosure of Interest: None Declared

P136
THE TRANSCRIPTIONAL REGULATION OF INTERLEUKIN-10 VERSUS INTERFERON GAMMA BY C-MAF AND BLIMP-1 IN INTERLEUKIN-27-DRIVEN CD4+ T-HELPER CELLS
L. S. Cox1, L. Gabryšová1, A. O’Garra1,2
1Laboratory of Immunoregulation and Infection, The Francis Crick Institute, 2National Heart and Lung Institute, Imperial College London, London, United Kingdom

Introduction: Type 1 T-helper (Th1) cells are crucial to protect against infection by intracellular pathogens via the expression of the pro-inflammatory cytokine interferon gamma (IFN-γ, encoded by Ifng). However, if left uncontrolled, Th1-mediated immune responses can cause immunopathology. To mitigate such events, Th1-cells co-express, alongside IFN-γ, the anti-inflammatory cytokine interleukin (IL)-10, in certain contexts, which acts as a brake to limit inflammation and prevent damage to the host by feedback inhibition of dendritic cells and macrophages. Previous RNA-seq analysis in the lab (L. Gabryšová, A. O’Garra) comparing in vitro differentiated Th1-cells which express IL-10 to those which do not, identified the transcription factors: proto-oncogene Maf (c-Maf, encoded by Maf) and B-Lymphocyte-Induced Maturation Protein 1 (Blimp-1, encoded by Prdm1) to highly correlate with Ifng expression. We therefore became interested in how c-Maf and Blimp-1 regulate IL-10 versus pro-inflammatory cytokine expression.

Methods: To investigate, naïve CD4+ T-cells with CD4-Cre mediated deletion of either Maf or Prdm1 were differentiated in vitro to Th1-cells under conventional conditions with IL-12 alone, but also in the presence of IL-27, a cytokine reported to induce IL-10 in multiple Th1-cell subsets. We then analysed the mRNA and protein expression of IL-10, pro-inflammatory cytokines and transcription factors under these conditions.

Results: Th1-cells driven with IL-12+IL-27 showed a dramatic increase in Ifng expression which peaked on Day 3, compared to Th1-cells driven with IL-12 alone. A similar, albeit smaller increase in Il10 was also seen on Day 3 in cells driven with IL-27 alone (Th1-cells). Notably, this pattern of increased Ifng expression by cells driven in the presence of IL-27 coincided with a similar increase in both Maf and Prdm1 expression. In contrast, Th1-cells driven with IL-12 alone expressed very little Il10 even by Day 7 of culture and displayed low expression of both Maf and Prdm1. All cells, regardless of driving cytokines used, expressed similar levels of T-box transcription factor 21 (Tbx21), the hallmark transcription factor of Th1-cells, but expressed differing levels of Ifng. Th1-cells driven with IL-12 alone or in conjunction with IL-27 expressed high levels of Ifng, whereas in Th1-cells Ifng expression was low. CD4-Cre mediated deletion of either Maf or Prdm1 revealed that both transcription factors are positive regulators of Il10 gene expression with absence of either transcription factor resulting in a decrease in Il10 gene expression in cells differentiated in the presence of IL-27. The converse was true for Ifng with both transcription factors negatively regulating ifng gene expression, most evidently in Th1-cells driven with IL-12+IL-27. Interestingly, Maf was found to be a positive regulator of Prdm1 only in Th1-cells. However, Prdm1 was a positive regulator of Maf in both Th1-cells and Th1-cells driven with IL-12+IL-27.

Conclusion: Together these results indicate roles for both c-Maf and Blimp-1 in the regulation of Il10 versus Ifng in Th1-cells driven in the presence of IL-27. Given that these cells may more closely reflect Th1-cells found in vivo, future work will use global analysis to unravel the molecular mechanisms by which c-Maf and Blimp-1 regulate cytokine gene expression these cells.

Disclosure of Interest: None Declared

P137
TGF-B1 SIGNALING PROMOTES IL-22 PRODUCTION BY TH17 CELLS
L. Garcia Perez1, P. Pelzcar1, J. Kempski1, N. Gagliani1, S. Huber1
1I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
**Introduction:** IL-22 is a key cytokine for the defense of mucosal barriers. The IL-22 receptor (IL-22r1) is expressed mainly in cells with epithelial origin in which IL-22 signaling promotes the production of anti-microbial peptides, proliferative and anti-apoptotic pathways, which together are crucial for tissue preservation. IL-22 can be produce by many cells in the immune system but Th17 and Th22 cells have been described to produce large amounts of this cytokine. The molecular regulation of IL-22 in T cells has been studied before. IL-23, IL-6, activation of STAT3, expression of RorT and Ahr activation are important factors promoting IL-22 production by T cells. TGFβ1, in contrary, inhibits Th22 cells through induction of the transcription factor cmaf. However, the effect of TGFβ1 signaling in Th17 on IL-22 production is unclear.

**Methods:** To investigate the factors that modulate IL-22 production in CD4+ T cells, in vitro differentiation of naive T cells from Foxp3eGFP/IL-17AeGFP/IL-22RFP reporter mice was performed followed by Flow Cytometry analysis.

To study the function of TGF-β signaling on IL-22 production by CD4+ T cells in vivo we used mice with impaired TGF-β signaling (dnTGF-βR2). Colitis-associated colorectal cancer was induced by the injection of the mutagen agent AOM followed by three cycles of DSS. Upon development of tumors, the production of IL-17 and IL-22 by CD4+ T cells from colon and tumor tissue were analyzed using flow cytometry.

**Results:** Based on in vitro experiments we demonstrated that, in contrast to what would have been expected, TGF-β1 promotes the production of IL-22 by Th17 cells. Mechanistically we found that TGF-β1 promotes Ahr expression, which in the presence of an Ahr ligand promoted IL-22 production by Th17 cells. Accordingly, we observed that mice with impaired TGF-β signaling in T cells have a reduced frequency of IL-17+IL-22+ T cells during colitis-associated colorectal cancer (CRC) in vivo. Of note, in further in vivo experiments, we could then demonstrate that TGF-β signaling in T cells via induction of IL-17+IL-22+ T cells promotes CRC.

**Conclusion:** We found that TGF-β1 signaling plays an essential role in the induction of IL-22 in Th17 cells, and thereby can promote CRC.

**Future outlook:** We are currently studying the molecular mechanism(s) by which TGF-β1 promotes IL-22 production. We specifically aim to identify the involved TGF-β1 signaling pathways and how these are linked to Ahr expression.

**Disclosure of Interest:** None Declared
P140
SINGLE-CELL RNA-SEQ REVEALS A DISTINCT TRANSCRIPTOMIC SIGNATURE OF CD32+ CD4+ T CELLS FROM HIV+ INDIVIDUALS ON SUPPRESSIVE THERAPY
M. Abdel-Mohsen1, A. Kossenkov1, C. Tomescu1, S. K. Vadrevu1, L. B. Giron1, K. M. Lynn1, K. Mounzer2, P. Tebas3, L. J. Montaner1

1The Wistar Institute, 2Philadelphia FIGHT, 3University of Pennsylvania, Philadelphia, United States

Introduction: CD32 was suggested as a marker of the replication-competent HIV reservoir. However, we recently reported that CD32 is expressed on cells with transcriptionally active HIV but does not enrich for HIV DNA in resting T cells [PMID 29669853; Sci Transl Med. 2018]. CD32 can represent a tool to better understand persistent HIV transcription during suppressive antiretroviral therapy (ART). We used single-cell RNA-sequencing to characterize the transcriptomic signature of CD32+ CD4+ T cells from HIV+ individuals on ART.

Methods: CD32+ CD4+ and CD32− CD4+ T cells were sorted from HIV+ ART-suppressed individuals using Fluorescence-activated cell sorting (FACS). The nanowell-based single-cell RNA-sequencing system, ICELL8, was used to characterize the single-cell transcriptomes of 151 CD32+ CD4+ T cells and 186 CD32− CD4+ T cells. Only cells with >100,000 reads were considered for subsequent analysis (131 CD32+ CD4+ T cells and 66 CD32− CD4+ T cells). False discovery rates (FDR) were calculated to account for multiple comparisons in statistical analyses. Ingenuity pathway analysis (IPA) was used to evaluate the functional significance of differentially expressed genes.

Results: All sorted cells expressed CD3 transcripts, suggesting a T cell lineage, as expected. 84 genes were differentially expressed between CD32+ CD4+ and CD32− CD4+ T cells (FDR < 0.05 and fold change > 2 fold), inclusive of genes associated with immune complex formation (especially between T and B cells), T cell activation via TCR signaling, and differential interferon response to viral infection. Pathway analysis showed that CD32+ CD4+ T cells are enriched with genes related to T cell activation via T cell receptor contact with antigen bound to MHC molecule on antigen-presenting cell. Only 17.6% of CD32+ CD4+ T cells expressed CD3b transcripts compared to 3% of CD32− CD4+ T cells.

Conclusion: Our data suggest that activated CD4+ T cells may express CD32; however, the majority of CD32 protein on CD4+ T cells, is probably originated by immunological complex between T cells and antigen-presenting cells (likely B cells), that drive T cell activation and latent HIV transcription via TCR signaling. Our data warrant further investigations on the role immune complex formation and activated cells in latent HIV transcription/reactivation and the survival of HIV RNA expressing cells during ART-suppressed HIV infection.

Disclosure of Interest: None Declared
reactivation of T lymphocytes in response to activating signals as part of the adaptive immune response.

**Methods:** We have studied the transcriptome of primary CD4+ T cells before and after transitioning into quiescence and following reactivation. We screened lncRNAs induced during T cell quiescence for proximity and correlated expression to protein-coding genes and binding to chromatin-associated proteins in CLIP-seq experiments.

**Results:** We observed that while most protein-coding genes were transcriptionally downregulated during transition to quiescence, the majority of expressed lncRNA genes were upregulated, a counterintuitive expression pattern which could have functional implications. As T cell quiescence is both transcriptionally and epigenetically regulated, we hypothesized that some of the lncRNAs that show increased expression during quiescence may play a role in establishment and/or maintenance of the quiescent state. This could be achieved by lncRNA-mediated recruitment of transcription factors or chromatin modifying proteins to key loci involved in regulation of the T cell activated state.

**Conclusion:** We have identified several lncRNA candidates which showed strongly correlated expression with their neighboring protein-coding genes which are known to play an important role in T cell function, including both transcription factors and members of chromatin modifying complexes. Interestingly, all our candidate lncRNAs, which will be subjected to in-depth characterization, showed binding to at least one known chromatin activator or repressor protein.

**Disclosure of Interest:** None Declared

---

**P141.A**

**ADMINISTRATION OF IL-12 AND IL-2 CORRECTS THE AGE-RELATED IMPAIRMENT OF CYTOTOXIC T CELL PRIMING IN LISTERIA MONOCYTOGENES INFECTED MICE**

M. Jergovic1,*, J. Uhrlaub1, M. Smithey1, J. Nikolich-Zugich1

1Immunobiology, University Of Arizona, Tucson, United States

**Introduction:** In response to infection with intracellular microorganisms, old mice mobilize decreased numbers of antigen-specific CD8 T cells with reduced production of effector molecules including granzyme B and IFN-g, decreased polyfunctionality (ability to produce multiple effector molecules), and impaired cytolytic activity. However, the naïve CD8 T cell-intrinsic vs. extrinsic contribution to, and molecular mechanisms behind, these defects remain unclear.

**Methods:** To address this question, we performed reciprocal transfer experiments with T cells from adult and old transgenic OT-1 mice. Recipient mice were infected with Listeria monocytogenes expressing Ovalbumin and antigen specific response was analyzed by flow cytometry.

**Results:** Our data shows that naïve adult OT-1 T cells fail to expand well in the old organism infected with Listeria-OVA. This defect is preceded by an impaired glycolytic switch when T cells are primed in the old organism, which is essential for effector function and proliferative burst. On the other hand, naïve CD8 T cells isolated from adult and old C57BL/6 mice proliferated and produced effector molecules to a similar extent when stimulated in vitro with polyclonal stimuli. Together these data indicate that old priming environment fails to efficiently prime and metabolically license cytotoxic T cells for massive proliferation and acquisition of effector function. Early after infection (day 1) with Listeria monocytogenes old mice exhibited lower expression of costimulatory molecules on DCs and a decrease in multiple inflammatory cytokines (IL-12, IFN-γ, IL-6, IL-1b) while basal levels of IL-2 were reduced irrespective of infection. These results point to a broad array of cellular defects in innate immunity that might be contributing to CD8 T cells receiving suboptimal signals during their recruitment in to the response. Here we show that these priming defects can be ameliorated by treatment of mice with inflammatory cytokines. Administration of high doses of IL-2 and IL-12 on days 4-6 of Listeria infection resulted in the reconstitution of the cytotoxic T cell response of aged mice to the level of adult.

**Conclusion:** We conclude that impairment of CD8 T cell response in aged mice is driven by the defects in the priming environment and not T cell intrinsic defects. Administration of cytokines IL-2 and IL-12 restores the antigen specific CD8 T cell response in aged mice to the level of the adult.

**Disclosure of Interest:** None Declared

---

**P141.B**

**THERAPEUTIC EX-VIVO EXPANDED CD8+CD25+FOXP3+TREG CELLS PROLONG THE ALLOGENEIC ISLET SURVIVAL**

J. S. Kim1,2,3,*, C. G. Park1,3,4,5, T.-N. Phuong1,5, H. Chung6, S. J. Hong6

1Seoul National University , Seoul, Korea, Republic Of, 2Institution of Endemic disease, 3xenotransplantation research center, 4Cancer Research Institute, 5Biomedical Sciences, Seoul National University , Seoul, Korea, Republic Of

**Introduction:** In addition to CD4+regulatory T cells(Tregs), adoptive transfer of CD8+CD25+FoxP3+T cells is emerging as an alternative adjunctive therapy to diminish current reliance on lifelong, nonspecific immunosuppression after transplantation. Here we evaluated the possible therapeutic application of consistently ex-vivo expanded mouse CD8+CD25+FoxP3+T cells to prevent the rejection of allogeneic islets.

**Methods:** B6 Mouse splenic CD8+CD25+T cells were enriched with FACSariaII and expanded in the presence of stimulatory aCD3 and aCD28Ab together with IL-2 (1,000units/ml) and TGF-beta (2.5ng/ml) for 9-12 days. Expanded CD8+CD25+T cells were adoptively transferred to the mouse transplanted with BALB/c islets (400 IEQs)
Clinical relevance of rejection by single administration. These results showed that expanded CD8 Tregs prolonged islet allograft survival for maximum 100 days. Co-transfer of ex-vivo expanded CD8+ Tregs resulted in suppression of IFN-γ producing cells in vivo.

Conclusion: Ex vivo expanded CD8 Treg retain regulatory activity in vitro and can protect islet allograft rejection by single administration. These results demonstrated that massively expanded CD8+ Tregs has clinical relevance of an adjunctive cellular therapy.

Disclosure of Interest: None Declared

P143
SPONTANEOUS DISEASE FEATURES IN SAVI MUTANT MICE OCCUR INDEPENDENTLY OF THE INTERFERON RECEPTOR

M. Motwani 1, S. Pawaria 1, J. Bernier 1, S. Moses 1, A. Rothstein 1, K. A. Fitzgerald 1

Introduction: Pattern recognition receptors detect pathogenic nucleic acids in the host cell and trigger a robust immune response as a first line of defense. In addition to the foreign nucleic acids, these receptors can also bind to the self or host nucleic acids resulting in autoimmune disorders. cGAS, a nucleotidyl transferase enzyme is a cytosolic DNA sensor that can detect both foreign and host DNA. Upon binding to the DNA, cGAS generates a second-messenger cGAMP, that binds to STING causing its activation and subsequent induction of Type I Interferons. Mutations in nucleic acid metabolizing proteins such as TREX1, RNASEH2, SAMHD1 cause accumulation of nucleic acids in the cells leading to autoimmune disorders categorized as Type I Interferonopathies. Recently, diseases like the SAVI...

Methods: We used experimental models of autoimmune uveitis (EAU), autoimmune encephalomyelitis (EAE), and glutamate-induced retinal neurotoxicity in IL-22−/− mice. We also examined the effects of intraocular injections of recombinant IL-22 or anti-IL-22 antibody in wild type animals during ocular inflammatory conditions.

Results: During EAU, IL-22 was produced in the eye by CD4+ eye-infiltrating T cells. EAU-challenged IL-22−/− mice, as well as WT mice treated systemically or intraocularly with anti-IL-22 antibody during the expression phase of disease, developed exacerbated retinal damage. Furthermore, IL-22−/− mice were more susceptible than WT controls to glutamate-induced neurotoxicity, whereas local IL-22 supplementation was neuroprotective, as judged by improved survival of retinal ganglion cells. Retinal glial Müller cells expressed IL-22ra1 in vivo, which was upregulated by inflammation, whereas in vitro, presence of IL-22 enhanced the ability of Müller cells to suppress proliferation of effector T cells. Finally, IL-22 injected into the eye concurrently with IL-1, inhibited the (IL-1-induced) expression of multiple proinflammatory and proapoptotic genes in retinal tissue and enhanced transcripts associated with new DNA synthesis.

Conclusion: In the aggregate, these findings suggest that IL-22 can function locally within the retina to reduce inflammatory damage and provide neuroprotection, by affecting multiple molecular and cellular pathways involving function of ocular resident cells and infiltrating inflammatory cells. Further study of the pathways regulated by IL-22 may enhance our understanding of neuroinflammation and instruct novel approaches to its modulation by augmenting natural neuroprotective mechanisms.

Disclosure of Interest: None Declared

P142
INTERLEUKIN 22 AMELIORATES NEUROPATHOLOGY AND PROTECTS FROM CENTRAL NERVOUS SYSTEM AUTOIMMUNITY

M. J. Mattapallil 1,*, J. L. Kielczewski 2, C. R. Zárata-Bladés 3, A. J. St.Leger 4, P. B. Silver 1, Y. Jittayasothorn 1, C. C. Chan 1, R. R. Caspi 1

1Lab. Immunology, 2Imaging Core Unit, National Eye Institute, N I H, Bethesda, United States, 3Lab. Immunoregulation, Federal University of Santa Catarina, Florianopolis, Brazil, 4Dept. Ophthalmology, Univ. Pittsburgh, Pittsburgh, United States

Introduction: Interleukin 22 (IL-22) is produced by cells of the immune system and acts on hematopoietic and non-hematopoietic cells, to modulate tissue responses during inflammation. IL-22 has opposing effects in different tissues, from pro-inflammatory (skin, joints) to protective (liver, intestine). Although PBMC from uveitis patients as well as T cells cloned from ocular samples of patients with Behçet’s uveitis produced IL-22, a causal link to disease is unclear. Therefore, we examined the effect of IL-22 in animal models of central nervous system (CNS) autoimmune diseases to understand its effects on neuroinflammation and modulation of disease severity.

Methods: We used experimental models of autoimmune uveitis (EAU), autoimmune encephalomyelitis (EAE), and glutamate-induced retinal neurotoxicity in IL-22−/− mice. We also examined the effects of intraocular injections of recombinant IL-22 or anti-IL-22 antibody in wild type animals during ocular inflammatory conditions.

Results: During EAU, IL-22 was produced in the eye by CD4+ eye-infiltrating T cells. EAU-challenged IL-22−/− mice, as well as WT mice treated systemically or intraocularly with anti-IL-22 antibody during the expression phase of disease, developed exacerbated retinal damage. Furthermore, IL-22−/− mice were more susceptible than WT controls to glutamate-induced neurotoxicity, whereas local IL-22 supplementation was neuroprotective, as judged by improved survival of retinal ganglion cells. Retinal glial Müller cells expressed IL-22ra1 in vivo, which was upregulated by inflammation, whereas in vitro, presence of IL-22 enhanced the ability of Müller cells to suppress proliferation of effector T cells. Finally, IL-22 injected into the eye concurrently with IL-1, inhibited the (IL-1-induced) expression of multiple proinflammatory and proapoptotic genes in retinal tissue and enhanced transcripts associated with new DNA synthesis.

Conclusion: In the aggregate, these findings suggest that IL-22 can function locally within the retina to reduce inflammatory damage and provide neuroprotection, by affecting multiple molecular and cellular pathways involving function of ocular resident cells and infiltrating inflammatory cells. Further study of the pathways regulated by IL-22 may enhance our understanding of neuroinflammation and instruct novel approaches to its modulation by augmenting natural neuroprotective mechanisms.

Disclosure of Interest: None Declared

P142
INTERLEUKIN 22 AMELIORATES NEUROPATHOLOGY AND PROTECTS FROM CENTRAL NERVOUS SYSTEM AUTOIMMUNITY

M. J. Mattapallil 1,*, J. L. Kielczewski 2, C. R. Zárata-Bladés 3, A. J. St.Leger 4, P. B. Silver 1, Y. Jittayasothorn 1, C. C. Chan 1, R. R. Caspi 1

1Lab. Immunology, 2Imaging Core Unit, National Eye Institute, N I H, Bethesda, United States, 3Lab. Immunoregulation, Federal University of Santa Catarina, Florianopolis, Brazil, 4Dept. Ophthalmology, Univ. Pittsburgh, Pittsburgh, United States

Introduction: Interleukin 22 (IL-22) is produced by cells of the immune system and acts on hematopoietic and non-hematopoietic cells, to modulate tissue responses during inflammation. IL-22 has opposing effects in different tissues, from pro-inflammatory (skin, joints) to protective (liver, intestine). Although PBMC from uveitis patients as well as T cells cloned from ocular samples of patients with Behçet’s uveitis produced IL-22, a causal link to disease is unclear. Therefore, we examined the effect of IL-22 in animal models of central nervous system (CNS) autoimmune diseases to understand its effects on neuroinflammation and modulation of disease severity.

Methods: We used experimental models of autoimmune uveitis (EAU), autoimmune encephalomyelitis (EAE), and glutamate-induced retinal neurotoxicity in IL-22−/− mice. We also examined the effects of intraocular injections of recombinant IL-22 or anti-IL-22 antibody in wild type animals during ocular inflammatory conditions.

Results: During EAU, IL-22 was produced in the eye by CD4+ eye-infiltrating T cells. EAU-challenged IL-22−/− mice, as well as WT mice treated systemically or intraocularly with anti-IL-22 antibody during the expression phase of disease, developed exacerbated retinal damage. Furthermore, IL-22−/− mice were more susceptible than WT controls to glutamate-induced neurotoxicity, whereas local IL-22 supplementation was neuroprotective, as judged by improved survival of retinal ganglion cells. Retinal glial Müller cells expressed IL-22ra1 in vivo, which was upregulated by inflammation, whereas in vitro, presence of IL-22 enhanced the ability of Müller cells to suppress proliferation of effector T cells. Finally, IL-22 injected into the eye concurrently with IL-1, inhibited the (IL-1-induced) expression of multiple proinflammatory and proapoptotic genes in retinal tissue and enhanced transcripts associated with new DNA synthesis.

Conclusion: In the aggregate, these findings suggest that IL-22 can function locally within the retina to reduce inflammatory damage and provide neuroprotection, by affecting multiple molecular and cellular pathways involving function of ocular resident cells and infiltrating inflammatory cells. Further study of the pathways regulated by IL-22 may enhance our understanding of neuroinflammation and instruct novel approaches to its modulation by augmenting natural neuroprotective mechanisms.

Disclosure of Interest: None Declared
**P144**

**GLYCA AS A NOVEL CANDIDATE BIOMARKER FOR RESPONSE TO INTERFERON-BETA AND NATALIZUMAB TREATMENT IN MULTIPLE SCLEROSIS**

D. M. Pineda, T. Dierckx, B. Pignolet, R. Liblau, D. Brassat, J. Van Weyenberg

1Ku Leuven, Leuven, Belgium, 2Rega Institute, KU Leuven, Leuven, Belgium, 3UMR 1043, INSERM, Toulouse, France

**Introduction:** Multiple sclerosis, an immune-mediated inflammatory demyelinating disease of the central nervous system. Both Interferon-beta and nataluzimab (NZ) have shown clinical benefit as first-line treatment in MS, although treatment response is not uniform and up to 40% of patients do not, or poorly respond to treatment. In search of biomarkers for therapeutic response, we have performed serum metabolomic analysis, including GlycA, a novel marker of chronic inflammation which has not been tested in MS. Large populational and cohort studies have demonstrated GlycA significantly correlated with increasing levels of serum inflammatory markers such as IL-6, C-reactive protein and TNF-α, but independently predicts overall mortality and severe infectious diseases. In addition, GlycA has shown promise in the stratification of disease severity in patients with other chronic inflammatory conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus and psoriasis.

**Methods:** A high-throughput metabolomics NMR platform (Nightingale Ltd.) was used to quantify 288 lipid and low-molecular-weight metabolite measures. Of those, GlycA is a biomarker of systemic inflammation and cardiovascular risk, summarizing the activation of multiple inflammatory pathways (Ritchie et al. 2015). Serum samples from healthy controls (n=50), patients with Clinically Isolated Syndrome (CIS, n=27) and MS, either untreated (n=60) or after treatment with IFN-beta (n=32) or Natalizumab (NZ, n=60), as well as RA patients (n=30) as a positive control.

**Results:** Although several plasma metabolome markers differed significantly between clinical groups (CIS, untreated MS, MS responders vs. non-responders, NZ responders vs. IFNbeta-responders), only GlycA faithfully reflected disease activity at all stages. First, GlycA was increased in CIS as compared to healthy controls and further increased from CIS to untreated MS patients (0.0026). Before treatment, GlycA levels among NZ nonresponders and responders did not differ, but a significant decrease was observed in NZ responders at 1 and 2 years following treatment (p=0.027). Strikingly, in IFN-beta responders GlycA decreased even further to levels indistinguishable from CIS patients. The greater decrease in GlycA levels observed in interferon-beta-treated vs NZ-treated patients is in agreement with the large transcriptomic effect of IFNbeta in MS, modulating hundreds of ISGs, whereas NZ specifically targets lymphocytes trafficking from the blood to the central nervous system, possibly resulting in a net increase in systemic inflammatory cells.

**Conclusion:** Several metabolomic markers differ between CIS, untreated and successfully treated MS, of which GlycA represents the most promising biomarker for clinical use, in monitoring disease progression and assessing treatment response among MS patients.

**Disclosure of Interest:** None Declared

---

**P145**

**PREFERENTIAL RECOGNITION OF ADVANCED GLYCATION END PRODUCTS BY SERUM**

---

**Cytokines 2018 – Abstracts**
ANTIBODIES AND LOW-GRADE SYSTEMIC INFLAMMATION IN DIABETES MELLITUS AND ITS COMPLICATIONS.
A. Raghavi1,*, J. Ahmad1
1RAJIV GANDHI CENTRE FOR DIABETES AND ENDOCRINOLOGY, ALIGARH MUSLIM UNIVERSITY, ALIGARH, India

Introduction: This study analyzes the detrimental effect of non-enzymatic glycation on human serum albumin (HSA) leading to the production of advanced glycation end products (AGEs).

Methods: HSA (20μM) incubated with glucose (400 mg/dL) formed AGEs confirmed by scanning electron microscopy. DNA-damage in subjects with diabetes mellitus was assessed with comet assay. Antibodies against in-vitro formed AGEs were evaluated in the sera of diabetic patients by enzyme-linked immunosorbent assay. Molecular docking to demonstrate affinity of native and glycosylated HSA with IgG. Low-grade systemic inflammation was quantified with IL-4, IL-6, TNF-α and NF-κB in serum and mRNA expression.

Results: The SEM showed the formation of aggregates in glycated-HSA. Serum auto-antibodies from diabetes patients with chronic kidney disease (CKD) showed appreciably high recognition of glycated-HSA compared to native HSA. Comet showed severe DNA damage in subjects with CKD compared to healthy. Molecular docking showed less affinity of glycosylated-HSA with IgG compared to native-HSA. Serum IL-4, IL-6, and TNF-α were found significantly higher in subjects with CKD compared to T2DM and healthy. mRNA expression of IL-4, IL-6 and NF-κB are also found significantly higher in CKD.

Conclusion: The non-enzymatic glycation-induced damage to the HSA and generate neo-epitopes that possess immunogenic response and low-grade systemic inflammation.

Disclosure of Interest: None Declared

P146
CROSSTALK BETWEEN TLR7 AND IFNG SIGNALING VIA STAT1 IN B CELLS CONTROLS SPONTANEOUS GERMINAL CENTER AND AUTOIMMUNE RESPONSES
Z. Rahman1,*, S. B. Chodisetti1, P. P. Domeier2, N. Choi1, T. Decker3
1Microbiology and Immunology, PennState University College of Medicine, Hershey, 2Immunology Program, Benaroya Research Institute, Seattle, United States, 3Max F. Perutz Laboratories, University of Vienna, Vienna, Austria

Introduction: Toll like receptor 7 (TLR7) is required for the development of spontaneous germinal center (Spt-GC) and lupus-like autoimmunity. Autoimmune-prone B6.Sle1b mice carrying an extra copy of TLR7 in the Yaa locus (named B6.Sle1b-Yaa mice) exhibit an exacerbation of lupus disease. However, the underlying molecular mechanisms in TLR7 driven acceleration of autoimmunity are not clearly defined.

Methods: We used TRL7 overstimulation model, in which we treated mice with TLR7 ligand imiquimod, and overexpression model, in which mice have two copies of TLR7 by expressing the Yaa locus. We have used bone marrow chimeras to determine the B cell-intrinsic role of serine 727 phosphorylation of Stat1 in elevated germinal center (GC) and autoantibody responses. Flow cytometry analysis and histology were used to evaluate the GC responses. ELISpot assay was used to quantify the autoantibody-producing AFCs.

Results: Here we report that overexpression of TLR7 or stimulation with its agonist, imiquimod increases IFNγR and STAT1 expression, and downstream STAT1 signaling in B cells. B6.Sle1b-Yaa mice deficient in IFNγR (designated Sle1b-Yaa(IFNgR-/-) mice) are unable to develop Spt-GCs, resulting in markedly reduced serum autoantibody (autoAb) titers and renal pathology. Further, mice in which B cells are deficient in IFNγR or STAT1 fail to generate TLR7-driven GC responses upon imiquimod treatment. Interestingly, in vitro TLR7 stimulation induces STAT1 phosphorylation at serine-727 in B cells. Consistently, lupus-prone B6.Sle1b mice with alanine substitution for serine-727 in STAT1 (Sle1b.STAT1-S727A mice) show significantly reduced Spt-GC responses, resulting in decreased number of autoAb-producing antibody forming cells (AFCs) and serum autoAb titers. B6.Sle1b-Yaa mice carrying the S727A allele also have substantially reduced splenomegaly, Spt-GC responses and autoAb-producing AFCs and serum ANA titers compared to B6.Sle1b-Yaa mice. Finally, using mixed bone marrow chimeras, we find that B cell-intrinsic STAT1-S727 phosphorylation is required for increased autoAb titers in B6.Sle1b mice. Mice with S727A mutation in STAT1, however, respond normally to foreign-Ag immunization.

Conclusion: These data highlight the importance of a crosstalk between IFNγ and TLR7 signaling in B cells via STAT1 in Spt-GC responses and lupus-like autoimmunity.

Disclosure of Interest: None Declared

P147
IFNAR SIGNALING IN RADIORESISTANT CELLS IS REQUIRED TO SUPPRESS ORGAN-SPECIFIC AUTOIMMUNITY IN VITILIGO.
R. L. Riding1,*, J. M. Richmond1, K. Fukuda1, J. E. Harris1
1Dermatology, University of Massachusetts Medical School, Worcester, United States

Introduction: Vitiligo is a common autoimmune skin disease that affects about 1% of the population. Patients develop white spots of depigmentation on the skin owing to autoreactive CD8+ T cell mediated destruction of the pigment producing cells of the epidermis, melanocytes. There are currently no FDA approved treatments for vitiligo and there is a great need to develop targeted and durable therapies. Type I interferons are a family of pleiotropic
cytokines that can drive pathogenic or protective responses depending on the cell type, timing and context in which it is present. In vitiligo, it is clear that type II interferon, IFNγ, and downstream IFN induced chemokines CXCL9 and CXCL10 drive disease pathogenesis. Since type I and type II interferon induce transcription of many overlapping gene targets and can drive similar cellular programs, we sought to determine the role of type I interferon in vitiligo.

Methods: To study the role of type I interferon in vitiligo we induced vitiligo in type I interferon receptor (IFNAR) deficient mice. To induce vitiligo in mice, we use transgenic mice that retain melanocytes in the epidermis due to expression of the melanocyte growth factor, stem cell factor. The day before vitiligo induction, we sublethally irradiate mice. The following day we transfer 1 million melanocyte-specific CD8+ T cells (PMEL) and activate the transferred PMEL in vivo by infecting mice with recombinant vaccinia virus that expresses their cognate antigen. The transferred PMEL proliferate, differentiate and become activated. Activated PMEL traffic into the skin where they target melanocytes for destruction. Over the next 5-7 weeks, spots of depigmentation develop on the mouse tail, ears, footpads and nose.

Results: We found that IFNAR-deficient mice developed accelerated and more severe vitiligo compared to wild type mice. These results suggest that in contrast to IFNγ signaling, which is required to drive disease pathogenesis, IFNAR signaling is required to suppress vitiligo. Further studies revealed that IFNAR signaling was not required on transferred autoreactive PMEL to mediate disease. To determine whether the hematopoietic compartment or radioresistant compartment requires IFNAR signaling to suppress vitiligo we performed bone marrow chimera studies. Results revealed that radioresistant cells require IFNAR to suppress vitiligo development. Flow cytometry analysis revealed that IFNAR deficiency in radioresistant cells leads to increased recruitment of autoreactive PMEL to the skin. PMEL in these hosts have reduced expression of the inhibitory molecule PD-1 and increased expression of effector cytokines TNFα and IFNγ.

Conclusion: Our studies conclude that IFNAR signaling in radioresistant cells is required to suppress vitiligo and is important to suppress the activation and effector function of autoreactive PMEL. Our studies of IFNAR signaling in the skin to suppress vitiligo will have broad implications to our understanding of mechanisms of peripheral tolerance and will shed light on the mechanistic role of IFNAR in other organ-specific autoimmune diseases, such as multiple sclerosis, in which the type I interferon pathway is targeted therapeutically.

Disclosure of Interest: None Declared

P148
IL-35 ADMINISTRATION PREVENTS THE DEVELOPMENT OF DIABETIC NEPHROPATHY
K. Singh1, Z. Luo1, M. Mejia-Cordova1, E. Enström1, S. Varli1, D. Espes1, P.-O. Carlsson1, M. Blixt1, L. Thorvaldson1, S. Sandler1 on behalf of Stellan Sandler

Introduction: Diabetes causes an elevation of the blood glucose level and a long-term hyperglycemia contributes to kidney damage, i.e. diabetic nephropathy (DN). DN exhibits signs of inflammation and infiltration of mononuclear cells in kidney. Regulatory T cells (Tregs), regulatory B cells (Bregs), tolerogenic antigen presenting cells (tolAPCs) maintain the homestasis of the immune system, specifically by producing anti-inflammatory cytokine IL-35. Despite intensive research the role of Tregs, Bregs and tolAPCs and IL-35 is not yet clear.

Methods: Herein, we determined the proportions of IL-35+ Tregs, IL-35+Bregs, IL-35(tolAPCs) and IL-17+Tregs and other immune cells in peripheral blood of patients with type 1 diabetes (T1D) with or with out DN and age, BMI and sex matched healthy controls by flow cytometry. The circulating levels of IL-35 was also measured in plasma samples of patients and healthy controls. We also studied the kinetics response of Treg, Bregs and tolAPCs in kidneys of multiple low dose streptozotocin and NOD mouse models of DN. Furthermore, immunostaining was performed on kidney tissue sections to determine the number of Foxp3+ cells and infiltrating mononuclear cells. Concentrations of albumin and creatinine were determined in urine samples of mice. IL-35 was systemically administrated in diabetic mice for 8 days. On the next day after the end of the treatment mice were sacrificed and kidneys were further investigated for immune response by using flow cytometry, immunostaining and in vitro immune assays. During the experimental duration the blood glucose levels were monitored and urine samples were collecte for determining the concentrations of albumin and creatinine.

Results: The circulating concentration of IL-35 was lower in T1D patients with DN and without DN than healthy controls. Proportions of IL35+Treg, IL35+Bregs, IL35+tolAPCs were lower but IL-17+Tregs were higher in patients with DN compared to healthy controls, suggesting a phenotypic shift of Tregs. The numbers of Tregs, Bregs and tolAPCs were higher in kidneys of diabetic mice. However, the proportions of IL35+Treg, IL35+Bregs, IL35+tolAPCs were lower in kidneys of diabetic mice. Furthermore, IFN-gamma+ Tregs were higher in kidneys of diabetic mice, indicating a phenotypic shift of Treg cells. Futhermore, the expression of transcription factor Eos was lower in Tregs of diabetic mice. The numbers of Foxp3+ cells were higher in kidney tissue sections of diabetic mice but the infiltrating mononuclear cells were also higher in those mice. The Albumin creatinine ratio (ACR) was higher in urine of diabetic mice.

After the administration of IL-35 in diabetic mice the mice became normoglycemic and had a lowered albumin creatinine ratio compared to PBS treated mice, illustrating that IL-35 prevents the development of DN. The IL-35+Tregs, IL-35+Bregs, IL-35+tolAPCs were increased in IL-35 treated mice. IFN-gamma+Tregs were decreased and the expression of transcription factor Eos was

1Medical Cell Biology, Uppsala University, Uppsala, Sweden
increased in Tregs of IL-35 treated mice. The number of Foxp3 cells was decreased, and mononuclear cells infiltration was also decreased in kidneys of IL-35 treated mice.

**Conclusion:** Thus our data suggest that IL-35+ regulatory immune cells are impaired in DN and systemic administration of IL-35 prevents the development of DN in mouse models of DN.

**Disclosure of Interest:** None Declared

P149

LOCAL PRODUCTION OF INTERFERON GAMMA AND FAS LIGAND IN THE SKIN ARE REQUIRED FOR MELANOCYTE CLEARANCE IN VITILIGO

J. P. Strassner1, J. M. Richmond3, V. N. Azzolino1, X. Fan1, J. E. Harris1
1Dermatology, University of Massachusetts Medical School, Worcester, United States

**Introduction:** Vitiligo is an autoimmune disease in which CD8 T cells selectively destroy melanocytes, leading to a patchy, disfiguring depigmentation of the skin. Nearly 1% of the population is affected, but there are no FDA approved therapies that result in repigmentation. Thus, there is an incredible unmet need to understand disease pathogenesis and develop targeted therapies that reverse depigmentation. Our group and others have highlighted the central role of IFNγ-dependent chemokines in the progression of disease; however, the mechanism of melanocyte destruction is not known. Since IFNγ is reported to have pleiotropic effects on melanocyte biology, we sought to examine if IFNγ has direct role in melanocyte killing.

**Methods:** We induced vitiligo in transgenic mice that develop black skin due to the enhanced expression of stem cell factor under a keratinocyte-specific promoter that leads to increased melanocyte retention in the epidermis. Depigmentation is easily quantified in these mice. To initiate disease, the mice are sub-lethally irradiated and subsequently injected with TCR-restricted CD8+ T cells and a recombinant vaccinia virus that expresses the cognate melanocyte-specific antigen, gp100. Depigmentation is assessed at weeks 5 and 7. To study the role of IFNγ in melanocyte killing, we crossed gp100-TCR restricted mice with IFNγ deficient animals. We crossed gp100-TCR restricted mice with perforin deficient and Fas Ligand (FasL) deficient animals to compare IFNγ mediated-killing against known putative effector molecules produced by cytotoxic CD8+ T cells.

**Results:** We found that disease incidence, disease severity and T cell accumulation in the skin was reduced in mice receiving adoptive transfer of either IFNγ deficient or FasL deficient gp100-specific T cells; however, perforin was dispensable and led to increased disease scores and T cell accumulation. Each T cell exhibited normal proliferation and migration phenotypes in the host mice. 50:50 co-injections of the deficient T cells with wild-type T cells rescued vitiligo scores, but not to the same level of disease as mice injected only with wild-type T cells. Intriguingly, FasL deficient and IFNγ deficient T cells accumulated at equal or greater numbers in the skin than co-injected wild-type T cells, suggesting that autoreactive T cells must produce IFNγ and FasL in the epidermal compartment to cause disease. Compared to wild-type gp100-specific T cells, MFI of IFNγ is higher in perforin deficient cells and greatly reduced in FasL deficient cells. To determine if IFNγ has a direct cytotoxic effect on melanocytes, we selectively deleted IFNγR using a conditional knockout. After inducing vitiligo in these mice, we did not detect any differences in disease development when mouse melanocytes lacked IFNγR.

**Conclusion:** Our studies conclude that IFNγ and FasL act locally in the skin to destroy melanocytes during vitiligo. Since FasL deficient cells produced very little IFNγ in the skin, we are not yet able to determine if FasL deficient cells fail to kill without FasL-induced apoptosis or IFNγ production. Targeting the IFNγ signaling is an attractive therapeutic approach since blockade would not only block progressive recruitment of autoreactive cells, but perhaps even melanocyte killing as well.

**Disclosure of Interest:** None Declared

P150

BCL-3 SUPPRESSES LUPUS-LIKE SYMPTOMS IN BL6/LPR MICE IN A TNFA-DEPENDENT MANNER

W. Tang1, H. Wang1, S. Saret1, H.-L. Ha1, H. Jaiswal1, E. Claudio1, U. Siebenlist1
1LMI/IAS, NIH/NIAID, Bethesda, United States

**Introduction:** Bcl-3 is an atypical member of the IkB family with unique properties. Unlike classical IkBs, Bcl-3 modifies NF-κB activity in nuclei by interacting with p50/NF-κB1 or p52/NF-κB2 homodimers on DNA. Depending on context, Bcl-3 may promote or inhibit gene expression. While recurrent translocations of Bcl-3 in some B cell tumors indicate a tumor-promoting role, we recently demonstrated that Bcl-3 may functions as a tumor-suppressor in the AOM/DSS-induced mouse colon tumor model, an inflammation-associated cancer model. We also previously reported that Bcl-3 is required for pathogenicity of T cells in T cell transfer-induced colitis and in EAE.

**Methods:** To further explore the biologic roles of Bcl-3 in inflammatory contexts, we investigated a lupus model. We generated BL6/lpr mice lacking Bcl-3 and studied the lupus-like symptoms in these mice.

**Results:** BL6/lpr mice carry the lpr mutation in Fas, resulting in functional loss of this death receptor, but unlike lpr mice on an MRL background, the BL6/lpr mice present with only very mild lupus-like symptoms. However Bcl-3 KO BL6/lpr (dKO) mice developed severe splenomegaly, dramatically increased numbers of double negative T cells - a hallmark of human lupus, ALPS and MRL/lpr mice - and exhibited inflammation in multiple organs, despite low levels of autoantibodies, similar to those in BL6/lpr mice. Mechanistically, elevated levels of TNFα in Bcl-3 KO BL6/lpr mice may promote lupus-like symptoms, since loss
of TNFα in these mice reversed the pathology that resulted from loss of Bcl-3.

**Conclusion:** These findings highlight the profound, yet highly context-dependent roles of Bcl-3 in the development of inflammation-associated pathology.

**Disclosure of Interest:** None Declared

### P151

**THE USE OF IL-10 TRANSFECTED CELLS TO FORM THE TOLEROGENIC ACTIVITY OF HUMAN MONONUCLEAR CELLS IN VITRO**

J. A. Shevchenko, V. P. Tereschenko, V. V. Kurlin, A. Z. Maksyutov, S. V. Sennikov

**Department of Molecular Immunology, Research Institute Of Fundamental And Clinical Immunology, Novosibirsk, Russian Federation**

**Introduction:** Immunotherapy with dendritic cells is considered to be an actual direction for the development of protocols for conducting clinical trials. In the therapy of autoimmune diseases and prevention of transplant complications, it is necessary to obtain the tolerogenic dendritic cells. To maintain the toxicogenic properties of dendritic cells, we obtained a DNA construct encoding IL-10 and tested its properties.

**Methods:** The adherent cell fraction from mononuclear cells was used to generate mature antigen-activated DCs. For this purpose, GM-CSF and IL-4 were added to the cells immature dendritic cells. Electroporation of DCs was performed on the 3rd day of the cultivation according to the protocol with a plasmid encoding human IL-10 (DCpIL-10). We determined the production of IL-10 in a culture of transfected dendritic cells, the expression level of surface dendritic cell markers, the level of suppression of lymphocyte proliferation in an autologous mixed culture, and the T-reg. Statistical data were processed using the GraphPadPrism 6.0 program.

**Results:** The production of IL-10 by transfected DC begins to increase 3 hours after transfection and increases within 24 hours. In the interval from 24 to 48 hours the production of IL-10 is preserved. The expression level of costimulatory molecules CD 86 and CD 83 is maximally reduced 48 hours after transfection, while retaining migration activity (CCR-7 expression). In the presence of IL-transfected DK, lymphocyte proliferation is suppressed and the content of T-regulatory cells is increased.

**Conclusion:** The use of electroporation of a pDNA construct encoding the IL-10 is an effective approach for inducing tolerogenic DCs and regulatory T cells in vitro. The production of IL-10 is stable and does not cause cell death in culture. This approach can be useful for the modulation of various cells in the cellular technologies of immunologic tolerance induction.

This work was supported by RSF. Agreement No. 16-15-00086 (01/11/2016)

**Disclosure of Interest:** None Declared

### P152

**EXTRACELLULAR VESICLES ARE INCREASED IN THE SERUM OF CHILDREN WITH AUTISM SPECTRUM DISORDER, CONTAIN MITOCHONDRIAL DNA, AND STIMULATE HUMAN MICROGLIA TO SECRETE IL-1BETA**

I. Tsilioni, T. C. Theoharides

**Immunology, Tufts University School of Medicine, Boston, United States**

**Introduction:** Autism Spectrum Disorder (ASD) has recently been shown to involve activation of microglia, but the triggers are not known. Extracellular vesicles (EVs) are secreted from many cells in blood or other biological fluids and carry molecules that could influence the function of target cells. EVs have been recently implicated in several diseases, but their presence or function in ASD has not been studied.

**Methods:** EVs were isolated from the serum of children with ASD and unrelated normotypic controls using the exoEasy Qiagen kit. They were characterized by determining the CD9 and CD81 EV-specific markers with Western blot analysis, while their morphology and size were assessed by Transmission Electron Microscopy (TEM). Human immortalized microglia SV40 were stimulated with EVs (1 or 5 µg/ml), quantified as total EV-associated protein, for 24 or 48 hrs and secretion of the proinflammatory cytokine IL-1β was measured by ELISA. Lipopolysaccharide (LPS) or the peptide neurotensin (NT) were used as “positive” controls. Cell viability was determined by Trypan blue (0.4 %) exclusion.

**Results:** EVs were shown to be spherical structures (about 100 nm) surrounded by a membrane. Total EV-associated protein was found to be significantly increased (p=0.02) in patients (n=20, 4-12 years old) as compared to age and sex-matched normotypic controls (n=8). Both 1 and 5 µg/ml of EVs isolated from serum of patients with ASD stimulated significant IL-1β secretion (p<0.0001 and p=0.008, respectively) from cultured human microglia (95.36 ± 5.31 pg/mL and 163.5 ± 13.34 pg/mL, respectively) as compared to unstimulated control microglia (59.76 ± 2.03 pg/mL and 117.7 ± 3.96 pg/mL, respectively). However, EVs from normotypic children did not have a significant effect (p=0.09) at either 1 or 5 µg/ml, as compared to control cells. Moreover, EVs from children with ASD contained increased amount (p=0.046) of mitochondrial DNA (mtDNA7S) as compared to normotypic controls.

**Conclusion:** These findings provide novel information that may help explain what triggers inflammation in the brain of children with ASD, and could lead to new effective treatments.

**Disclosure of Interest:** None Declared

### P153

**EXTRACELLULAR ADP/P2Y1 AXIS MEDIATES CXCL-2 INDUCED NEUTROPHIL CHEMOTAXIS IN RHEUMATOID ARTHRITIS**

X. Zhang, M. Qian

**Cytokines 2018 – Abstracts**

**P151**

**THE USE OF IL-10 TRANSFECTED CELLS TO FORM THE TOLEROGENIC ACTIVITY OF HUMAN MONONUCLEAR CELLS IN VITRO**

J. A. Shevchenko, V. P. Tereschenko, V. V. Kurlin, A. Z. Maksyutov, S. V. Sennikov

**Department of Molecular Immunology, Research Institute Of Fundamental And Clinical Immunology, Novosibirsk, Russian Federation**

**Introduction:** Immunotherapy with dendritic cells is considered to be an actual direction for the development of protocols for conducting clinical trials. In the therapy of autoimmune diseases and prevention of transplant complications, it is necessary to obtain the tolerogenic dendritic cells. To maintain the toxicogenic properties of dendritic cells, we obtained a DNA construct encoding IL-10 and tested its properties.

**Methods:** The adherent cell fraction from mononuclear cells was used to generate mature antigen-activated DCs. For this purpose, GM-CSF and IL-4 were added to the cells immature dendritic cells. Electroporation of DCs was performed on the 3rd day of the cultivation according to the protocol with a plasmid encoding human IL-10 (DCpIL-10). We determined the production of IL-10 in a culture of transfected dendritic cells, the expression level of surface dendritic cell markers, the level of suppression of lymphocyte proliferation in an autologous mixed culture, and the T-reg. Statistical data were processed using the GraphPadPrism 6.0 program.

**Results:** The production of IL-10 by transfected DC begins to increase 3 hours after transfection and increases within 24 hours. In the interval from 24 to 48 hours the production of IL-10 is preserved. The expression level of costimulatory molecules CD 86 and CD 83 is maximally reduced 48 hours after transfection, while retaining migration activity (CCR-7 expression). In the presence of IL-transfected DK, lymphocyte proliferation is suppressed and the content of T-regulatory cells is increased.

**Conclusion:** The use of electroporation of a pDNA construct encoding the IL-10 is an effective approach for inducing tolerogenic DCs and regulatory T cells in vitro. The production of IL-10 is stable and does not cause cell death in culture. This approach can be useful for the modulation of various cells in the cellular technologies of immunologic tolerance induction.

This work was supported by RSF. Agreement No. 16-15-00086 (01/11/2016)

**Disclosure of Interest:** None Declared

**P152**

**EXTRACELLULAR VESICLES ARE INCREASED IN THE SERUM OF CHILDREN WITH AUTISM SPECTRUM DISORDER, CONTAIN MITOCHONDRIAL DNA, AND STIMULATE HUMAN MICROGLIA TO SECRETE IL-1BETA**

I. Tsilioni, T. C. Theoharides

**Immunology, Tufts University School of Medicine, Boston, United States**

**Introduction:** Autism Spectrum Disorder (ASD) has recently been shown to involve activation of microglia, but the triggers are not known. Extracellular vesicles (EVs) are secreted from many cells in blood or other biological fluids and carry molecules that could influence the function of target cells. EVs have been recently implicated in several diseases, but their presence or function in ASD has not been studied.

**Methods:** EVs were isolated from the serum of children with ASD and unrelated normotypic controls using the exoEasy Qiagen kit. They were characterized by determining the CD9 and CD81 EV-specific markers with Western blot analysis, while their morphology and size were assessed by Transmission Electron Microscopy (TEM). Human immortalized microglia SV40 were stimulated with EVs (1 or 5 µg/ml), quantified as total EV-associated protein, for 24 or 48 hrs and secretion of the proinflammatory cytokine IL-1β was measured by ELISA. Lipopolysaccharide (LPS) or the peptide neurotensin (NT) were used as “positive” controls. Cell viability was determined by Trypan blue (0.4 %) exclusion.

**Results:** EVs were shown to be spherical structures (about 100 nm) surrounded by a membrane. Total EV-associated protein was found to be significantly increased (p=0.02) in patients (n=20, 4-12 years old) as compared to age and sex-matched normotypic controls (n=8). Both 1 and 5 µg/ml of EVs isolated from serum of patients with ASD stimulated significant IL-1β secretion (p<0.0001 and p=0.008, respectively) from cultured human microglia (95.36 ± 5.31 pg/mL and 163.5 ± 13.34 pg/mL, respectively) as compared to unstimulated control microglia (59.76 ± 2.03 pg/mL and 117.7 ± 3.96 pg/mL, respectively). However, EVs from normotypic children did not have a significant effect (p=0.09) at either 1 or 5 µg/ml, as compared to control cells. Moreover, EVs from children with ASD contained increased amount (p=0.046) of mitochondrial DNA (mtDNA7S) as compared to normotypic controls.

**Conclusion:** These findings provide novel information that may help explain what triggers inflammation in the brain of children with ASD, and could lead to new effective treatments.

**Disclosure of Interest:** None Declared

**P153**

**EXTRACELLULAR ADP/P2Y1 AXIS MEDIATES CXCL-2 INDUCED NEUTROPHIL CHEMOTAXIS IN RHEUMATOID ARTHRITIS**

X. Zhang, M. Qian
Introduction: Rheumatoid arthritis (RA) is a common autoimmune disease that causes millions of adults to be disabled every year. The extracellular nucleotides, alternatively called ‘alarmins’ which is an endogenous non-infective factor released by injured tissues during the inflammation and identified by PRRs to involve in joint damage. They are considered as the important markers of local inflammation and disease progression in RA. The immunological mechanisms responsible for the precise roles of ‘alarmin’ ADP plays in RA have not yet been understood.

Methods: Using collagen-induced arthritis mouse model, we aim to investigate the roles of ADP/P2Y1 axis in inflammatory arthritis. Then, we performed the migration assays, like Transwell to show the ADP-induced neutrophil chemotaxis, whereas specific CXCL-2 antibody was used to block the CXCL-2 to confirm its biological function. Western blot and bioinformatics methods were involved in the experiments to certify the possible signal pathway of ADP induced CXCL-2 production.

Results: We report here that eADP/P2Y1 play an important role in the pathogenesis of RA by recruiting neutrophils via CXCL2 production. Interestingly, ADP can be released in large quantities not only in the joint tissues of RA model mice, consisting with high expression of ADP receptor P2Y1 in RA patients. We then find that ADP treated RA mice are significantly exacerbated with more neutrophils in inflamed joints, whereas P2Y1 deficiency and pharmacologic inhibition results in restoring the severity of arthritis. In addition, extracellular ADP/P2Y1 axis enhances CXCL2 mediated neutrophil recruitment both in vitro and in vivo, which increase the pathogenesis of RA. Moreover, we also provide evidence that intracellular Ca2+ involved ADP-dependent activation of NF-kB signaling pathway significantly, which in turn associated with CXCL2 production.

Conclusion: Overall, this study reveals the nonredundant role for ADP/P2Y1 axis as an alternative inflammatory mediator in CXCL-2 mediated neutrophil recruitment and tissue inflammation in rheumatoid arthritis.

Disclosure of Interest: None Declared

P154
SPATIOTEMPORAL ACTIVATING OF A MAMMARY PRIMARY SUPER-ENHANCER CONTROLS SECONDARY ENHANCERS AND GENE EXPRESSION DURING PREGNANCY
H. K. Lee1,*
1NIDDK, NIH, Bethesda, United States

Introduction: Cytokines activate genetic programs dependent on regulatory elements that recruit lineage-specific transcription factors, thereby generating super-enhancers. We have investigated how spatiotemporal cytokine signaling regulates mammary gene expression up to several thousand-fold in a complex locus with nine genes, 22 putative enhancers and four super-enhancers during pregnancy.

Methods: We uncovered an establishment and roles of primary super-enhancer that precedes other enhancers within the locus during pregnancy using chromatin profiles and mouse genetics.

Results: The primary super-enhancer with four constituent enhancers sets at the early stage of pregnancy. The individual or combinational deletion of each enhancer within the primary super-enhancer uncovered a functional hierarchy between constituent enhancers, and the regulation of secondary enhancers and promoters by that reduces target gene expression up to 99%. We also identified two genes with a different regulatory mechanism under the influence of primary super-enhancer, unlike five genes in the locus. Our studies unveil mechanistically the primary super-enhancer is significant in activating the secondary enhancers and promoters and inducing lineage-specific gene expression in the locus by cytokine signaling.

Conclusion: The combination of in vivo chromatin and genetic analyses provided us with a high-resolution map of primary and secondary regulatory elements that govern exceptional gene activation during pregnancy.

Disclosure of Interest: None Declared

P155
A HIGH FAT DIET RESCUES SHORTENED LIFESPAN AND CACHEXIA-LIKE PHENOTYPES BY MODULATING INSULIN SIGNALING IN SIRT6 DEFICIENT MOUSE
Z. Li1,*; K. Xu1, Q. Liu1, Z. Wang1
1School of pharmaceutical sciences, Tsinghua University, Beijing, China

Introduction: Cachexia as a complex metabolic syndrome has been found associated with many end-stage diseases, especially cancer. Cachexia is characterized by severe loss of body weight, as a result of loss of fat and muscle, which worsens prognosis of many illness and leads to increased mortality. Although it has been reported that exercise, protein-affluent diet or some molecular could improve some of cachexia phenotype, more effective intervention in cachexia is still in need. Sirt6 as a NAD+ dependent histone deacetylase is involved in the regulation of many essential processes. Sirt6 deficient mice displayed severe loss of fat, muscle and bone density, which are typical phenotypes of cachexia.

Methods: The Sirt6 KO mice and littermates were treated with high fat diet or normal diet since 3 weeks old for one week. Appearance, body weight, and lifespan were monitored constantly. Metabolic cages were used to test the metabolic changes. Feces were collected for the analysis of gut microflora. We mainly focused on fat tissue, muscle tissue, and liver to compare the metabolic changes. The total proteins used for western-blots analysis were extracted from above tissues. Total mRNA were extracted from liver and used for transcriptomic analysis.
**Results:** In our results, we found the treatment of high fat diet could significantly increase lifespan at around 4 folds and rescue fat loss, muscle atrophy, hypoglycemia and idiopathic enteritis in SIRT6 deficient mice. Furthermore, gut microflora and gene expression pattern have also improved under the treatment of high fat diet. In addition, excessive activation of AKT signaling and glycolysis induced by SIRT6 deficiency was attenuated, on the contrary, fatty acid biosynthesis was increased by high fat diet in SIRT6 deficient mice. Inhibition of insulin signaling and IGF-1 signaling was the key step of high fat diet effect since PI3K inhibition could ameliorate the saving effect of high fat diet on SIRT6 deficient cells.

**Conclusion:** We found that the high fat diet could effectively save the aberrant phenotype of SIRT6 deficient mice. The modification of insulin signaling under high fat diet is the key reason of this process. Those results suggest that high fat diet could be an effective treatment for cachexia and other metabolic disorder diseases.

**Disclosure of Interest:** None Declared

---

**P157**  
**THE STABILITY OF INTERLEUKIN-11 IS COMPROMISED BY THE CODING SNP RS4252548 (R112H)**

J. Lokau¹, S. Göttert¹, P. Arnold², S. Düsterhöft¹, D. Massa López¹, J. Grötzingr¹, C. Garbers¹  
¹Institute of Biochemistry, ²Institute of Anatomy, Kiel University, Kiel, Germany

**Introduction:** Human height is a complex phenotype which is influenced by a high number of genetic variances. Recently, an uncommon single nucleotide polymorphism (SNP) in the IL11 gene (rs4252548), which translates into the exchange of Arg-112 into His-112 in Interleukin-11 (IL-11), was described to strongly correlate with a reduction in height. The pleiotropic cytokine IL-11 has important functions in bone remodeling, and loss of IL-11 signaling has been associated with impaired bone formation, decreased osteoclast numbers, and craniofacial malformations. Therefore, we aimed to understand how the R112H variation alters IL-11 biology.

**Methods:** We recombinantly produced IL-11 wildtype (wt) and IL-11_R112H in E. coli and determined their secondary structure. We analyzed the biological activity of the variants with an IL-11-dependent cell line and used a combination of molecular modeling and in vitro experiments to examine receptor binding and thermal stability. Finally, we performed ex vivo differentiation of osteoclasts in order to detect effects on osteoclastogenesis.

**Results:** We found that the R112H mutation does not interfere with solubility or folding of IL-11. Likewise, the receptor binding and biological activity of IL-11_R112H is indistinguishable from wt IL-11. However, we detected a higher flexibility within the IL-11_R112H molecule compared to wt and, as a consequence, a diminished thermal stability. The reduced stability is caused by the loss of the positively charged arginine side chain.

Furthermore, due to the decreased stability, IL-11_R112H failed to support the survival of osteoclast progenitor cells.

**Conclusion:** Our results show that IL-11_R112H is biologically active, but thermally less stable. The compromised stability might be relevant in osteoclastogenesis and bone turnover, which offers an explanation for the reduction in height observed in carriers of the minor allele.

**Disclosure of Interest:** None Declared

---

**P158**  
**EPIGENETIC REGULATION OF IL-17 SIGNALING PATHWAY IN AIRWAY EPITHELIAL CELLS**

J. Luo¹,², A. Ferguson¹, X. An¹, Z. Sun², T. Wang³, Q. Zhang⁴, W. Chen⁵, J. Kolls¹  
¹Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, United States, ²Department of Pathology, the Second Xiangya Hospital, Central South University, Changsha, China, ³Division of Pediatric Pulmonology, Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, ⁴Department of Integrative Biology and Physiology, University of California Los Angeles, Los Angeles, ⁵Center for Translational Research in Infection and Inflammation, Tulane School of Medicine, New Orleans, United States

**Introduction:** Airway epithelial cell is the first barrier to the inhaled environmental particles, playing vital roles in host defense and the pathophysiology of some Airway diseases, such as Cystic Fibrosis (CF), asthma, and COPD. It has been reported that IL-17 signaling pathway is involved in chronic airway infection and airway autoimmune regulation. Our preliminary data indicates that IL-17 induced proinflammatory chemokines and cytokines are spontaneously released by Human Bronchial Epithelial (HBE) cells established from CF patients and the spontaneous release can be abolished by a bromodomain inhibitor, a small molecule that inhibits epigenetic enhancement of gene transcription, suggesting that potential epigenetic regulations of these inflammatory mediators might be involved. We hypothesize that the enhanced production of proinflammatory mediators observed in the airway are in part controlled by an epigenetic regulatory program.

**Methods:** Transcriptomic analysis was carried out on the airway epithelial cells including the airway epithelium from CF patients underwent clinical bronchoscopy, Normal Human Bronchial Cells (NHBEs), as well as human bronchial epithelial cell line HBE1. To conduct an integrative epigenomic analysis on airway, based on RNA-seq, we further chose a newly established assay for transposaseaccessible chromatin using sequencing (ATAC-seq), a simple two-step protocol provides information on nucleosome positioning, chromatin accessibility and transcription factor binding simultaneously. Chemokine production at RNA and protein level was also confirmed by qPCR and ELISA. The bromodomain and extraterminal domain (BET) inhibitor, histone acetyltransferase (HAT) inhibitor and DNA
methyltransferase inhibitor were applied to further determine the specific regulatory mechanism.

**Results:** RNA-seq analysis revealed a dramatic upregulation of chemokine genes in the airway epithelium from CF patients, which correlated with downregulation of histone deacetylases, suggesting chemokine expression in CF airway can be modified by histone acetylation. ATAC-seq analysis on HBE cells derived from control and CF donors was conducted and more peaks were observed in CF cells at the promoter regions of several chemokine genes suggesting these loci are more accessible compared to control HBE cells. In vitro, qPCR results revealed a dramatic upregulation of chemokine genes in the epithelial cell line HBE1 cells after IL-17A stimulation, and the upregulation effect could be overwhelmingly blocked by bromodomain inhibitor, and partially suppressed by HAT inhibitor. Furthermore, this IL-17 mediated upregulation can also be enhanced by DNA methyltransferase inhibitor, suggesting chemokine expression in the epithelial cells can be regulated by both histone acetylation and DNA methylation.

**Conclusion:** These data strongly implicate that the activation of IL-17 mediated signaling in the airway promotes chemokines production through repressing histone deacetylases, and the upregulation can be further enhanced by DNA methylation inhibition, suggesting that therapeutic approaches through modifying histone acetylation or DNA methylation can be explored to control the IL-17 mediated pathways in patients with airway inflammation.

**Disclosure of Interest:** None Declared

---

**P159**

**MAPPING IL-6 SIGNALING NETWORKS IN HUMAN CD4 T CELLS**

J. Martinez-Fabregas1, E. Pohler1, S. Mitra2, S. Wilmes1, P. Fyfe1, I. Moraga1

1Cell Signalling and Immunology, University of Dundee, Dundee, United Kingdom, 2Sahlgrenska Translational Melanoma Group, University of Gothenburg, Gothenburg, Sweden

**Introduction:** Interleukin-6 (IL6) is a very pleiotropic cytokine that acts as a central regulator of the immune response. IL-6 controls the extent of the pro-inflammatory response by inducing differentiation of Th-17 cells and inhibiting the differentiation of Treg and Th-1 cells. IL-6 is also involved in the regulation of other immune cell populations including B cells, monocytes and macrophages, as well as exhibiting hormone-like functions involved in metabolic control, regeneration, bone metabolism and control of pain1. IL-6 elicits its large plethora of biological responses by engaging a surface receptor comprised of IL-6Ra and gp130 receptor subunits, which triggers the activation of JAK1 and STAT1/STAT3 signaling pathway2. Additional non-STAT signaling pathways are also activated by IL-6, but their contribution to IL-6 biological responses are not well defined. How IL-6-induced signaling networks are integrated by cells to produce functional diversity is currently not fully understood.

**Methods:** Here we set to identify cellular determinants contributing to IL-6 functional pleiotropy by characterizing the complete signalosome engaged by IL-6 in human CD4 T cells using a combination of high-throughput flow cytometry and quantitative proteomic studies.

**Results:** In a first step, we used an antibody array comprised of 25 antibodies targeting components of major signaling pathways, i.e. PI3K, MAPK, JAK/STAT, …. Surprisingly, we only detected activation of STAT1 and STAT3 by IL6 in both resting and activated CD4 T cells, in strike contrast to anti-CD3 stimulation, which resulted in activation of a more complex signaling program. We further confirmed this initial observation by immunoblotting. Interestingly, STAT3 was not only phosphorylated on Y705 (JAK1 mediated) but also on S727, indicating that additional pathways to JAK/STAT were engaged by IL-6. To identify these pathways, we used STAT3 S727 phosphorylation as a proxy and tested the ability of IL-6 to promote this phosphorylation in the presence of inhibitors targeting pathways previously reported to control STAT3 S727 phosphorylation in other systems, i.e. (TORC, GSK3, MEK, PI3K, CDK, SRC). Only Torin, a TORC inhibitor, was able to decrease STAT3 S727 phosphorylation. Since rapamycin, a TORC1 specific inhibitor, did not have the same effect we concluded that S727 STAT3 phosphorylation by IL-6 on CD4 T cells was dependent on the TORC2 complex. Currently, we are performing phospho-proteomics studies to identify networks engaged by IL-6 that lead to TORC2 activation and chromatin immunoprecipitation assays to characterize the impact that S727 phosphorylation has on STAT3 transcriptional activity.

**Conclusion:** Our data highlight the large complexity of the signaling network engaged by IL-6 in human CD4 T cells and the need to fully characterize this network to understand and manipulate IL-6 biological responses.

**Disclosure of Interest:** None Declared

---

**P160**

**RNA BASE MODIFICATIONS REGULATE THE TYPE I INTERFERON RESPONSE**

M. Mcfadden1, A. McIntyre2, N. Gokhale1, C. Mason2, S. Horner1

1Molecular Genetics & Microbiology, Duke University Medical Center, Durham, 2Physiology & Biophysics, Weill Cornell Medicine, 3Tri-Institutional Program in Computational Biology and Medicine, New York, 4Department of Medicine, Duke University Medical Center, Durham, United States

**Introduction:** Type I interferon (IFN) drives 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 (m6A) has emerged as a potent
regulator of RNA function. We hypothesized that m^6^A could act as a regulator of the expression of ISGs activated in response to viral infection. Indeed, we have previously shown that the m^6^A machinery regulates viral infection, however how m^6^A could regulate the dynamic and coordinated expression of ISGs in response to viral infection is still unknown.

**Methods:** To determine whether m^6^A regulates ISG expression, we perturbed the levels of the m^6^A machinery in human cells prior to treatment with type I IFN. The m^6^A machinery includes the methyltransferase complex METTL3 and METTL14 and the demethylase FTO. We either depleted this m^6^A machinery using siRNAs or ectopically expressed these proteins in human cells. Then, we performed a number of experiments designed to determine the changes in RNA levels, transcription, and translation of ISGs in response to type I IFN. These included differential gene expression analysis by using either RNA-seq or quantitative real-time PCR (qRT-PCR), analysis of transcription by metabolic labeling of nascent RNA with 4-thiouridine coupled with qRT-PCR, and immunoblotting. Additionally, we mapped m^6^A in the transcriptome during the IFN response using m^6^A RNA immunoprecipitation followed by sequencing (meRIP-seq).

**Results:** Our RNA-seq analysis revealed that after IFN treatment, loss of FTO resulted in upregulation of a subset of ISGs, whereas METTL3/14 depletion did not significantly affect the mRNA levels of ISGs. Interestingly, we found that FTO depletion increased the transcription of this subset of ISGs, as measured by metabolic labeling of nascent RNA. However, METTL3/14 depletion, which did not affect mRNA levels of ISGs, decreased protein abundance of specific ISGs. This was supported by our results that METTL3/14 overexpression increased the protein abundance of these same ISGs. To determine whether m^6^A may be involved in the regulation of candidate ISGs, we used meRIP-seq to map m^6^A in the IFN-induced transcriptome. We found that many ISGs are modified by m^6^A during the IFN response, and these included the same ISGs that were regulated by METTL3/14. For example, IFITM1 was found to contain m^6^A within its 3'UTR and is regulated by METTL3/14, while PKR did not contain m^6^A and was not regulated by METTL3/14. Future studies will address the mechanisms by which m^6^A regulates ISG expression.

**Conclusion:** Taken together, these data implicate FTO as a transcriptional regulator of the type I IFN response, and suggest that METTL3/14 contribute to the production of certain ISGs at the protein level, possibly through the addition of m^6^A to the mRNAs of these genes. Overall, these studies reveal new insights into the mechanisms by which the m^6^A machinery coordinate the dynamic production of ISGs in response to viral infection and may have broad implications for understanding the dysregulated gene expression that can lead to inflammatory diseases.

**Disclosure of Interest:** None Declared

---

**CELL-TYPE- AND STIMULUS-SPECIFIC LANDSCAPE OF THE TNF LOCUS.**

A. Nambu\(^1\), L. D. Jasenosky\(^1\), A. E. Goldfeld\(^1\)

\(^1\)Program in Cellular and Molecular Medicine (PCMM), Boston Children’s Hospital/Harvard Medical School, Boston, United States

**Introduction:** TNF (Tumor Necrosis Factor), a pleiotropic cytokine produced from a variety of cell types induced by many stimuli, plays a significant role in host defense and autoimmunity. Our lab has previously demonstrated that TNF transcription is controlled in a cell-type- and stimulus-specific manner via recruitment of distinct enhanceosomes to the TNF promoter and via a long-range TNF/LT locus enhancer element. Here, using CRISPR-based modification of human cell lines and targeting mice in vivo, we show that two independent and distinct activator binding sites within a distal TNF/LT locus enhancer element controls cell-type and stimulus specificity of TNF transcription via distinct patterns of chromatin modification in T and monocyteic cellular lineages.

**Methods:** CRISPR/Cas9 editing of human cell lines and mice in vivo, were combined with DNase hypersensitivity, ChIP, ATAC-seq, ChIP-seq and 3C-based Hi-C approaches.

**Results:** We detected cell-type- and stimulus-specific chromatin structure, modification, and transcription factor binding to a distal TNF locus enhancer element, which forms distinct 3D structures dependent on cell-type and stimulus. Cellular modification of these distal sites in cell lines and from primary cells from in vivo CRISPR-edited mice demonstrate that these TNF/LT locus sites control TNF gene expression in T cells and macrophages.

**Conclusion:** We have discovered that cell-type- and stimulus specific TNF gene expression and its distinct landscape of chromatin structure in T and monocyteic cellular lineages is controlled via two activator binding sites in a distal TNF/LT locus enhancer element. These results also provide a potential target for modification of TNF-related pathologies associated with cell-type-specific expression of TNF.

**Disclosure of Interest:** None Declared

---

**P162**

**CD206 M2-LIKE MACROPHAGES REGULATE WHITE AND BEIGE PROGENITORS**

A. Nawaz\(^1\), Y. Igarashi\(^1\), T. Kado\(^2\)

\(^1\)First Department of Internal Medicine, University of Toyama, University of Toyama, toyama, \(^2\)First Department of Internal Medicine, University of Toyama, University of Toyama, Toyama shi, Japan

**Introduction:** Obesity and type 2 diabetes is increasing rapidly worldwide and is accompanied by many complications, including impaired muscle regeneration. One of the characteristic features of obesity is a chronic inflammation in adipose tissue associated with a phenotypic transformation of macrophages, from anti-inflammatory M2- to pro-inflammatory M1-like

---

**P161**
macrophages, thereby causing insulin resistance and type 2 diabetes. Several lines of evidence suggest that adipose tissue-resident macrophages (ATMs) are involved in maintaining insulin sensitivity in adipocytes along with improvement in metabolic genes. Much is known about the role of M1-like ATMs in the inflammatory process in obese adipose tissue, which is closely related to the development of insulin resistance both in humans and animals. In contrast, M2-like ATMs in the lean state enhance insulin sensitivity. Nonetheless, it is largely unknown how depletion of M2-like ATMs regulate insulin sensitivity and adipocyte progenitor (AP) proliferation.

Methods: we utilized CD206DTR and Tgfb1 knock out mice to investigate the role of CD206 M2-like macrophages and CD206-secreted cytokines in the regulation of APs behavior.

Results: Here, I show that depletion of CD206 M2-like macrophages resulted in the generation of smaller adipocytes, upregulated expression of metabolically favorable genes and enhanced insulin sensitivity in both chow and high-fat diet-fed CD206-reduced mice. In vivo and in vitro studies revealed that TGFβ, abundantly expressed in CD206 M2-like macrophages, regulate AP differentiation and proliferation. To validate this hypothesis, I generated genetically engineered mice in which CD206 specific TGFβ1 was knocked out after tamoxifen treatment. Increased number of APs and smaller adipocytes were observed in the CD206 specific TGFβ1 knockout mice. Our study also shows that the expressions of UCP1 and other browning related genes were significantly upregulated after depletion of CD206 M2-like macrophages. Immunofluorescence and flow cytometry analysis further confirmed that beige progenitors are enhanced in the WAT of CD206-reduced mice, suggesting that depletion of CD206 M2-like macrophages promotes cold-induced browning of WAT.

Conclusion: Collectively, I show that CD206 M2-like macrophages in adipose tissues constitute an important microenvironment in which they regulate both white and beige AP growth/differentiation and thereby control adiposity and systemic insulin sensitivity.

Disclosure of Interest: None Declared

P163
ENHANCED BINDING OF THE SOCS2-SH2 DOMAIN TO PHOSPHORYLATED TARGETS VIA A NOVEL EXOSITE
S. E. Nicholson1,2, E. M. Linossi1,2, G. Veggian3, D. Calleja1, C. Tan1,2, C. Walters1,2, L. F. Dagley1,2, S. S. Li4, N. J. Kershaw1, J. J. Babon1,2, S. S. Sidhu3
1Walter & Eliza Hall Institute of Medical Research, 2Department of Medical Biology, University of Melbourne, Melbourne, Australia, 3The Donnelly Centre, University of Toronto, Toronto, 4University of Western Ontario, London, Canada

Introduction: The Suppressor Of Cytokine Signaling Proteins (CIS and SOCS1-7) are important negative regulators of cytokine and growth factor signaling. They act as substrate adaptors for an E3 ubiquitin ligase complex (Cullin-Ring E3 ligase), binding to tyrosine phosphorylated target proteins via their central Src Homology 2 (SH2) domain. In particular, SOCS2 is a negative regulator of growth hormone signaling (Metcalf et al., Nature 2000) and when induced by interferon gamma, acts as an immune checkpoint in dendritic cells, dampening anti-tumor immunity to melanoma (Nirschl et al., Cell 2017). While the study by Nirschl et al. suggests that inhibition of SOCS2 activity might improve dendritic cell priming of CD8 T cells, they did not identify the pathway or protein targets for SOCS2 action.

Methods: We have utilized phage-display technology to explore alternative binding surfaces on the SH2 domain of the SOCS family of proteins and affinity-purification/mass spectrometry to identify novel SOCS-interacting proteins.

Results: Several non-phosphorylated peptides were identified that specifically bound the SOCS2-SH2 domain with low micromolar affinity (Kd 1.5-5.0 μM), similar to that of the known SOCS2 binding site in the growth hormone receptor (GHR pY595, Kd 1.0 μM). Surprisingly, these peptides did not compete with phosphopeptide binding, but instead significantly enhanced affinity of the SOCS2-SH2 domain for the GHR pY595 peptide (20-fold; Kd 0.05 μM). Ongoing biochemical and structural studies aim to characterize the binding site of these exosite peptides on SOCS2 and to examine the functional impact of enhanced SOCS2 affinity for its phosphorylated targets. Parallel studies identified a novel SOCS2 interacting protein from dendritic cells.

Conclusion: We hypothesize that proteins which share sequence homology with the exosite peptides, will modulate SOCS2-SH2 binding, providing a previously unappreciated level of regulation to SOCS protein function. By extension, this work may identify similar sites on other SH2-containing proteins that augment their binding to phosphorylated targets. The identification of a new candidate SOCS2 target protein may explain how this SOCS family member acts as an immune checkpoint.

Disclosure of Interest: None Declared

P164
INTERFERON STIMULATION MARKS CHROMATIN AND CREATES EPIGENETIC MEMORY
K. Ozato1, R. Kamada2, W. Yang2, J. Zhu3, R. Oda1, T. Fujita1, A. Dey1
1DDB, NICHD, NATIONAL INSTITUTES OF HEALTH, Bethesda, United States, 2Biological Chemistry, Hokkaido University, Sapporo, Japan, 3NHBLI, NATIONAL INSTITUTES OF HEALTH, Bethesda, United States, 4Institute for Viral Research, Kyoto University, Kyoto, Japan

Introduction: Histone modifications shape chromatin landscape and provide a basis of epigenetic regulation. It has been shown that interferon (IFN) stimulation causes large scale chromatin alterations over IFN stimulated genes (ISGs).
Methods: We studied whether IFN and other innate immune stimuli create epigenetic memory.

Results: We found that cells previously exposed to IFN respond faster and more robustly to a second IFN stimulation, suggestive of epigenetic memory. This memory phenotype was observed in mouse embryonic fibroblasts (MEFs) and BM derived macrophages previously stimulated by IFN-beta or IFN-gamma, respectively. In MEFs, this memory response was somatically inherited, and seen after cycles of cell divisions. Moreover, cells previously exposed to IFN showed greater anti-viral resistance against viral infection, indicating the biological significance of the memory. RNA-seq analysis revealed that, of total ISGs induced in MEFs (approximately 2,000), more than half exhibited memory phenotype, while the rest of ISGs did not.

Conclusion: This memory was not readily attributed to changes in IFN receptor expression or JAK/STAT signaling. Rather, our data indicate that it is linked to specific chromatin marks created on ISGs. Together, the memory response detected here may be part of "Trained Innate Immunity", an emerging concept, where innate immunity possesses the ability to mount an adaptive behavior in ever shifting pathogen environments.

Disclosure of Interest: None Declared

P165
DIVERSE SUPERANTIGENS TRIGGER B7/CD28 COSTIMULATORY RECEPTOR ENGAGEMENT CRITICAL FOR INDUCTION OF INFLAMMATORY CYTOKINE STORM
A. Popugailo1,*, Z. Rotfogel1, E. Supper1, D. Hillman1, R. Kaempfer1
1Biochemistry and Molecular Biology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

Introduction: Staphylococcal and streptococcal superantigens are virulence factors that cause toxic shock by hyperinducing inflammatory cytokines. Effective T-cell activation requires interaction between the principal costimulatory receptor CD28 and its coligands, B7-2 (CD86) and B7-1 (CD80). To elicit an inflammatory cytokine storm, bacterial superantigens must bind directly into the homodimer interfaces of CD28 and B7-2. We reported recently that by engaging CD28 and B7-2 directly at their dimer interfaces, staphylococcal enterotoxin B (SEB) potently enhances intercellular synapse formation mediated by B7-2 and CD28, resulting in T-cell hyperactivation.

Methods: Costimulatory receptor B7-2/CD28 and B7-1/CD28 interactions were analyzed by flow cytometry. Induction of inflammatory cytokines in human peripheral blood mononuclear cells.

Results: Here, we addressed the question, whether diverse bacterial superantigens share the property of triggering B7-2/CD28 receptor engagement and if so, whether they are capable of enhancing also the interaction between B7-1 and CD28, which occurs with an order of magnitude higher affinity. To this end, we compared the ability of distinct staphylococcal and streptococcal superantigens to enhance intercellular B7-2/CD28 engagement. Each of these diverse superantigens promoted B7-2/CD28 engagement to a comparable extent. Moreover, they were capable of triggering the intercellular B7-1/CD28 interaction, analyzed by flow cytometry of co-cultured cell populations transfected separately to express human CD28 or B7-1. Streptococcal mitogenic exotoxin Z (SMEZ), the most potent superantigen known, was as sensitive as SEB, SEA and toxic shock syndrome toxin-1 (TSST-1) to inhibition of cytokine induction by CD28 and B7-2 dimer interface mimetic peptides.

Conclusion: Thus, superantigens act not only by mediating unconventional interaction between MHC-II molecule and T-cell receptor but particularly, by strongly promoting engagement of CD28 by its B7-2 and B7-1 coligands, a critical immune checkpoint, forcing the principal costimulatory axis to signal excessively. Our results show that the diverse superantigens use a common mechanism to subvert the inflammatory cytokine response, strongly enhancing B7-1/CD28 and B7-2/CD28 costimulatory receptor engagement.

Disclosure of Interest: A. Popugailio: None Declared, Z. Rotfogel: None Declared, E. Supper: None Declared, D. Hillman: None Declared, R. Kaempfer Grant / Research support from: NIAID grant 2U54AI057168.

P166
STRUCTURAL CHARACTERIZATION OF A PROTEIN-PROTEIN INTERACTION REQUIRED FOR IL1B GENE TRANSCRIPTION USING COMBINED COMPUTATIONAL AND EXPERIMENTAL APPROACHES
S. H. Pulugulla1,*, R. Workman1, N. W. Rutter1, E. X. Esposito2, Z. Yang3, J. Adamik4, D. L. Galson4, P. E. Auron1
1Duquesne University, Pittsburgh, 2exeResearch, LLC, East Lansing, 3Pfizer, Cambridge, 4University of Pittsburgh, Pittsburgh, United States

Introduction: Interleukin 1β (IL-1β) is a pro-inflammatory cytokine that is normally expressed and released by monocytes in response to infection and injury. Previous reports by our group have demonstrated that transcription of the human IL1B gene that codes for Interleukin 1β depends upon chromatin looping mediated by a mutual interaction between the DNA-binding domains (DBD) of two transcription factors, Spi1-PU.1 (Spi1) at the promoter and C/EBPβ at a far-upstream enhancer.

Methods: Glutathione-S-transferase (GST) protein–protein pull down assays were used to map interacting regions between Spi1 and C/EBPβ using a collection of Spi1 deletions. The C/EBPβ-Spi1 interaction was independently generated using ZDOCK and HADDOCK online docking servers, followed by LowMode and Nanoscale Molecular Dynamics (NAMD) simulations. A docked structure derived by combination of the above

in
vitro and in silico approaches revealed an interaction pocket that was screened with Molecular Operating Environment’s Amber10:EHT force field induced fit small molecule docking software for identifying interaction-inhibiting compounds. One such potential compound was evaluated for effects on both C/EBPβ:Spi1 interaction and activity at the IL1B gene promoter using Chromatin Immunoprecipitation (ChIP) and mRNA expression (qPCR) in LPS-activated THP-1 cells.

Results: The in vitro (GST) interaction studies revealed that the recognition helix and part of the β-sheet wing of the winged-Helix-Turn-Helix DBD of Spi1 are necessary for its interaction with C/EBPβ. Initial computational docking methods suggested that the Spi1 DBD interacts with the C-terminal C/EBPβ leucine zipper in the absence of carboxy-terminal extra-zipper tail, via complementary polar/aliphatic residues, similar to that reported for a C/EBPβ:c-Myb interaction. NAMD revealed a strong ionic interaction between Cys 345 at the carboxyl terminus of C/EBPβ with Arg 232 of Spi1. This is consistent with our previous report that this arginine residue in the Spi-1 DNA recognition helix is critical for C/EBPβ:Spi1 association. L-Arginine, a known anti-inflammatory, was computationally identified to bind in the C/EBPβ:Spi1 interaction pocket containing Arg 232, suggesting a potential mode for disruption. Subsequent studies using cultured LPS-activated THP-1 cells, incubated with L-Arginine, demonstrated a significant decrease in C/EBPβ association with Spi1 on the IL1B promoter in parallel with a reduction in IL1B mRNA transcription without any significant effect on direct binding of either protein to cognate DNA.

Conclusion: This study provides reinforcement of the critical and unique role for Spi1 in monocyte IL1B gene expression by using a combined computational-experimental approach. More importantly it demonstrates specific drug targeting at a gene promoter, a structure that has by virtue of its homogeneity defied pharmaceutical interest. The IL1B promoter, with its dependence on a unique targetable protein-protein interaction, may define it as the first example of a specifically druggable promoter.

Disclosure of Interest: None Declared

P167
DEMONSTRATION OF THE REGULATORY ROLE OF LEUKEMIA INHIBITORY FACTOR (LIF) AND ONCOSTATIN M (OSM) IN THE RESPONSE OF HUMAN TROPHOBLASTS TO PRO-INFLAMMATORY SIGNALS M. Ravelojaona1,*, J. Hamelin-Morrisette1, J. Girouard1, C. Vaillancourt2, C. Reyes-Moreno1

1Medical biology, University of Quebec at Trois-Rivières, Trois-Rivières, 2Institut Armand-Frappier, Centre INRS, Laval, Canada

Introduction: Over-activation of pro-inflammatory pathways at the maternal-fetal interface would be detrimental to uterine reception of the embryo but also to fetal survival, which may result in early abortion or pre-eclampsia in pregnant women [1]. Therefore, the production of anti-inflammatory factors is essential for the modulation of inflammation during pregnancy. The cytokine LIF is also a key factor in the regulation of inflammatory response and embryo implantation [2-3]. Since LIF is known to stimulate the production of OSM during implantation and the process of resolution of inflammation [1-4], we propose that LIF and OSM may be involved in the regulation of inflammation in the gestation period. Our studies aim to determine the intracellular mechanisms induced by LIF and OSM to modulate the inflammatory response of trophoblastic cells to proinflammatory gestational factors IFNγ and GM-CSF.

Methods: BeWo and HTR8/SVneo cells are two established trophoblast cell lines commonly used for studying placental function. BeWo cells are derived from a choriocarcinoma [5], HTR-8/SVneo cells are a transformed first trimester human extravillous trophoblast cell line [6]. Experimental procedures include western blot, microinvasion assays, MTT viability/proliferation assay, and human chorionic gonadotropin (HCG) dosage.

Results: To study the effect of the LIF and OSM on pro-inflammatory factors, the cells were pre-treated with either LIF or OSM for 48 hours. Subsequently, activation of the pro-inflammatory IFNγ/STAT1 and GM-CSF/STAT5 pathways were induced at increasing time intervals. Our results indicate that LIF inhibits IFNγ-induced STAT1 activation and GM-CSF-induced STAT5 activation, but inversely sustain STAT3 activation in response to IFNγ and GM-CSF. Moreover, LIF reverses the inhibitory effect of IFNγ on trophoblast motility, and reduces GM-CSF-induced HCG production by BeWo cells. OSM increases trophoblast cell proliferation and induces phosphorylation of STAT3 in human trophoblastic cells BeWo and HTR8/SVneo. In the BeWo model, a 48 h pretreatment period with OSM showed a clear reduction in IFNγ activity by a significant decrease in STAT1 phosphorylation, compared with control untreated cells.

Conclusion: These in vitro studies are the first to describe the anti-inflammatory activity of LIF and OSM in the process of trophoblast function in response to pro-inflammatory cytokines IFNγ and GM-CSF. The mechanism involved inhibition of STAT1 and STAT5 activation whilst sustained STAT3 activation.

Disclosure of Interest: None Declared

P168
CALCITRIOL INHIBITS TRIPLE NEGATIVE BREAST CANCER CELL PROLIFERATION THROUGH INDUCING THE SYNTHESIS OF IL-1 BETA AND TNF-ALPHA

I. M. Reza1,2,*, R. A. G. Becerra1,3, L. D. Nieto1, M. S. Mendoza1,4, D. B. Hernández1, F. L. Gallo1

1Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán”, 2Toxicología, 3Instituto de Investigaciones Biomédicas, México, Mexico, 4Inmunología, Instituto de Investigaciones Biomédicas, México, Mexico
Introduction: Triple negative breast cancer (TNBC) is one of the most aggressive subtypes, with poor prognosis and high metastatic capacity. Its aggressive behavior may involve inflammatory process characterized by deregulation of molecules related to the immunologic response such interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Calcitriol, as immunomodulatory agent, modulates the synthesis of these immunological mediators in different tissues. However, the regulation of IL-1β and TNF-α by calcitriol in TNBC is still little known.

Methods: Cell proliferation was evaluated in TNBC SUM-229PE and HCC1806 cells, and estrogen receptor positive MCF7 cells with different treatments to evaluate the effect of calcitriol, EB1089, IL-1β and TNF-α or the combination of calcitriol with TNF-α. The cells were treated with the antibodies anti-IL-1R1 and anti-TNFFR1 alone or in combination with calcitriol or the cytokines by the XTT method. Using Western blotting the IL-1R1, IL-1R2, TNFR1, TNFR2 and VDR protein expression was determined. Gene expression was evaluated by real time polymerase chain reaction. IL-1β and TNF-α quantification by the ELISA method. Statistical analyses were determined by one-way ANOVA followed by the Holm-Sidak method.

Results: The SUM-229PE and HCC1806 TNBC cell lines expressed receptors for vitamin D, IL-1β and TNF-α. Moreover, calcitriol, its analogue EB1089, IL-1β and TNF-α inhibited or did not change established cell lines proliferation. Also, we observed that synthesis both IL-1β and TNF-α cytokines was stimulated by calcitriol and EB1089 treatment. Interestingly, the antiproliferative activity of calcitriol was significantly reversed when the cells were treated with anti-IL-1β receptor 1 (IL-1R1) and anti-TNF-α receptor type 1 (TNFFR1) antibodies. Besides, we demonstrated that the combination of calcitriol with TNF-α resulted in a greater antiproliferative effect than with either of the agents alone in the two TNBC cell lines and an estrogen receptor positive cell line.

Conclusion: Calcitriol exerts its antiproliferative effects in part by inducing the synthesis of IL-1β and TNF-α thought IL-1R1 and TNFFR1, respectively in TNBC cells. In addition, the simultaneous treatment of calcitriol and TNF-α resulted in a greater antiproliferative effect than either compound alone, in breast cancer cells with triple negative and estrogen receptor positive phenotype.

Disclosure of Interest: None Declared

P169
IFN-G AND TNF-A SYNERGISE TO KILL INTESTINAL EPITHELIAL CELLS THROUGH A JAK1/2 DEPENDENT PATHWAY
N. Saini1*, P. Stamou1, M. Bustamante-Garrido1, P. Flood1, S. Rajaram1, J. A. Woznicki1, P. Aza-blanc1, K. Nally1
1APC Microbiome Institute, University College Cork (UCC), Cork, Ireland, 2Functional Genomics Resources, Sanford-Burnham-Prebys Medical Discovery Institute, La Jolla, CA, United States

Introduction: Inflammatory bowel diseases (IBD) consisting of Crohn’s disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders of the gut driven by multiple factors. Dysregulation of host cytokine networks is a key molecular feature associated with immunopathology to the intestinal epithelium in IBD. Specifically, the Th1 cytokines IFN-γ and TNF-α are central drivers of disease pathogenesis potentially due to their ability to independently and synergistically induce cell death of intestinal epithelial cells (IEC). Here, we investigated the molecular mechanisms underpinning killing of IEC by the combination of IFN-γ/TNF-α.

Methods: We performed an RNAi screen targeting 768 human kinases and a drug library screen of FDA approved drugs and Phase II/III compounds (approx. 2500) to identify modulators of IFN-γ/TNF-α killing in HT29 colonic epithelial cells. The top hits from these screens – JAK1/2 and JAKinibs – were validated with multiple siRNAs and a panel of JAKinibs in a panel of colonic cell lines. We analysed the role of TNFFR1 and canonical TNFFR1-associated complexes (I, Ila, Iib and ripoptosome) in IFN-γ/TNF-α induced killing using RNAi and a panel of apoptotic and necroptotic inhibitors. Involvement of the Bcl2 family and mitochondria in IFN-γ/TNF-α killing was assessed by RNAi mediated knockdown of BAK and BAX. Finally, we validated the role of JAK1/2 in IFN-γ/TNF-α mediated killing of primary human colonic organoids using JAKinibs.

Results: A human kinome RNAi screen, a drug library screen and follow-up validation work identified JAK1 and JAK2 as the main modulators of IFN-γ /TNF-α induced killing of HT29 cells. RNAi-mediated knockdown of the JAK1/2 target STAT1 also rescued the cells. Inhibitors of TNF-α-induced apoptosis or necroptosis did not block IFN-γ /TNF-α induced killing. RNAi mediated double knockdown of pro-apoptotic Bcl2 family members BAK and BAX did not block cell death. IFN-γ and TNF-α synergised to kill primary human colonic organoids and this was blocked by JAKinibs.

Conclusion: These data demonstrate that IFN-γ and TNF-α synergise to kill intestinal epithelial cells through a JAK1/2 and STAT1 dependent pathway independently of canonical TNF-α-death receptor pathways.

Disclosure of Interest: None Declared

P170
INTERLEUKIN-6 AND INTERLEUKIN-11 CLASSIC, TRANS- AND CLUSTER SIGNALING BY SOLUBLE AND MEMBRANE-BOUND HYPER-CYTOKINES
L. Lamertz1*, J. Scheller1
1Institute of Biochemistry and Molecular Biology II, Medical Faculty, Heinrich-Heine-University, Duesseldorf, Germany

Introduction: Endogenous soluble (s)gp130 and the much more potent dimerized fusion protein sgp130Fc
were originally described as specific Interleukin (IL)-6 trans-signaling inhibitors, which leave classic IL-6 signaling intact. Why sgp130Fc did not interfere with classic-signaling is completely unclear but might be due to sterical hindrance, as sgp130Fc might simply not be able to access and bind membrane-bound IL-6:IL-6R complexes.

**Methods:** We designed membrane-bound Hypercytokines, in which IL-6 or IL-11 was connected to IL-6R or IL-11R by flexible peptide linkers. Membrane-bound Hyper-IL-6/IL-11 cytokines were efficiently expressed on the surface of Ba/F3 cells, and tested for their binding to sgp130Fc as determined by flow cytometry.

**Results:** We demonstrated that sgp130Fc binds to membrane-bound IL-6:IL-6R and IL-11:IL-11R complexes. As a consequence, sgp130Fc was able to inhibit the recently described IL-6 trans-activation/cluster signaling. Moreover, we demonstrated that also IL-11 is able to activate cells by trans-activation. Moreover, autocrine IL-6 and IL-11 classic and trans-signaling in cells endogenously producing membrane-bound or soluble Hyper-cytokines were not inhibited by extracellular inhibitors such as sgp130Fc or the neutralizing gp130 antibody B-R3, highlighting mechanistical differences between autocrine and paracrine IL-6 and IL-11 signaling. Since autocrine Hyper-IL-6-induced signaling was inhibited by co-expression of sgp130Fc, our explanation for this observation is that autocrine signaling is initiated within the cell.

**Conclusion:** We have shown that sgp130Fc binds to membrane-bound IL-6:IL-6R complexes and thereby inhibits IL-6 and IL-11 trans-presentation, but does not inhibit autocrine trans-signaling or para- and autocrine classic signaling. Finally, our findings reinforce the long-standing question why sgp130(Fc) does not interfere with classic signaling.

**Disclosure of Interest:** None Declared

**P171**

**TYROSINE KINASE 2 IS NOT REQUIRED FOR INTERFERON-LAMBDA MEDIATED SIGNALING AND PROTECTION AGAINST LETHAL INFLUENZA A VIRUS INFECTION IN MICE**

D. Schneppf1,2, L. Polcik1, M. Stanifer2, A. Ohnemus1, H. H. Gad4, R. Hartmann4, B. Strob6, S. Boulan6, P. Stäheli6

1Institute of Virology, Medical Center University of Freiburg, Freiburg im Breisgau, 2Spatmann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs University Freiburg, Freiburg, Germany, 3Department of Infectious Diseases, Heidelberg University, Heidelberg, Germany, 4Department of Molecular Biology and Genetics, Structural Biology, Aarhus, Denmark, 5Institute of Molecular Genetics, University of Veterinary Medicine, Vienna, Austria

**Introduction:** Binding of interferons (IFN) to their respective surface receptors activates JAK kinases such as JAK1, JAK2 and TYK2. Upon activation these kinases mediate the phosphorylation of STAT molecules, which eventually promotes the expression of a large number of IFN-stimulated genes (ISGs) encoding antiviral resistance factors. JAK1 is an essential component of signaling complexes activated by type I (α/β), type II (γ) and type III (λ) interferons. The role of JAK2 and TYK2 is less clear. TYK2 is associated with both the IFNAR1 and the IL-10Rβ receptor chain, each forming a subunit of either the type I or type III IFN receptor complex. However, recent work with a human derived haploid cell line indicated that TYK2 was dispensable for a type III IFN mediated antiviral state, although it played a key role for type I IFN signaling.

**Methods:** Here we aimed to clarify the role of TYK2 in the signaling pathways of type I and type III IFNs using primary airway epithelial cells and primary mini-gut organoids derived from mice harboring defective or functional alleles for Tyk2. We stimulated these primary cultures with increasing amounts of the respective IFNs and measured the transcriptional induction of several ISGs via RT-qPCR. Further we performed survival experiments with mice expressing or lacking Tyk2 which were treated with saline, IFN-α or IFN-λ prior to an infection with a lethal dose of a highly virulent variant of the influenza A virus strain PR8.

**Results:** Primary airway epithelial cells derived from the trachea of mice as well as mini-gut organoid cultures readily responded to IFN-λ by upregulating ISG expression irrespective of whether they carried functional or defective Tyk2 alleles. In contrast, IFN-α mediated ISG induction was severely diminished in cells with no functional TYK2. When mice were treated with IFN-α or IFN-λ before intranasal infection with a lethal dose of influenza A virus, IFN-λ efficiently protected from disease irrespective of whether the mice carried functional or defective Tyk2 alleles. In contrast, IFN-α was only protective in animals carrying a functional Tyk2 gene, while Tyk2-deficient animals succumbed to the infection.

**Conclusion:** Tyk2 deficiency severely limits IFN-α signaling but does not affect IFN-λ mediated ISG induction in respiratory and intestinal epithelial cells or protection against a lethal infection of the respiratory tract.

**Disclosure of Interest:** None Declared

**P172**

**RE-VISITING THE MOLECULAR BASIS FOR TYPE I INTERFERON SIGNALING USING CRISPR KNOCKOUTS**

G. Schreiber1,2, V. Urni1, M. Shemesh1

1Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel

**Introduction:** Type I interferons (IFN-1) are best known for their role in innate immunity, but they are also involved in other functions including immunomodulation, restricting proliferation, cancer surveillance, and the regulation of the adaptive immune response. All these responses are mediated through the interaction with a single cell surface receptor, albeit at different ligand and receptor concentrations, ligand subtypes, and time of activation. This functional plasticity of IFN signaling is a result of variations in different ingredients of the system, whether
controlled or stochastic. This leads to differential IFN-1 responses, which are important for cells to maximize efficiency while minimizing detrimental effects.

**Methods:** To investigate the molecular mechanism of IFN-1 signaling we generated a string of knockout (KO) cell lines on the basis of HeLa wild-type cells. Together with reconstitution of the deleted genes, functional assays and RNAseq these provided us with a clear view on the basic requirements of IFN-1 for signaling.

**Results:** In HeLa cells both IFNAR1 and IFNAR2 receptors are absolutely required to obtain any IFN-1 induced signaling. The KO of one of the receptors completely eliminated phosphorylation of downstream activators, gene induction (as determined on a genome wide level by RNAseq) and any observed phenotype. Reconstitution experiments using mutated receptors shows that no single tyrosine on IFNAR1 eliminates the activity, but removal of the Tyk2 binding site does. The deletion of either STAT1 or STAT2 had only a partial effect on the IFN-1 induced antiviral activity or gene induction. Conversely, the deletion of both genes completely abrogated any IFN-1 induced activity. So did also the double STAT2-IRF1 KO, which suggest that in the absence of STAT2, signaling proceeds through the activation of IRF1 by the STAT1 homodimer. Knockdown of IRF9 on background of STAT1 KO eliminates most residual gene induction observed in the STAT1 KO, suggesting that STAT2-IRF9 dimer is the main signaling pathway in the absence of STAT1. The KO of either STAT1, STAT2, STAT3 or STAT1-STAT2 double KO had no effect on the phosphorylation of any of the other STATs, thus their phosphorylation is not dependent on the binding of other STATs to the receptors. As the STAT1-STAT2 KO did not promote any IFN-1 induced activity despite normal phosphorylation of STAT3 and STAT6 it questions their role in this system. This was verified by the STAT3 KO, which had no effect on IFN-1 activation.

**Conclusion:** IFN-1 signaling in human HeLa cells is promoted by the binding of IFN-1 to IFNAR1 and IFNAR2, which promotes their dimerization. This is required for any IFN-1 induced activity. IFN-1 induced signaling goes only through STAT1 and STAT2, although not both are required. No function for activated STAT3 was observed in this system.

**Disclosure of Interest:** None Declared

**P173**

**THE ROLE OF TYPE I INTERFERON RECEPTORS IN MEDIATING DIFFERENTIAL ACTIVITY**

_**M. Shemesh**1, G. Schreiber*
  1Weizmann Institute Of Science, Rehovot, Israel

**Introduction:** Type I interferons are a family of cytokines central to the activation of innate immunity and to the connection between innate and acquired immunity. This family has 16 members in human, all bind the same cell surface receptors – IFNAR1 and IFNAR2. Though all can initiate an anti-viral response (robust response), only some members are capable of initiating other response, such as control of cell proliferation or immunomodulation (tunable response). How this differential activity is possible through binding the same receptors? Here, I investigated the cytoplasmic domain of the receptors to identify regions driving differential activity.

**Methods:** To this end, we created IFNAR1 and IFNAR2 knocked-out (KO) HeLa cells and characterized them. In addition we created mutated versions of the receptors and transfected them into the KO cells. We assessed the IFN activity in each condition by using qPCR for genes expression levels and western blot for STATs phosphorylation levels.

**Results:** In HeLa cells both IFNAR1 and IFNAR2 receptors are absolutely required to obtain any IFN-1 induced signaling. The KO of one of the receptors completely eliminated phosphorylation of downstream activators, gene induction (as determined on a genome wide level by RNAseq) and any observed phenotype. Reconstitution experiments using mutated receptors shows that no single tyrosine on IFNAR1 eliminates the activity, but removal of the Tyk2 binding site does. The deletion of the membrane proximal area on IFNAR1 (AA 463 – 483) or the membrane distal area on IFNAR2 (Δ365) resulted in the expression of only robust genes (though decreased), but no expression of the tunable genes. STAT1/2 phosphorylation was also decreased. These two areas are suspected to be STAT2 binding sites.

**Conclusion:** We suspect that the mediator for the IFN differential signaling is STAT2. We therefore concentrate our current efforts to elucidate its temporal role in IFN activity and its binding partners.

**Disclosure of Interest:** None Declared

**P174**

**CYTOKINES BOOST TRANSCRIPTIONAL OUTPUT IN NK CELLS VIA CHROMATIN REMODELING AND RAPID REDISTRIBUTION OF TRANSCRIPTION FACTOR BINDING**

_H.-Y. Shih1, G. Sciume2, J. O'Shea*
  1NIAMS, NIH, Bethesda, United States

**Introduction:** Innate lymphoid cells defend the host against invading pathogens through rapid boost of immune responses that require precise programming. Previous literatures, including ours, have shown that innate lymphoid cells developmentally acquire defined chromatin landscapes that are poised for instant firing. However, how external stimuli trigger the switches of genes from poised to active states in a short period remains puzzling.

**Methods:** To investigate the cytokine-mediated chromatin remodeling for rapid gene induction in innate lymphoid cells, we treated murine NK cells with interleukin -2 and -12 for six hours followed by ATAC-seq and ChIP-seq analysis. In ChIP analysis, we targeted both lineage-determining and signal-dependent transcription factors, as well as enhancer and promoter markers including histone acetyltransferase p300, H3K27 acetylation, H3K4 mono-, di- and tri-methylation. To understand the relationships between epigenomic changes and
transcriptomic output, we also performed RNA-seq for NK cells under the same condition. The high-throughput sequencing data were further integrated and digested by stringent analytic protocol.

**Results:** Using computational approaches we identified rapid formation of super-enhancers (SEs) that facilitate the high magnitude of gene induction in NK cells upon cytokine stimulation. Such dramatic chromatin remodeling events involve the composition of de novo enhancers (~25%) on top of activating poised enhancers those are primed during development. Notably, these neo-synthesized enhancers are enriched with motif sequences recognized by signal-dependent transcription factors (e.g. STATs) rather than lineage-determining transcription factors (e.g. T-bet), even though these regions recruit both types of molecules.

**Conclusion:** Our data revealed distinct regulatory circuits for lineage-determining genes and rapidly inducible genes and argued hierarchical roles of lineage-determining and signal-dependent transcription factors in regulation of different types of enhancers and transcriptional activities.

**Disclosure of Interest:** None Declared

---

**P176**

**SERINE 727 PHOSPHORYLATION OF STAT1 MODULATES A TYPE I INTERFERON DRIVEN NEUROINFLAMMATORY DISEASE**

P. Songkhunawej1,2,*, M. Hofer1

1School of Life and Environmental Sciences, The University of Sydney, Sydney, Australia

**Introduction:** While the central nervous system (CNS) is considered an immune privileged site, many resident cells including astrocytes and microglia are able to respond to a number of cytokines such as interferon alpha (IFN-α). IFN-α is critical for a normal antiviral response in the CNS. However, chronic production of IFN-α has been implicated in a number of neurological disorders such as Aicardi-Goutières syndrome, Cree encephalitis, and congenital calcification which lead to premature death. Transgenic mice with astrocyte targeted production of IFN-α (GIFN mice) are the only existing mouse model that recapitulates the only existing mouse model that recapitulates the characteristics of IL-6 in naïve, and identifies a regulatory role for cytokine mediated neuroinflammatory diseases.

**Disclosure of Interest:** None Declared

---

**P177**

**PROTEIN TYROSINE PHOSPHATASES ACT AS RHEOSTAT REGULATORS OF STAT1 ACTIVITY IN CYTOKINE TREATED MEMORY CD4 T-CELLS.**

J. P. Twohig1,2,*, A. C. Figueras1,2, R. Andrews3, B. C. Cossins1,2, A. D. Soria1,2, D. Hill1,2, J. U. Fernandez1,2, D. Millrine1,2, N. M. Williams3, T. Tiganis4, G. W. Jones1,2, S. A. Jones1,2

1Dept of Infection and Immunity, School of Medicine, 2System Immunity Research Institute, School of Medicine, 3Division of Psychological Medicine and Clinical Neurosciences, School of Medicine, Cardiff, 4Department of Biochemistry and Molecular Biology, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, United Kingdom

**Introduction:** As a lymphokine interleukin-6 (IL-6) controls T-cells recruitment, activation, differentiation and proliferative survival. These properties are primarily controlled by the latent transcription factors STAT1 and STAT3. While naïve CD4 T-cell express the IL-6 receptor, activated T-cells isolated from sites of inflammation display altered IL-6 receptor expression and show impaired STAT1 phosphorylation following cytokine stimulation. In this study, we have combined transcriptomic approaches and chromatin-immunoprecipitation sequencing (ChiP-seq) to compare the transcriptional profile of IL-6 in naïve, activated and memory CD4 T-cells. These investigations show that prior T-cell activation re-tunes the signaling characteristics of IL-6, and identifies a regulatory role for

---

*Disclosure of Interest:* None Declared
protein tyrosine phosphatases in controlling IL-6 driven STAT1 signaling in activated and memory CD4 T-cells. **Methods:** Purified resting and activated CD4 T-cells in a series of differentiation states from WT, Il6ra<sup>−/−</sup>, Ptpn22<sup>−/−</sup> and Lck-Cre/Ptpn2<sup>−/−</sup> mice were examined. Cytokine receptor signaling was monitored by intracellular flow cytometry, imaging flow cytometry, qPCR and immunohistochemistry of inflamed tissues. Transcriptomic and ChIP-seq analysis were combined with bioinformatic and molecular pathway analysis to evaluate gene expression changes and associated transcriptional control mechanisms.

and associated transcriptomic control mechanisms. **Results:** Whilst IL-6 driven STAT3 activation was a stable characteristic of CD4<sup>+</sup> T cells, prior TCR stimulation induced epigenetic changes, which restricted cytokine-induced STAT1 phosphorylation in activated and memory T cells. This level of STAT1 regulation is controlled by protein tyrosine phosphatases that were selectively induced following naïve CD4 T-cell activation. Specifically, PTPN2 (and to a lesser extent PTPN22) were found to bind STAT1 and restricted the tyrosine phosphorylation of STAT1 in activated and memory CD4 T-cells. This mechanism accounted for an alteration in the transcriptional profile of IL-6 responses in effector memory T-cells, and illustrated how changes in the regulation of STAT1 modified the IL-6/STAT3 control of cell identity, immune function, proliferative survival and metabolism. **Conclusion:** Naïve T-cell activation re-tunes STAT1 signaling to deliver unique cytokine responses in memory CD4 T-cells. Such alterations in cytokine responsiveness may explain why naïve, activated and memory T-cells display fundamental differences in proliferation, survival, sensitivity to antigen, and alterations in T-cell homing to lymphoid tissues.

**Disclosure of Interest:** None Declared

---

**P179**

**TUMOR NECROSIS FACTOR ALPHA AND INTERLEUKINS IL-1, IL-4, IL-6, IL-7, IL-8, IL-10, AND IL-12 WITH COMPLEX INFLAMMATORY, ANTI-INFLAMMATORY AND REGULATORY FUNCTIONS ARE CONTRIBUTING TO THE PATHOGENESIS OF COPD IN LUNG Airways**

Z. Vitenberga<sup>1</sup>, M. Pilmane<sup>1</sup>, A. Babjoniseva<sup>2</sup>

<sup>1</sup>Institute of Anatomy and Anthropology, Department of Morphology, Riga Stradins University, <sup>2</sup>Pauls Stradins Clinical University Hospital, Riga, Latvia

**Introduction:** Chronic obstructive pulmonary disease (COPD) is a progressive disease leading to airway luminal obstruction and airflow limitations. Intercellular signalling networks with high complexity, interrelations and overall wide distribution of various cytokines and chemokines cause a spectrum of mechanisms achieving COPD. Numerous biomarkers from investigations of non-invasive samples have been evaluated in most of the COPD studies, whereas the local findings are limited due to the invasive nature of procedures to acquire airway biopsies and tissue samples. Hereby local findings within the airway wall provide valuable information and support the relevance of biomarkers in the pathogenesis of COPD. The aim of this study was to determine the local distribution of cytokines and chemokines in COPD affected lung tissue and to compare findings with control group.

**Methods:** In COPD group, lung tissue specimens were obtained during flexible bronchoscopy from 37 patients. In control group, lung tissue material obtained during an autopsy from 49 healthy control subjects was evaluated. Tissue samples were examined by haematoxylin and eosin staining. Immunoreactive cells positive for interleukins (IL) IL-1α, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, and tumor necrosis factor-α (TNF-α) were detected by biotin-streptavidin immunohistochemistry method.

**Results:** Overall higher numbers of IL-7, IL-8 and IL-10 (mostly from few (0/+)) to almost abundance (+++/+++) and overall less numbers of IL-1α and IL-6 (mostly from no positive (0) to numerous to abundance (+++/++++)) immunoreactive cells were marked in airway bronchial epithelium and mucosal connective tissue of COPD affected lung. Furthermore, statistically significant (P < 0.05) higher numbers of immunoreactive cells located in COPD group airway epithelium for IL-1α, IL-4, IL-6, IL-7, IL-8, and IL-10 were evaluated. No statistically significant difference between the numbers of IL-12 and TNF-α immunoreactive cells was observed in epithelium and connective tissue of COPD affected lung. In comparison with the control group, we found statistically significant (P < 0.05) higher numbers of immunoreactive cells positive for TNF-α and all examined interleukins in COPD group.

**Conclusion:** Increased numbers of IL-1α and IL-10 immunoreactive cells within the background of IL-4, IL-6, IL-7, IL-8, IL-12, and TNF-α supports the extensive presence and correct immune response in COPD. Moreover, the pattern with dominance of immunoreactive cells in COPD affected airway epithelium over connective tissue is highlighting the essentials of epithelium in inflammatory signalling. The cytokine IL-4, IL-6, IL-7, and IL-10 distribution and appearance suggest persistent stimulation of cytokine expression in lung tissue and indicate the significance of local modulating and regulatory pattern in COPD, whereas IL-1α, IL-8, IL-12, and TNF-α – pro-inflammatory pattern.

**Disclosure of Interest:** None Declared

---

**P179**

**MAPPING THE INTERLEUKIN-27 SIGNALOSOME – FROM CELL SURFACE RECEPTORS TO T-CELL DIFFERENTIATION**

S. Wilmes<sup>1</sup>, M. Hafer<sup>2</sup>, J. Martinez-Fabregas<sup>1</sup>, E. Pohler<sup>1</sup>, P. Fyfe<sup>1</sup>, C. Gorby<sup>1</sup>, J. Piehler<sup>2</sup>, I. Moraga-Gonzalez<sup>1</sup>

<sup>1</sup>School of Life Sciences, Division of Cell Signalling and Immunology, University of Dundee, Dundee, United Kingdom, <sup>2</sup>Department of Biology/Chemistry, Division of Biophysics, University of Osnabrück, Osnabrück, Germany
Introduction: A hallmark of cytokine biology is functional selectivity - the ability to elicit differential cellular responses through the same cell surface receptor. IL-27 and IL-6 are two clear examples of this, both of them key players for regulation of inflammatory responses. The heterodimeric cytokine IL-27 (p28 & EBI3) engages a cell surface receptor formed by IL-27Ra and gp130, triggering the activation of JAK1/JAK2 and STAT1/STAT3. This results in an anti-inflammatory phenotype spearheaded by production of IL-10, differentiation of Tregs and inhibition of Th-17 responses. IL-6, on the other hand, engages a hexameric complex comprised of two copies each of gp130, IL-6Ra and IL-6, which triggers activation of JAK1 and STAT1/STAT3. In contrast to IL-27, IL-6 is considered a paradigm cytokine for pro-inflammatory responses, including promotion of Th-17 cells and suppression of Tregs. How activation of the same signaling pathways by these two cytokines through a shared receptor (gp130) results in opposite biological responses is not well defined.

Methods: How do IL-27/IL-6 early signaling events ultimately lead to opposite cellular decisions on T cells? We have used a multidisciplinary approach encompassing single-molecule fluorescence imaging, detailed signaling studies via high-throughput phospho-flow cytometry, in vitro differentiation of human CD4+ and high resolution proteomic studies to cover the multiple layers of signaling.

Results: We performed a series of signaling studies in human naïve CD4+ and CD8+ T cells to study the kinetics of STAT1/STAT3 phosphorylation. While IL-6 and IL-27 activated very similar profiles of STAT3, IL-27 stimulation led to a stronger and more sustained activation of STAT1 than IL-6. This is compatible with the observation that mainly STAT1 is responsible for the signaling differential between IL-27 and IL-6 (1). To gain mechanistic insight into the preferential activation of STAT1 by IL-27 we performed dual-colour fluorescence imaging studies. Correlated diffusion of IL-27Ra and gp130 was only observed upon stimulation with IL-27, indicating the formation of a receptor hetero-dimer, in line with the model of ligand-induced receptor assembly. Similar results were found with IL-6, suggesting that receptor dynamics was not the source of STAT1 differential activation. Next, we mutated tyrosines present in the IL-27Ra intracellular domain. The mutant Y613F almost completely abrogated the pSTAT1 response, indicating that this tyrosine is the main residue for STAT1 activation. To understand the functional consequence of the more sustained STAT1 activation by IL-27, we cultured human CD4+ T cells under Treg or Th-17 polarizing conditions. While IL-6 stimulation resulted in expansion of TH17 cells and suppression of Tregs, we observed the opposite for IL-27.

Conclusion: We conclude that the functional selectivity of IL-27 towards anti-inflammatory responses must be encoded by the stronger and more sustained activation of STAT1. The molecular basis for this functional selectivity needs to be elucidated by further experiments.

Disclosure of Interest: None Declared

Cytokines 2018 – Abstracts

P180
UTX IS AN EPIGENETIC REGULATOR OF IFN-GAMMA AND TNF-ALPHA SYNERGY IN INTESTINAL EPITHELIAL CELLS
J. A. Woznicki1,*, J. Velmurugan1, P. Flood1, M. Bustamante-Garrido1, M. Aguileran1, V. Rossoni1, M.-L. Hammarström2, E. Brint1, A. Houston1, F. Shanahan1, S. Melgar1, K. Nally1
1APC Microbiome Ireland, University College Cork, Cork, Ireland, 2Department of Clinical Microbiology, Umeå University, Umeå, Sweden

Introduction: Inflammatory bowel diseases (IBD) consisting of Crohn’s disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders of the gut driven by multiple, often co-existing factors. Dysregulation of host cytokine networks is one of the key molecular features associated with immunopathology in these heterogeneous conditions. Pro-inflammatory Th1 cytokines IFN-γ and TNF-α occupy critical nodes within these networks, potentially due to their ability to synergistically boost mucosal inflammation and death of intestinal epithelial cells (IEC). Here, we investigated whether histone methylation contributes to generation of these disease-relevant responses to IFN-γ/TNF-α in human IEC.

Methods: We performed an RNAi perturbation screen targeting 81 human histone methyltransferases (HMT) and demethylases (HDM) to identify modulators of CXCL10 secretion and cell death induced by IFN-γ/TNF-α in HT-29 colonic epithelial cells. The top hit – UTX – was validated by siRNA pool deconvolution and re-screened in a panel of colonic cell lines. We analysed the global effect of UTX knockdown on the transcriptional response to IFN-γ/TNF-α by RNA-Seq and confirmed key results by RT-qPCR. To further validate UTX, we tested the effect of two small-molecule catalytic inhibitors of UTX methylstat and GSK-J4 – on expression of UTX-regulated genes. We investigated the efficacy of GSK-J4 in a DSS-induced colitis mouse model of IBD. Additionally, we studied expression of UTX and its regulated chemokines in colonic pinch biopsies and purified enterocytes from patients with inactive/active UC. Finally, we tested the effect of GSK-J4 on inflammatory responses of ex vivo anti-CD3/28-activated UC biopsies and primary human colonic organoids treated with IFN-γ/TNF-α.

Results: An HMT/HDM-focused RNAi screen identified the H3K27 demethylase UTX/KDM6A as a positive regulator of synergistic induction of CXCL10 by IFN-γ/TNF-α in human IEC. RNA-Seq of UTX-depleted cells coupled with gene set enrichment and pathway analyses revealed that UTX is required for expression of T-cell chemokines and anti-viral genes in response to IFN-γ/TNF-α. This UTX-dependent transcriptional effect was partially phenocopied by methylstat and GSK-J4, two small-molecule inhibitors of UTX demethylase function. In a pre-clinical model of IBD, GSK-J4 attenuated DSS-induced colitis in mice. In human IBD, the mRNA/protein expression of UTX was reduced in whole biopsy tissue from patients with active UC compared to non-IBD.

Disclosure of Interest: None Declared
individuals. UTX levels were, however, significantly increased in a purified enterocyte population from UC tissue and they positively correlated with high T-cell chemokine expression in active UC biopsies. Finally, GSK-J4 reduced secretion of the same T-cell chemokines in ex vivo anti-CD3/28-activated biopsies from active UC. Within a non-cytotoxic window, the inhibitory effect of GSK-J4 on UTX-dependent genes was lost in IFN-γ/TNF-α-treated human colonic organoids.

Conclusion: We identified UTX as an epigenetic regulator of IFN-γ/TNF-α synergy in IEC and highlight its potential function in shaping Th1 immune responses in the gut via regulation of T-cell chemokine expression.

Disclosure of Interest: None Declared

P181
CHARACTERIZATION OF A SMALL PEPTIDE WITH UNKNOWN FUNCTION THAT IS LIKELY INVOLVED IN THE HOST INNATE AND ADAPTIVE IMMUNE RESPONSE
H. Young1, N. Tarasova1, M. Sanford1, W. Hu2
1NCI AT FREDERICK, Frederick, 2Mayo Clinic, Rochester, United States

Introduction: Analysis of gene expression in a mouse model of chronic inflammation (based on persistent IFN-g gene expression) has revealed high expression of a gene of unknown function. The gene, located on mouse chromosome 19, is only found in mouse and rats although functional homologues in other species cannot be ruled out. Originally classified as a long non-coding RNA, this gene has been found to have an open reading frame of 78 amino acids.

Methods: Utilizing an antibody derived against a portion of the gene, we have confirmed via Western blot, that the peptide is likely expressed.

Results: Structural analysis of the peptide has not revealed significant similarity to any known proteins although the structure does suggest that it may be excreted. Based on RNA expression analysis, the gene appears to be most highly expressed in NK cells and cells in the myeloid/DC lineage and is more highly expressed in mature but not naïve T cells. In addition, it is inducible by IFN and LPS and Type 1 IFN appears to be required for enhanced IFN-g induced expression in a mouse macrophage cell line.

Conclusion: A KO mouse has been derived and we are currently characterizing the impact of gene deletion on host immune function. Based on RNA seq data from a number of different sources, we believe that this peptide has a role in both the murine innate and adaptive immune responses.

Disclosure of Interest: None Declared

P181.A
DISSECTING IMMUNE CELL VARIABILITY IN CYTOKINE SIGNALLING BY SINGLE CELL MASS CYTOMETRY AND COMPUTATIONAL MODELLING
R. Mukherjee1,2*, G. Altan-Bonnet1

Introduction: Signalling via cytokines is the primary mode of communication between cells of the immune system. Principal among cytokine signalling pathways is the JAK-STAT signalling cascade. Mammalian cells have seven STAT proteins, namely STATs 1, 2, 3, 4, 5a, 5b, and 6. Current understanding of the biology of STAT response revolves around the notion of a select group of cytokines specifically activating a particular STAT which, in turn leads to the transcription of a class of effector molecules in subsets of responding cells. In the present study, we employed single cell mass cytometry coupled with machine learning algorithms to probe variabilities in JAK-STAT signalling response between immune cell types.

Methods: Harvested splenocytes from adult female C57BL/6 mice were stimulated in vitro with recombinant cytokines. The cells were then fixed, permeabilized and stained with heavy-metal isotope tagged antibodies against cell-surface and pSTAT antigens. The cells were then analysed on a Helios CyTOF mass cytometer.

Results: Our results indicate huge diversity in pSTAT response among immune cell types. We demonstrate that all cytokines lead to activation of all pSTATs, but at different levels which is again highly variable among different immune cells. Interestingly, there seems to be a clear dichotomy among immune cells with respect to preferential activation of either pSTAT1 or pSTAT4. Partial least squares discriminant analysis (PLS-DA) revealed that a cocktail of all cytokines elicited quantitative and qualitatively different responses than the individual responses added together in some cell types, suggesting that the combined action of a group of cytokines is not equal to a linear combination of responses elicited by individual cytokines. Moreover, observing the factor weights across the two latent variables revealed that pSTATs 3, 4, and 5 had opposite signs to that of pSTAT1 on Latent Variable 1, the axis which best reflected the differences in cytokine treatment among cell types. This hints at possible preferences among immune cells to respond in a particular way that may be encoded in the phenotypic features of the cell itself.

Conclusion: Our next efforts are targeted at obtaining a mechanistic understanding of the observed differences in STAT phosphorylation between immune cell types. By extending the findings of this study to a large number of possible cytokine combinations, we plan to develop a quantitative model of the network of cellular interactions within the hematopoietic compartments in both mice and humans.

Disclosure of Interest: None Declared
P181.B
MODULATION OF TREG BY IL-2 AS A REVIVED THERAPEUTIC STRATEGY FOR AUTOIMMUNE DISEASES
L. Xu1, X. Song1, L. Su1, Y. Zheng2, J. Sun1.∗
1School Of Pharmaceutical Science & Technology, Tianjin University, Tianjin, 2State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing, China

Introduction: Interleukin 2 (IL-2) is a multifunctional cytokine that keeps the homeostasis of T cells in immune system. The mechanism is that at low-dosage, IL-2 can promote the development and maintenance of regulatory T cells (Treg) which is critical to immune tolerance, while enhance the functions of effector T cells (Teff) at high-dosage. Treg is sensitive to low level of IL-2 because Treg expresses high-affinity IL-2 receptors (IL-2Rαβγ) constitutively. Currently, clinical trials demonstrate that low-dosage IL-2 selectively expands Treg without much impact on Teff. Therefore, administration of low-dosage IL-2 is considered as a new therapeutic strategy for autoimmune diseases. In addition, antibodies against IL-2 and IL-2 muteins have also been demonstrated to selectively expand Treg. Therefore, IL-2, IL-2-antibody and IL-2 muteins are three strategies of IL-2 therapy.

Methods: The functional deficiency of Treg cells is tightly associated with autoimmune diseases. Clinical trials of low dosage of IL-2 have also improved the symptoms of patients with autoimmune diseases. We will review the processes of IL-2 therapy as a revived strategy for autoimmune disease treatments.

Results: Application of low dosage of IL-2 has been demonstrated in many autoimmune disease treatments. Patients with SLE were administered recombinant human IL-2 at a dose of 1 million IU for 3 cycles, once every two days for 2 weeks. Patients with T1D were administered IL-2 at 3 MIU/day for a 5-day course, and followed up for 60 days. Other diseases like Chronic graft-versus-host disease (GVHD), Hepatitis C virus-related autoimmunity, Alopecia Areata (AA) and immune thrombocytopenia (ITP) have also been treated with low-dosage IL-2 in clinical trials.

Antibodies against IL-2 is another strategy for autoimmune disease treatments. JES6-1, an antibody against mouse IL-2 can blocks IL-2/IL-2Rα interaction and selectively expand T cells with high-expression of IL-2Rα. Application of IL-2/JES6-1 complexes proved to expand Treg selectively, result in a normal balance of the Teff:Treg ratio in animal model. Based on these data, IL-2/antibody complexes could prove valuable for the treatment of autoimmune disorders.

IL-2 muteins can be designed to stimulate Treg selectively by enhancing the affinity for IL-2Rα or reduce the affinity for IL-2Rβγ. AMG 592 is an investigational IL-2 mutein designed for expanding Treg selectivity. It increases the Treg: Teff ratio with no directional change in NK cell numbers and minimal increase in Teff.

Conclusion: Clinical trials have demonstrated that low-dose of IL-2 can expand and maintain Treg in patients with autoimmune diseases like SLE and T1D. The application of IL-2, its muteins and IL-2/antibody complex will become new strategies in the treatment of autoimmune diseases.

Disclosure of Interest: None Declared

P181.C
SIMULTANEOUS QUANTIFICATION OF INFLAMMATORY DISEASE BIOMARKERS USING COMMON LAB FLOW CYTOMETERS
P. Rughwani1, B. Sun1, J. Lehmann1, S. Ji1, W. Jiang1.∗
1BIOLEGEND INC., San Diego, United States

Introduction: Inflammation is associated with a vast variety of human diseases ranging from cardiovascular, pulmonary, neurodegenerative, and autoimmune diseases. A large number of soluble receptors, growth factors, cytokines, chemokines and other factors possess pro-inflammatory and/or anti-inflammatory properties and are important players in immune-regulation. They are pivotal in inflammatory processes and serve as promising inflammatory disease biomarkers.

Methods: We have developed a new LEGENDplex™ Human Inflammation Panel 2 (13-plex) targeting inflammation and immune response biomarkers involved in inflammatory and autoimmune diseases, using fluorescence-encoded beads that are suitable for use on general lab flow cytometers. The panel allows simultaneous quantification of 13 related analytes, namely TGF-β1, PAI-1, sTREM-1, PTX3, sCD40L, sCD25 (IL-2Ra), CXCL12 (SDF-1), sST2, sTNF-RI, sTNF-RII, sRAGE, CX3CL1 (Fractalkine), sCD130 (gp130).

Results: Each antibody pair was carefully optimized for assay specificity, sensitivity, accuracy and reproducibility. The 13-plex panel has been validated by detecting expected changes in biological samples.

Conclusion: Together with the previously released LEGENDplex™ Human Inflammation Panel that measures IL-1β, INFα2, INF-γ, TNF-α, MCP-1 (CCL2), IL-6, IL-8 (CXCL8), IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33, the high quality, low cost and ease of use panel will provide an excellent multiplex solution to enable legendary discovery in inflammation and associated processes.


P182
PYROPTOSIS IS A NOVEL MECHANISM OF IL-33 RELEASE FROM AIRWAY EPITHELIAL CELLS
O. Bernard1; M. Lachowicz-Scroggins1, L. Sharp2, E. Gordon2
1PCCM-SABRE, UCSF, 2PCCM-SABRE, Ucsf - Gordon Lab, San Francisco, United States

Introduction: IL-33 is among the most replicated asthma-associated genetic loci, and in mice, this secreted cytokine promotes the development of type 2 inflammation. However, uncertainty remains about the basic biology of
this important cytokine. IL-33 is robustly expressed in the human airway, but without a signal sequence and a nuclear localization, it remains unknown how IL-33 is secreted. We have previously demonstrated that multiple splice variants are expressed in human airway epithelial brushings. Interestingly, only one transcript is associated with airway type 2 inflammation in a cohort of non-exacerbating asthmatics. This IL-33 variant, lacking exons 3 and 4 (Δ exon 3,4 IL-33), confers cytoplasmic localization and facilitates extracellular secretion upon ionomycin stimulation, contrary to full-length IL-33.

We hypothesize that, similar to other IL-1 family members, secretion of Δ exon 3,4 IL-33 by airway epithelial cells may rely on a recently described cell-death pathway called pyroptosis. Induced by intracellular lipopolysaccharide, this pathway activates inflammatory caspases (-4 and -5 in humans) which results in the activation of the pore-forming protein gasdermin D. It is through interaction with membrane phosphoinositides, PtdIns(4,5)P2 and PtdIns(4,5)P2, that gasdermin D facilitates the extracellular release of IL-1 beta.

Methods: A human lung epithelial cell line, Beas2B, was cultured in vitro and transfected using lentivirus to overexpress full length IL-33 or the Δ exon 3,4 IL-33 variant, tagged with GFP. Cells were then exposed to media, LPS, LPS + Fugene, and Fugene alone. Supernatant was collected after 16 hours and GFP was measured in the conditioned media (as a surrogate for IL-33 release). Purified Δ exon 3,4 was used for protein-lipid overlay assay using PIP strips.

Results: Airway epithelial cells that overexpress Δ exon 3,4-IL-33 robustly secrete IL-33 in response to pyroptosis induced by intracellular LPS. Extracellular LPS and Fugene alone do not induced IL-33 secretion. Full-length IL-33 is not secreted in response to pyroptotic stimuli in airway epithelial cells. Moreover, we find that Δ exon 3,4 IL-33 binds strongly to membrane phosphoinositides, including those bound by gasdermin D.

Conclusion: Together these data support a novel mechanism for release of IL-33 from airway epithelial cells. First, full length IL-33 transcript must be spliced to form the Δ exon 3,4-IL-33 transcript. Then, pyroptosis results in IL-33 secretion. This pathway may be facilitated by activated gasdermin D binding to IL-33 and membrane phosphoinositides.

Disclosure of Interest: None Declared

P183

TYPE 2 INFLAMMATORY CELLULAR IMMUNE MECHANISMS IN CANINE ATOPIC DERMATITIS

S. Frueh1*, J. Eule1, M. Saikia1, L. M. Webb1, O. Oyesola1, D. Shiroor1, R. L. Cubitt1, M. G. Castelhano2, W. H. Miller1, E. D. Tait Wojno1

1Baker Institute for Animal Health and Department of Microbiology and Immunology, Cornell University College of Veterinary Medicine, Ithaca, United States

Introduction: Canine atopic dermatitis (AD) is a multifactorial, chronic, pruritic skin disease with high prevalence that is associated with significant morbidity. This disease bears striking similarities to human AD, suggesting that the dog is an excellent naturally occurring AD disease model. Excessive immunological activation in response to normally innocuous environmental antigens is characteristic of canine AD, but the mechanisms underpinning allergic conditions in dogs are still unclear. Studies in murine models and humans show that allergic disease is associated with a Type 2 inflammatory response in which CD4+ T helper Type 2 (Th2) cells and group 2 innate lymphoid cells (ILC2s) produce the Type 2 cytokines interleukin (IL)-4, IL-5, and IL-13 to drive disease. However, the significance of these cell types and factors in canine AD has not been fully investigated. We hypothesize that Th2 cells and ILC2s orchestrate allergic reactions in dogs and can be found systemically in allergic subjects.

Methods: To test this hypothesis, we recruited healthy, non-allergic control dogs and age- and sex-matched allergic dogs. For each dog, we collected extensive clinical information and utilized novel flow cytometric assays to analyze blood samples for the systemic immune cell phenotype. Differences in the allergic vs. control groups were analyzed by parametric comparison while controlling for sex, age and time of blood draw. In addition, FACS-purified canine CD4+ T cells were subjected to a novel in vitro T helper subsets polarization assay to investigate the factors and cytokines that promote polarization and activation of canine Th2 cells.

Results: We observed a significant increase in Th2 cells, identified by staining for the transcription factor GATA3, in the blood of allergic dogs compared to non-allergic controls. We also report for the first time the existence of a canine cell type that shares characteristic markers with human and mouse ILC2s, though no significant differences were observed in ILC2 populations between healthy and allergic dogs. Purified dog CD4+ T cells could be activated and induced to produce effector cell cytokines characteristic for T helper cell subsets Th1, Th2 and Th17, providing a tool to study additional factors that contribute to effector cell functions of canine T cells.

Conclusion: Together, our data suggest that Th2 cells are associated with canine allergic disease and may play an important role in disease pathogenesis. We are currently working to assess Th2 cell functionality and gene regulation using flow cytometry and in vitro assays including single-cell RNA transcriptomic profiling of allergic dogs of a single breed. This study provides better understanding of the immunological mechanisms that underlie atopic conditions in canine patients, further characterizing the dog as a disease model for human allergies and will informing the development of new diagnostic tools and improved treatments for dogs and humans.

Disclosure of Interest: None Declared
P184
AMPHIREGULIN-PRODUCING PATHOGENIC MEMORY T HELPER-2 CELLS DRIVE AIRWAY FIBROSIS VIA ACTIVATION OF EOSINOPHILS

K. Hirahara1,2,*, Y. Morimoto1, M. Kiuchi1, M. Okano1, K. Kokubo1, A. Onodera1, D. Sakura3, Y. Okamoto3, T. Nakayama1
1Department of Immunology, Graduate School of Medicine, Chiba University, 2AMED-PRIME, AMED, 3Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan

Introduction: Memory helper T (Th) cells provide longstanding host defense against microbial pathogens. However, distinct subpopulations of memory Th cells cause chronic inflammatory diseases such as asthma. Asthma is a chronic allergic inflammatory disease with pathogenic airway remodeling including fibrotic changes in the lung. The immunological mechanisms that induce tissue fibrotic changes in allergic airway inflammation remain unknown.

Methods: Mice were received OVA specific memory Th2 cells followed by multiple antigen challenges. We also set up the experimental system of chronic airway inflammation induced by repetitive HDM exposure.

Results: We found that epithelial cytokine, Interleukin-33 (IL-33) enhanced Amphiregulin production by ST2(high) memory Th2 cells, and Amphiregulin reprogrammed the transcription of eosinophils toward an inflammatory state with enhanced production of Osteopontin, a major profibrotic immunomodulatory protein. Both Amphiregulin-producing CRTH2(high)CD161(high)CD45RO+CD4+ Th2 cells and Osteopontin-producing eosinophils were massively accumulated in polyps from patients with eosinophilic chronic rhinosinusitis.

Conclusion: The IL-33-ST2-Amphiregulin-EGFR-Osteopontin axis directs fibrotic responses in eosinophilic airway inflammation.

Disclosure of Interest: None Declared

P185
TEMPORAL ABROGATION OF IL-4R-SIGNALING IS DETRIMENTAL FOR AMELIORATION OF TH2 AND TH17 INFLAMMATION IN OVALBUMIN INDUCED ALLERGIC ASTHMA.

J. Khumalo1, F. Kirstein2, S. Hadebe3, M. Scibiorek1,4, F. Brombacher1,4
1Pathology, Division of Immunology, South African Medical Research Council Immunology of infectious diseases, Faculty of Health Sciences, University of Cape Town, 2Pathology, Division of Immunology, South African Medical Research Council Immunology of infectious diseases, Faculty of Health Sciences, University of Cape Town, 3Pathology, Division of Immunology, South African Medical Research Council Immunology of infectious diseases, Faculty of Health Sciences, University of Cape Town, 4Pathology, International centre for Genetic Engineering and Biotechnology, Infectious Diseases and Molecular Medicine, Division of Immunology, Health Sciences, University of Cape Town., Cape Town, South Africa

Introduction: Airway hyperresponsiveness (AHR), pulmonary inflammation, eosinophilia and mucus hyperplasia are the hallmarks of allergic asthma. This chronic disease is driven predominantly by a Th2 immune response with pathology largely caused by IL-4 and IL-13 receptor signalling which share a common pleiotropic receptor subunit, IL-4Ra1,2. The IL-4/IL-13 axis has been the target of treatment, however, anti-Th2 based therapies for asthma have seen limited success in clinical trials with the reciprocal regulatory mechanisms remaining elusive3. The therapy conundrum is further compounded by the phenotypic heterogeneity among the asthma cases, limited treatment of steroid resistant cases and possible non-Th2 inflammatory mechanisms involved in the asthma pathogenesis4. Therefore, more effective therapeutic options are necessary for established Th2 effector responses.

Methods: Abrogation of the IL-4Ra in allergen sensitized mice mitigates allergen induced AHR, eosinophilia and mucus hyperplasia in vivo5. We developed a novel, tamoxifen induced, conditional IL-4R knockdown murine model (Rosaα creERT2/IL-4Ra flox) and sort to investigate the temporal role of the receptor signalling during the effector phase (therapeutic) and priming/sensitization phase (prophylactic). Rosaα creERT2/IL-4Ra flox, IL-4Ra flox and IL-4Ra+ mice were sensitised with OVA/Alum complex intraperitoneally and challenged them with OVA intranasally.

Results: We found that partial deletion of the IL-4Ra during the priming and effector phase was protective from ovalbumin induced airway hyperreactivity, eosinophilia and goblet cell metaplasia in the Rosaα creERT2/IL-4Ra flox/mice compared to its littermate controls. Interestingly, there was an increased proliferation of CD4+CD25+FoxP3+ T regulatory cells and reduced Th1/Th17, neutrophilic inflammatory phenotype in the conditional knockout (Rosaα creERT2/IL-4Ra flox/mice) compared to the IL-4Ra+ mice

Conclusion: We thus conclude that abrogation of IL-4Ra during both effector and sensitisation phase would have significant therapeutic benefit both for Th2 and non-Th2 (steroid resistant) type asthma. Strikingly, IL-4Ra signaling is detrimental prior to sensitisation for potentiating a Th17 inflammatory response.

Disclosure of Interest: None Declared

P187
FIZZ-1/RELMA AND IL-33 REGULATION BY ONCOSTATIN M IN MOUSE LUNG

C. D. Richards1,*, F. Botelho1, L. Ho1, A. Yip1, F. Lao1, A. Dubey1, R. Park1, A. Humbles2, R. Kolbeck2
1McMaster University, Hamilton, Canada, 2MedImmune, Gaithersburg, United States

Disclosure of Interest: None Declared
**Introduction:** Oncostatin M (OSM) is a gp130 cytokine family member that we have shown induces Th2-skewed cytokine profile, Arginase1+ M2-like macrophages, IL-33 expression in alveolar type II epithelial cells, and more recently FIZZ1/RELMα upon overexpression in C57Bl/6 mouse lungs. FIZZ1/RELMα is a cysteine rich product found at mucosal sites and is associated with Th2-skewed immunity, alternatively activated macrophage (M2) populations and exacerbation of extracellular matrix (ECM) accumulation in mouse models of lung inflammation. IL-33 is an Interleukin-1 family member that acts as an alarmin and is also involved in facilitation of Th2-skewed inflammation and ECM in mouse models. Here we assess the requirement of IL-33 in the induction of FIZZ1/RELMα.

**Methods:** Transient overexpression of OSM in vivo was induced using Adenovirus vector encoding OSM (AdOSM) administered endotracheally in WT or IL-33KO mice (C57Bl/6 background), and compared to control vector (AdDel70) or naive mice. Responses by cell cultures of mouse macrophages were measured by ELISA of supernatants.

**Results:** Total lung lysates at Day 7 showed high induction of protein and mRNA for RELMα and IL-33 after pulmonary administration of AdOSM in comparison to naive or AdDel70 treated mice. RELMα protein levels (ELISA) in broncho-alveolar lavage (BAL) were <100ng/ml in control mice, but markedly increased upon AdOSM treatment to >10,000ng/ml at Day 7 (n=3/group in 3 separate experiments). RELMα was reduced >50% at the mRNA and protein level in IL-33KO mice in comparison to WT in response to AdOSM. This was associated with ablation of Th2 (IL-4/IL-13) cytokine levels and eosiophils (in BAL) as well as Arginase1+ M2-like macrophage accumulation (immunohistochemistry) in IL-33KO mice. Mouse bone marrow derived macrophages (BMDM) skewd in vitro toward M2-like cells (IL-4/IL-13 stimulation) showed significantly elevated RELMα in supernatants, whereas BMDM stimulated with IL-33 did not.

**Conclusion:** Thus, maximal induction of FIZZ1/RELMα by OSM requires IL-33 in vivo, however this is not due to direct action of IL-33 on macrophages, may involve other cell types, and is likely a consequence of indirect action by the reduction of Th2-skewed cytokines (IL-4/IL-13) in the absence of IL-33. (Supported by Canadian Institutes of Health Research, operating grant # 137013).

**Disclosure of Interest:** None Declared

**P188**

**IL-36G PROMOTES AIRWAY INFLAMMATION AND HYPERRESPONSIVENESS DURING RESPIRATORY INFECTION**

H. L. Tay1,*, A. Hsu1, T. Nguyen2, C. Donovan2, A. Collison2, A. Lochrin2, N. Bartlett2, J. Mattes2, P. Wark2, P. Hansbro2, M. Yang2, P. S. Foster1

1Immunology & Microbiology, 2The University of Newcastle, Newcastle, Australia

**Introduction:** Asthma is an inflammatory disorder that was once treated as a single disease. Conventional asthma treatment involves the use of inhaled glucocorticosteroids and bronchodilators to inhibit inflammation and airway constriction. However approximately 20% of asthma patients are poorly responsive to steroid treatment and have increased risk of hospitalization and death. Respiratory infections are thought to be one of the major risk factors that trigger the steroid resistant exacerbations. In these patients, innate inflammatory mediators and cells are linked to pathogenesis and disease severity. Our aim is to determine the novel roles of interleukin (IL)-36 family cytokines in regulating airway inflammation and lung function during respiratory infection.

**Methods:** Naïve mice were administered with recombinant IL-36γ intranasally to characterize its functional role in lungs. As endpoints, airway hyperresponsiveness were measured using Flexivent (SCIREQ). Airway inflammation were assessed by histological changes, BALF, and lung cells infiltration, cytokines and chemokines production. Microarray analysis were used to determine differential gene expression following IL-36γ treatment. In a separate model, mice were infected with Rhinovirus (RV) and are treated with anti-IL-36R or Isotype control and endpoint assessed.

**Results:** Expression of IL-36γ was significantly increased by pathogen-associated molecular patterns derived from bacteria and viruses in macrophages. Single exposure to IL-36γ in vivo increased inflammatory genes expression (gene profiling), neutrophils recruitment and airway hyperresponsiveness (AHR). Interestingly, repeated exposure to IL-36γ also increased eosinophils recruitment and IL-13 production by ILC2. We next sought to determine the importance of IL-36γ in respiratory infection (RV). We demonstrated that blockade of IL-36γ signalling with anti-IL-36R decreased neutrophilic inflammation and airway hyperresponsiveness during RV infection.

**Conclusion:** Infection can augment inflammation in lungs of asthmatics by increasing inflammatory cytokines production, or inflammatory cell recruitment, which worsen the symptoms of asthma. In our model, we show that infections that trigger asthma exacerbations are linked to IL-36 production and that independently delivery of this cytokine to the airways results in AHR and neutrophilic inflammation. Our results suggest that IL-36γ play a role in RV induced airway inflammation and asthma.

**Disclosure of Interest:** None Declared

**P189**

**CHRONIC ETHION EXPOSURES AND ENDOTOXIN INTERACTION INDUCE PULMONARY DAMAGE AND GENOTOXICITY IN MICE**

G. Verma1,2,*, C. S. Mukhopadhyay2, R. Verma2, B. Singh2, R. S. Sethi1

1Experimental Medicine and Biotechnology, Post graduate Institute of Medical Education and Research, Chandigarh, 2Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, LUDHIANA, India
Introduction: Ethion, an organophosphate insecticide, is extensively used on vegetables, fruits, cotton, and ornamental crops. Ethion enters the environment leading to exposure of animals and humans. Ethion causes moderate to high toxicity along with lung and respiratory disorders in animals. Endotoxins are normally present in the environment especially around the agricultural operations. The combined effects of endotoxins with insecticides on animals and humans, which likely occur in agricultural settings, are poorly understood. Specifically, there is a paucity of data on the pulmonary and genotoxic effects of ethion especially during co-exposure to microbes or microbial molecules such as endotoxin. Therefore, we investigated the effects of ethion alone or in conjunction with endotoxin in a mouse model.

Methods: The animals were divided into two treatments and one control groups (n=10; each group). The treatment groups were administered ethion (4 and 2mg kg⁻¹/animal/day, orally) dissolved in corn oil for 90 days while the control group was administered corn oil only. Following treatment, five animals from each group were challenged with 80 μg E. coli lipopolysaccharide (LPS) intranasally and remaining five animals from each group were treated with normal saline solution via the same route. Blood was collected and subjected to comet assay to assess genotoxicity. Histopathology on lungs was performed to observe inflammation. The mRNA expression of Toll-like receptor (TLR) 4, TLR 9, Tumor necrosis factor (TNF)α and interleukin (IL)-1β was assessed by Real Time PCR. Protein expression of TLR4, TLR9, TNFα and IL-1β was observed by immunohistochemistry.

Results: Ethion @4mg kg⁻¹ caused significant (p<0.05) leukocytopenia and lymphocytopenia. Ethion @4 mg kg⁻¹ caused lung inflammation and altered the mRNA expression of TLR-9 and IL-1β in lungs. Further, ethion at both doses alone or with LPS resulted in lung inflammation. Ethion @4 mg kg⁻¹ or LPS increased (p < 0.05) the tail length, tail DNA percent and olive moment, and decreased (p < 0.05) the head DNA percent compared to the normal saline control. Further, ethion @4 mg kg⁻¹ along with LPS showed an increase (p < 0.05) in tail length and olive moment compared to ethion alone and increase (p < 0.05) in tail DNA percent and decrease (p < 0.05) in head DNA percent compared to LPS alone. Ethion @2 mg kg⁻¹ did not change the tail parameters compared to normal saline control. But its combination with LPS increased (p < 0.05) the tail parameters and decreased (p < 0.05) head DNA percent compared to control.

Conclusion: Taken together, these data show ethion exposure before administration of LPS resulted in genotoxicity and increased pulmonary expression of TLR-9 and IL-1β. These data have implications for agricultural workers who may get exposed to both insecticides and microbial molecules such as endotoxin and bacterial DNA

Disclosure of Interest: None Declared
to alter the cellular metabolism. Recent reports showed that IFN-I is also used to cure the allergic diseases including mastocytosis. However, the molecular mechanism or the role of type I IFN in allergic responses is poorly understood.

Methods: We studied the effect of type I interferon signaling on the IgE-mediated anaphylaxis by activating the specific signaling using the recombinant IFN-a, blocking antibody against IFNAR1, or STAT3 inhibitor. To analyze what type I interferon signaling activates in the mast cells, bone-marrow derived mast cells from WT or Ifnar1/-/- mice were tested.

Results: We found that Ifnar1/-/- mice showed the severer systemic anaphylaxis than WT, producing higher amount of histamine. Consistently, blockade of IFN-I signaling by anti-IFNAR1 monoclonal antibody exacerbated the IgE-mediated anaphylaxis. Interestingly, Stat1-/- or Stat2/-/- mice did not show the severer anaphylactic response to WT, indicating that other signaling molecule(s) than STAT1 or STAT2 was required. We identified that STAT3 works in concert with STAT1 during anaphylaxis, as Ifnar1/-/- mast cells failed to activate STAT3, whereas Stat1/-/- mast cells were able to do, or pharmacological inhibition of STAT3 in Stat1/-/- mice but not in WT exacerbated anaphylaxis.

Conclusion: Our findings suggest that both STAT1 and STAT3 play as key regulators for the proper regulation of allergic response.

Disclosure of Interest: None Declared

P190
IFNL4-DG ALLELE IS ASSOCIATED WITH AN INTERFERON SIGNATURE IN TUMORS AND SURVIVAL OF AFRICAN-AMERICAN MEN WITH PROSTATE CANCER
1Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, 2Advanced Biomedical Computing Center, Leidos Biomedical Research, National Cancer Institute, Bethesda, 3Pathology, Lerner Research Institute, and Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, 4Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, 5Cancer Prevention and Control Program, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, United States

Introduction: Men of African ancestry experience an excessive prostate cancer mortality which could be related to an aggressive tumor biology. We previously described an immune-inflammation signature in prostate tumors of African-American patients. Here, we further deconstructed this signature and investigated its relationships with tumor biology, survival, and a common germline variant in the interferon lambda 4 (IFNL4) gene.

Methods: We analyzed gene expression in prostate tissue datasets and performed genotype and survival analyses. We also overexpressed IFNL4 in several human prostate cancer cell lines.

Results: We found that a distinct interferon signature that is analogous to the previously described “Interferon-related DNA Damage Resistance Signature” (IRDS) occurs in prostate tumors. Evaluation of two independent patient cohorts revealed that IRDS occurs about twice as often in prostate tumors of African-American than European-American men. Furthermore, analysis in The Cancer Genome Atlas (TCGA) showed an association of IRDS with early recurrence of prostate cancer. To explain these observations, we assessed whether IRDS is associated with a germline variant within the IFNL4 gene (rs368234815-dG, IFNL4-dG) that controls production of IFNλ-4, a type-III interferon, and is most common in subjects of African ancestry. We show that the IFNL4-dG allele was significantly associated with IRDS in prostate tumors and overall survival of African-American patients. Moreover, IFNL4 expression in 22Rv1 and PC-3 human prostate cancer cells induced IRDS.

Conclusion: Our study links a germline variant that controls production of IFN-λ-4 to the occurrence of a clinically relevant interferon signature in prostate tumors that may predominantly affect men of African ancestry. Together, these observations indicate that IRDS and IFNL4-dG allele may have an important function in the tumor biology and survival of African-American patients, and may influence immune therapy outcomes, which should be examined in future studies.

Disclosure of Interest: None Declared

P191
THE RXR AGONIST LG100268 ALters POLARIZATION OF BONE MARROW-DerIVED Macrophages
N. Raychaudhuri1, K. Liby1
1Pharmacology and Toxicology, Michigan State University, East Lansing, United States

Introduction: Macrophages and their polarization status are important factors in the tumor microenvironment. Macrophages can change from a pro-inflammatory, tumor-suppressive M1 phenotype to an anti-inflammatory, tumor promoting M2 phenotype as tumors advance. M1/M2 macrophage polarization in vitro is phenotypically and functionally plastic in response to various cytokines and the milieu of growth factors. Mouse bone marrow derived macrophages (BMDM) were differentiated by GMCSF or M-CSF to skew them towards M1 or M2 phenotypes, respectively. Rexinoids, selective ligands for RXR nuclear receptors, suppress carcinogenesis in a variety of animal models. The rexinoid LG100268 inhibits inflammation in vitro, but its effects on macrophage polarization are unknown.

Discussion: We investigated Macrophage polarization in vitro induced by LG100268 and compared the effects on M1 and M2 macrophages. We have previously shown that LG100268 inhibits inflammation in vitro, but its effects on macrophage polarization are unknown.

Disclosure of Interest: None Declared
Methods: Cell Culture: BMDMs were isolated from femur and tibia of mice, plated with Complete RPMI. 10 ng/mL GMCSF or MCSF were added into the culture media and incubated for 7 days to differentiate into M1 or M2 macrophages. NO Production: Macrophages were stimulated with 100 ng/ml of LPS±LG100268 (100 nM) for 48 hrs. NO levels in culture sup were measured by the Griess reaction. RNA isolation and real-time PCR: M2 macrophages were treated with LG100268 and LPS for 24 hrs. mRNA was isolated by Trizol extraction and cDNAs were generated by reverse transcription. RT-PCR was performed with iQ SYBR Green Supermix (Bio-Rad). The RT2 qPCR primers for IL-6, TNF-α, IL-1β, VEGFα and β-actin (internal control) were from Qiagen. IL-10 primers used were forward 5′-ATTGTATTCCTGGGTGAGAAG -3′ and reverse 5′-CACAGGGGAGATTCGATGACA -3′; RT-PCR reactions were performed in triplicate on a Real-Time PCR system (Agilent MX300P). Relative quantification of amplicons was performed using the CT method. Quantification of secreted proteins: Confluent cultures in 96-well plates were treated with LPS or LPS+LG100268. Cell supernatant was collected and assayed by Quantikine ELISA (R&D Systems). Data analysis: Data are presented as mean ± S.D. Statistical differences were determined with the two-way ANOVA and Student's t-test. Level of significance was considered at p<0.05.

Results: LPS-stimulated M2 macrophages increased production of TNF-α, IL-1β and IL-6 mRNA and proteins when treated with LG100268, suggesting redirection to an M1 phenotype. In addition, VEGFα and IL-10 mRNA and protein production were reduced in M2 macrophages treated with LG100268. Nitric Oxide (NO) secretion was induced in LPS-stimulated BMDMs in a dose-dependent manner. There was a significant reduction in NO secretion when the LPS-stimulated M1 and M2 BMDMs were treated with LG100268.

Conclusion: This data suggest that LG100268 can repolarize macrophages from an M2 to M1 phenotype.

Disclosure of Interest: None Declared

P192
IL-6 TRANS-SIGNALING AND ADAM17 REQUIRED FOR EGF-R–INDUCED INTESTINAL TUMORS: NOVEL THERAPEUTIC PERSPECTIVES

S. Rose-John1,2
1Biochemistry, UNIVERSITY OF KIEL, Kiel, Germany
2Immunology, UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER, Houston

Introduction: Cytokines receptors exist in membrane bound and soluble form. The IL-6/soluble IL-6R complex stimulates target cells not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. We have named this process ‘trans-signaling’. The soluble IL-6R is generated via ectodomain shedding by the membrane bound metalloprotease ADAM17. Soluble gp130 is the natural inhibitor of IL-6/soluble IL-6R complex responses. The dimerized recombinant soluble gp130Fc fusion protein is a molecular tool to discriminate between gp130 responses via membrane bound and soluble IL-6R responses.

Methods: We have generated hypomorphic ADAM17 mice which express only 5% ADAM17 as compared to wt mice. Furthermore, we have generated sgpl30Fc transgenic mice in which IL-6 trans-signaling is blocked. These mice were used in models of colon cancer.

Results: Colorectal cancer is treated with antibodies blocking epidermal growth factor receptor (EGF-R), but therapeutic success is limited. EGF-R is stimulated by soluble ligands, which are derived from transmembrane precursors by ADAM17-mediated proteolytic cleavage. In mouse intestinal cancer models in the absence of ADAM17, tumorigenesis was almost completely inhibited, and the few remaining tumors were of low-grade dysplasia.

DNA sequencing analysis demonstrated downregulation of STAT3 and Wnt pathway components. Because EGF-R on myeloid cells, but not on intestinal epithelial cells, is required for intestinal cancer and because IL-6 is induced via EGF-R stimulation, we analyzed the role of IL-6 signaling. Tumor formation was equally impaired in IL-6−/− mice and sgpl30Fc transgenic mice, in which only trans-signaling via soluble IL-6R is abrogated. ADAM17 is needed for EGF-R–mediated induction of IL-6 synthesis, which via IL-6 trans-signaling induces β-catenin–dependent tumorigenesis. Our data reveal the possibility of a novel strategy for treatment of colorectal cancer that could circumvent intrinsic and acquired resistance to EGF-R blockade.

Conclusion: Therefore, sgpl30Fc is a novel therapeutic agent for the treatment of chronic inflammatory diseases and cancer and it underwent phase I clinical trials as an anti-inflammatory in 2013/2014. Phase II clinical trials in patients with autoimmune diseases such as inflammatory bowel disease have recently started in China, Taiwan and South Korea.

Disclosure of Interest: S. Rose-John Shareholder of: Conaris Research Institute, Consultant for: Consultant and speaker for AbbVie, Chugai, Genentech Roche, Pfizer and Sanofi.

P193
UNIQUE MECHANISMS OF ACTION OF PEGYLATED HUMAN IL-15 (NKTR-255)

K. S. Schluns1, T. O. Robinson1, S. M. Hegde1, A. Gangadharan1, T. Miyazaki2
1Immunoology, UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER, Houston, 2Nektar Therapeutics, San Francisco, United States

Introduction: IL-15 has anti-tumor activity but with limited efficacy due to its short in vivo half-life. Nektar Therapeutics has developed a PEGylated human IL-15 (NKTR-255) that exhibits a prolonged in vivo half-life and enhanced potency, which is currently being examined as a potential cancer immunotherapy. Since responses by IL-15 can be mediated by transpresentation via the IL-15Ra, as soluble IL-15/IL-15Ra complexes, or by cis-presentation, we investigated the role of IL-15Ra in driving enhanced IL-15Ra-dependent tumorigenesis. Our data reveal the possibility of a novel strategy for treatment of colorectal cancer that could circumvent intrinsic and acquired resistance to EGF-R blockade.

Conclusion: Therefore, sgpl30Fc is a novel therapeutic agent for the treatment of chronic inflammatory diseases and cancer and it underwent phase I clinical trials as an anti-inflammatory in 2013/2014. Phase II clinical trials in patients with autoimmune diseases such as inflammatory bowel disease have recently started in China, Taiwan and South Korea.

Disclosure of Interest: S. Rose-John Shareholder of: Conaris Research Institute, Consultant for: Consultant and speaker for AbbVie, Chugai, Genentech Roche, Pfizer and Sanofi.
NKTR-255 responses by naïve and memory CD8 T cells and NK cells in mice.

Methods: NKTR-255 responses were examined by adoptive transfer of CFSE-labeled naïve ovalbumin-specific CD8 T cells (OT-I) or established memory OT-I T cells followed by systemic treatment with NKTR-255. To assess responses by central and effector memory T subsets, sorted CD44hi memory phenotype CD8 T cells were transferred into wildtype (Wt) recipients followed by NKTR-255 treatment. Additionally, NK cell responses to NKTR-255 were analyzed in IL-15Ra bone marrow (BM) chimeras by BrdU incorporation.

Results: Although NKTR-255 can bind cell surface IL-15Ra in mice, we found that naïve CD8 T cells transferred into WT and IL-15Ra/- recipients proliferated similarly and acquired a central memory phenotype after NKTR-255 treatment. Interestingly, IL-15Ra/- naïve OT-I T cells had a deficient response to NKTR-255 but not to rIL-15 or soluble IL-15 complexes compared to WT OT-I T cells. In contrast, NKTR-255 induced a similar level of proliferation by memory IL-15Ra/- and WT OT-I T cells. Furthermore, WT memory CD8 T cells transferred to IL-15Ra/- recipients proliferated in response to NKTR-255. Sorted memory CD8 T cells maintained the proportion of CD62L+ and negative subsets after NKTR-255. Since NK cells are heavily dependent on IL-15Ra for development, BM chimeras were generated with either IL-15Ra/- or WT BM in WT recipients. In this system, IL-15Ra/- NK cells incorporated similar amounts of BrdU as WT NK cells after NKTR-255 treatment.

Conclusion: These findings suggest cis-presentation, but not trans-presentation, of NKTR-255 is important for naïve CD8 T cell responses. Conversely, memory CD8 T cells do require trans-presentation or cis-presentation for NKTR-255 responses and both central and effector memory CD8 T subsets responded similarly to NKTR-255. Lastly, NK cell responses to NKTR-255 are not driven by cis-presentation. These findings demonstrate that PEGylated IL-15 has altered requirements for IL-15Ra that permits unique mechanisms to be employed. Moreover, the mechanisms utilized by NKTR-255 vary depending on the responding cell type. Overall, PEGylation of IL-15 has a potential to modify IL-15Ra dependency leading to different mechanisms of actions and unique therapeutic effects.

This study was supported by Nektar Therapeutics, San Francisco, CA, USA.


P195
DARK SIDE OF INTERFERON GAMMA SIGNALING IN TRIPLE NEGATIVE BREAST CANCER
S. Singh1, R. Chakrabarti1
1School Of Veterinary Medicine, Philadelphia, United States

Introduction: Triple Negative Breast Cancer (TNBC) is one of the most aggressive types of breast cancer, with patients showing high rates of recurrence and metastasis. While the biology of TNBC is poorly understood, a consistent observation showed strikingly high degree of immune infiltration into TNBC tumors, suggesting a critical role for the immune system in augmenting the growth and metastasis of TNBC. Kaplan Meier analysis indicated that high ELF5 expression is correlated with better distant metastasis free survival of TNBC patients.

Methods: In this study, we have used preclinical mouse model for basal TNBC with heterozygous loss of Elf5. We compared tumor initiation and metastasis in wild type (Wt) and Elf5-Het (Het) tumor bearing mice. Tumor cells harvested from these tumors were subjected to RNA sequence analysis. Interferon associated gene signatures were found to be significantly upregulated in Elf5-Het tumors. Using flow cytometry, we observed that Elf5-Het tumors have high amount of infiltrating NK cells, which was further confirmed through immunohistochemistry (IHC). Through quantitative PCR analysis, NK cells were found to express interferon gamma (IFN-γ). Additionally, IHC of tumors from Wt and Het mice showed high interferon gamma receptor 1 (IFN-γR1) in Elf5-Het tumors. Preliminary IHC analysis of patient tumors suggest high IFN-γR1 along with low Elf5 in TNBC patients as compared to Non-TNBC patients.

Results: To understand how Elf5 regulates immune infiltration, we generated a pre-clinical TNBC mouse model and found that loss of Elf5 amplifies tumorigenesis and lung metastases. Enhanced infiltration of macrophages, NK cells and regulatory T cells (Tregs) was observed in primary tumors from Elf5-Het mice. Upregulation in IFN-γ associated genes was observed in tumor cells of Elf5-Het mice compared to WT tumors. Elf5 reporter mice analysis strongly suggested direct regulation of IFN-γR1 protein expression by Elf5. In vitro tumorsphere assay showed that Elf5-Het tumor cells do form for tumorspheres (indication of cancer stem cell activity) compared to WT and this increased tumorsphere number could be rescued by blocking IFN-γR1 in Elf5 Het tumor cells. Our data suggests that Elf5 mediated alteration of IFN-γ signaling is responsible for modulation of immune associated pathways, facilitating growth of tumor cells and metastasis. In vitro treatment of murine TNBC cells, EpRas with IFN-γ and their subsequent injection into mice leads to an increased lung metastasis. This indicates towards existence of a memory behavior in IFN-γ treated TNBC cells. Corroborating preclinical mouse model data, we observed that IFN-γR1 is elevated in TNBC patient samples as compared to adjacent normal tissue or non-TNBCs.

Conclusion: Our study will delineate how Elf5 driven immune infiltration results in high Interferon-γ (IFN-γ) in tumor microenvironment promoting tumor progression and metastasis in basal TNBCs. In a nutshell, clinical
implications of these results would lead to a paradigm shift in treatment strategies involving IFN-γ and would challenge the existing modality of use of IFN-γ in clinical trials. Administration of Anti-IFN-γR1 antibody along with chemotherapeutic drugs to Elf5-low patients may result in a better clinical outcome than chemotherapy alone, which is the current standard care.

Conflict of Interest: The authors have no competing interest.

Disclosure of Interest: None Declared

P196
NLRS ARE POTENTIAL BIOMARKERS FOR CARCINOGENESIS, AND ERS PARTICIPATE IN NLRS-ASSOCIATED SIGNALING PATHWAYS BY DIRECTLY REGULATING NLRS

W. Fan¹, X. Gao¹, S. Song¹
¹Nanjing Agricultural University, Nanjing, China

Introduction: Increasing evidence indicates that the nucleotide-binding domain and leucine-rich repeat containing gene family (NLRs) may act as critical back-up defenses and provide synergistic responses when confronted with persistent danger[1]. However, the exact contribution of most NLRs to disease and the precise regulatory mechanism of NLRs are still unknown.

Methods: We explored the rapidly expanding area of research regarding the function, expression and regulation of NLRs by comprehensive data mining[2]. And using Chip-qpcr, EMSA, and dual luciferase reporter assays to identified one transcription regulator of NLRs. We also observed inflammasome assembly by immunofluorescence microscopy.

Results: The analysis indicated that NLRA, NOD1-2, NLR3-5, NLRP2, NLRP4, NLRP8, and NLRP12-14 were highly associated with neoplasms, inflammation, and infectious diseases. Furthermore, NLRs were differentially expressed in different cancer tissues. Such expression discrepancies might influence inflammatory regulation and tumorigenesis. Importantly, estrogen receptors (ERs), which were first identified as NLR transcription regulation factors in this study, both regulate NLR expression and promote inflammasome assembly after stimulation with different ER agonists (ERβ and ERα).

Conclusion: In short, the research demonstrates that NLRs are potential biomarkers for numerous important diseases, especially cancer, and ERs participate in the NLR-associated signaling pathway by directly regulating NLRs. Our results provide novel insight into ERs as therapeutic targets in NLR-related inflammation and cancer[3].

Disclosure of Interest: None Declared

P197
BLYS ANTAGONIST DESIGNED BY CADD AMELIORATES COLLAGEN-INDUCED ARTHRITIS

W. Zhu¹, X. Sun², Z. Lf³, R. Lf³, J. Sun¹
¹Cellular & Molecular Pharmacology, School Of Pharmaceutical Science & Technology, Tianjin University, Tianjin, ²People’s Hospital, Peiking University, Beijing, China

Introduction: B lymphocyte stimulator (BLyS), a member of tumor necrosis factor (TNF) family, is a critical factor for B cell maturation and survival, and its overexpression contributes to the development of autoimmune diseases and BLyS antagonists have been developed for the treatment of autoimmune disorders. Currently, most BAFF antagonists are antibodies against BAFF or soluble BAFF receptor-IgG1 Fc fusion proteins. Small molecules such as peptides as antagonists to block the biological activity of cytokine have been investigated intensively. Based on the crystal structures of the protein–protein complexes, protein mimics can be designed as antagonists with computer-aided drug design (CADD) to block the cytokine-receptor interactions.

Methods: We designed a novel BLyS antagonist, TC, by computer-aided drug design. We constructed TC and a IgG Fc fusion protein, TC-Fc peptibody, and investigated the efficacy of TC-Fc peptibody on collagen-induced arthritis (CIA). CIA mice were randomly divided into three groups, treated with TC-Fc, Fc and PBS respectively. Clinical scores associated with the severity of arthritis were assessed on alternate day from the first day. Histopathological scores, B and T cells changes and autoantibody levels were measured at the end of the experiment.

Results: TC-Fc peptibody could bind to BLyS and inhibit BLyS-TACI interaction in a dose-dependent manner. CIA mice treated with TC-Fc had the lowest incidence among the groups during the development of arthritis. Compared with Fc group, treatment of TC-Fc significantly reduced the numbers of B cell and T helper cell subsets, reduced the pathogenic T helper cells, including Th1, Th2 and Th17. TC-Fc peptibody was able to alleviate swelling of the paws significantly, as demonstrated by the lower arthritis scores compared with Fc and PBS group.

Conclusion: These studies demonstrated that TC-Fc could inhibit the interaction of BLyS with its receptor, reduce the B cells and T helper cells ratios, ameliorate CIA development, and decrease serum levels of CIA-specific antibodies, which showed the prophylactic effects of TC-Fc peptibody on experimental arthritis.

Disclosure of Interest: None Declared

P198
PREDICTING CANCER SURVIVAL: IFN PRODUCTION VALUES DIFFER IN SHORT-TERM AND LONG-TERM CANCER SURVIVORS

K. Uno¹, K. Yagi¹
¹Basic Research, LOUIS PASTEUR CENTER FOR MEDICAL RESEARCH, Kyoto, Japan
Introduction: The human type I interferon (IFN) system performs basic host-defense functions, such as infection prevention and cancer immunological surveillance. Our previous analysis of IFN production tests in patients with various diseases showed that in spite of the cause (infectious disease, cancer, metabolic disease and nephritis), IFN production is impaired by the onset of diseases and this impairment leads to a higher risk of infectious diseases and cancer development.

Methods: This study compares the individual mean (type I) IFN production value of healthy subjects and cancer survivors over 5 years and survivors less than 5 years. Participants IFN production was quantified more than 5 times within 2 years. Heparinized peripheral blood samples were used within 8 h of drawing the blood. Samples were cultured with Sendai virus (HVJ) and incubated at 37°C for 20 h. Supernatants were harvested and IFN titer value in the supernatants was determined by bioassay; the titer value represents the IFN production value. Periodical log transformed IFN-α production values were quantified more than five times and the mean value was determined for all subjects. Patients’ periodical log transformed IFN values (y) were then plotted versus age (x) and fitted to a linear formula (y=mx+n).

Results: The mean IFN value in patients who survived more than 5 years cancer were significantly higher than in patients who survived less than 5 years; in fact, they had similar IFN levels as healthy subjects. The slope of graph (m) were healthy subjects (-0.007), over 5-year survivors (0.021) and patients who survived less than 5 years (-0.218). Patients who died within 5 years had a significantly negative slope; over 5-year survivors had a positive slope and healthy subjects had a slightly less negative slope. These results suggest that cancer patients with a high IFN production value or values that result in a positive slope may have a better survival that those with low IFN values. We also found that in stomach and colon cancer patients, the mean IFN production values are significantly different between short and long-term survivors.

Conclusion: Periodical measurement of IFN production is useful to make prognostic predictions for cancer patients.

Disclosure of Interest: None Declared

P199
CROSS-REGULATION OF IFNG AND IL-27 ENHANCES IMMUNOTHERAPY-INDUCED IMMUNE-RELATED ADVERSE EVENTS IN A PRE-CLINICAL MOUSE MODEL OF AUTOIMMUNE DISEASE.
J. C. Valencia1*, R. Erwin-Cohen1, M. Sandford1, K. Tsuneyama2, H. Young 1
1NCI-Frederick, Frederick, United States, 2Pathology and Laboratory Medicine, Tokushima University, Tokushima, Japan

Introduction: Immune-related adverse events (IrAEs) decrease cancer survival in patients with pre-existing autoimmune diseases (AD) receiving immune agonists or checkpoint inhibitors (CPI) therapy. Currently, anti-tumor immunotherapy associated cytokine cross-regulation and IrAEs has not been explored in hosts with pre-existing autoimmunity.

Methods: Using the ARE-del pre-clinical model of lupus-like disease exhibiting chronic moderately high systemic IFNg levels, we evaluated the effectiveness of intraperitoneal single or combined immunotherapy with anti-PD1 and anti-CD40 agonist antibodies against 7-day established B16F10 subcutaneous grafts in ARE-del heterozygous (HET) mice with mildly-active lupus-like disease.

Results: Only the combined anti-PD1 with anti-CD40 (anti-PD1/CD40) therapy and anti-CD40 alone decreased tumor burden (p=0.002, ANOVA) relative to isotype treated controls. No mice were tumor free. Mimicking clinical data, only the anti-PD1/CD40 arm improved survival (p=0.034, log rank test) only in B16F10-bearing WT mice. Gross and histological examination determined that anti-PD1-based therapies (anti-PD1 or anti-CD1/40) elicited distant (lungs) or local (lymph nodes) metastases in 20 to 30% of HET and WT mice. Comprehensive systemic and local histological and cytokine profile analysis demonstrated that anti-PD1/CD40 worsened the histological scores for pre-existing liver and lupus nephritis pathology in HET mice consistent with IrAEs. In fact, severe lupus nephritis was strongly associated with mortality in anti-PD1/CD40-treated HET mice. Further analysis showed that worsening of histological scores correlated with increases of the endogenous levels of IL-27, TNF-alpha, Cxcl9, and Cxcl10 in anti-PD1/CD140-treated HET mice relative to controls. IFNg levels remain unchanged compared to isotype treated mice suggesting that synergy the increased cytokines advanced disease. Immunoblotting analysis of spleen lysates confirmed PD1-based therapies increased the protein levels and phosphorylation of common IFNg based therapies incr...
P200
MURINE GUANYLATE-BINDING PROTEIN-2 (MGBP-2) INHIBITS TRIPLE-NEGATIVE BREAST CANCER MIGRATION IN VITRO.
D. Vestal1, G. Nyabuto1
1Biological Sciences, University of Toledo, Toledo, United States

Introduction: The Guanylate-Binding Proteins (GBPs) are a family of 67-71 kDa interferon-induced GTPases. hGBP-1 alters the proliferation, migration, and invasion of different cancer cell types. At least two GBPs have been associated with better prognosis in breast cancers. Because hGBP-2 correlates with better metastasis-free survival, we asked whether GBP2 inhibited cell migration/invasion.

Methods: The murine TNBC cell lines, 4T1 and 67NR, were isolated from a single murine mammary tumor that arose spontaneously in a Balb/c mouse. To determine whether these TNBC cell lines would be useful to characterize the role of mGBP-2 in breast cancer migration/invasion, 4T1 and 67NR cell lines were assessed for mGBP-2 expression levels, migration, and proliferation.

Results: Highly migratory 4T1 cells express little or no mGBP-2, while poorly migratory 67NR cells do. Treatment of 4T1 cells with IFN-g to increase mGBP-2 levels inhibited their migration. Conversely knockdown of mGBP-2 in 67NR cells increased their migration. mGBP-2 did not alter TNBC cell proliferation.

Conclusion: mGBP-2 may contribute to improved prognosis in breast cancer by inhibiting tumor cell migration/invasion. Future studies will examine the role of mGBP-2 on invasion/metastasis in vivo.

Disclosure of Interest: None Declared

P201
EXPLORING THE POSSIBLE ROLE OF IFNL4 IN LIVER CANCER
F. Wang1, O. O. Onabajo1, A. Obajemu1, O. Florez-Vargas1, L. Prokunina-Olsson1
1Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, United States

Introduction: Chronic infection with hepatitis C virus (HCV) can lead to fibrosis and cirrhosis of the liver and eventually, to liver cancer. A dG allele of a genetic variant rs368234815-dG/TT (IFNL4 genotype), which creates an open reading frame for interferon lambda 4 (IFNL4), is of strong clinical significance because of its association with decreased clearance of HCV infection but reduced liver fibrosis. However, no link between IFNL4 genotype and liver cancer has been reported. Based on our previous observation that IFNL4 causes reduced proliferation and increased cell death in human hepatic cells, we now focused on exploring the mechanisms of these antiproliferative effects of IFNL4, as they can be relevant for liver cancer.

Methods: We used HepG2-IFNL4-GFP cells with Dox-inducible production of IFNL4. Starting from this model, we eliminated the interferon lambda receptor (IFNLR1) by CRISPR/Cas9 gene editing. Cell viability, colony formation assays, cell apoptosis and cell cycle assays were used to evaluate the effects of IFNL4 on cell proliferation. Global transcriptome analysis was done by RNA-sequencing to investigate the changes in these cell lines with or without Dox induction. Ingenuity Pathway Analysis (IPA) and iPathwayGuide software were used to identify the pathways significantly enriched in each condition. We also analyzed the association between expression of select genes identified by pathway analysis, and rs12980275 (a proxy SNP for rs368234815), r² = 0.72 and D’ = 0.93 by multivariate linear regression models in liver hepatocellular carcinoma (LIHC) dataset (n = 364) of The Cancer Genome Atlas (TCGA).

Results: A stable IFNLR1 knockout cell line (HepG2-IFNL4-GFP-IFNLR1KO) was generated and verified based on the loss of IFNLR1 protein expression and JAK/STAT signaling. Using this cell model, we observed a number of IFNL1-dependent effects caused by IFNL4: inhibition of cell proliferation through induction of apoptosis and S phase cell cycle arrest, and induction of mitogen-activated protein kinases (MAPK) and cell apoptosis-related pathways. iPathwayGuide analysis of RNA-sequencing data, followed by qRT-PCR validation, showed that mineral absorption pathway, represented by metallothioneins (MTs) genes, which are considered tumor suppressors, was significantly induced by IFNL4 in an IFNL1-dependent manner. In TCGA liver cancer dataset, expression of MTs was gender-dependent; in stratified analysis IFNL4 genotype was significantly associated with MT gene expression in tumors from female patients.

Conclusion: In summary, our results could provide an explanation why IFNL4-dG allele is not associated with risk of liver cancer, even though it is strongly associated with risk of establishing chronic HCV infection. By inhibiting cell proliferation through inducing cell apoptosis and S phase cell cycle arrest and inducing MT genes, IFNL4 might be counter-acting negative effects leading to liver cancer development. These findings may suggest new mechanisms of IFNL4 function related to liver carcinogenesis and treatment.

Disclosure of Interest: None Declared

P201.A
IFNL4-DG PREDISPOSES TO PROSTATE CANCER AMONG MEN AT INCREASED RISK OF SEXUALLY TRANSMITTED INFECTIONS
1Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, 2Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, 3Cancer Prevention and Control Program, Lombardi Comprehensive Center,
Introduction: Sexually transmitted infections (STIs) can reach the prostate gland, where their harmful effects may be mediated by the innate immunity, including interferons. Humans are polymorphic for the germline dinucleotide variant, rs368234815-TT/dG, in the IFNL4 gene encoding interferon λ4. Since IFNL4-dG allele has been linked to impaired viral clearance, we hypothesized that potential exposure to sexually transmitted pathogens may increase prostate cancer risk in an IFNL4-dG-dependent manner.

Methods: The study included 976 prostate cancer patients and 1034 population controls, with equal representation of individuals of European and African ancestry. Genetic variant rs368234815 was genotyped in genomic DNA of patients and controls with a TaqMan assay. Possible exposure to STIs was assessed by a questionnaire on sexual history, based on self-reported number of sexual partners at the age of 20-30 or 30-40 years, and over lifetime. Induction of IFNL4 mRNA and protein expression in two prostate cancer cell lines (PC3 and DU145) was tested after infection with Sendai virus (SeV).

Results: We detected statistically significant positive interaction between the number of lifetime sexual partners and IFNL4-dG in the development of aggressive prostate cancer (P-interaction=0.004). Among men who had 20 or more sexual partners vs. <5 partners in their lifetime, the odds ratios for prostate cancer increased from 1.00 (95% CI: 0.59, 1.68) for TT/TT genotype to 1.97 (95% CI: 1.10, 3.51) for dG/TT genotype to 1.64 (95% CI: 1.06, 2.53) for dG/dG genotype. Induction of IFNL4 mRNA expression in vivo and protein expression in two prostate cancer cell lines (PC3 and DU145) was tested after infection with Sendai virus (SeV).

Conclusion: Based on these findings, we conclude that a gene-environment interaction between IFNL4-dG and sexual activity might increase the risk of prostate cancer. The proposed synergistic mechanism of prostate cancer pathophysiology is relevant for all populations but could disproportionately affect men of African ancestry, in which this genetic variant and was induced in both cell lines.

Disclosure of Interest: None Declared

P201.B
MIGRATION AND PROLIFERATION OF HEP3B CELLS IN 3D IN RESPONSE TO THE PRESENCE OF GROWTH FACTORS PRINTED ON A 384-PILLAR PLATE WITH SIDEWALLS
N. Janto1, A. Roth1, M.-Y. Lee1
1Biomedical Engineering, Cleveland State University, Cleveland, United States

Introduction: Hepatocellular carcinoma (HCC) is an aggressive liver cancer that is very widespread globally and its incidence is increasing among the developed and undeveloped world. Scientists and clinicians are looking to better understand the mechanisms which govern the metastasis of HCC tumors. The goal of this work is to develop a high-throughput 3D HCC cell migration assay on a 384-piller plate with sidewalls as a means to better understand the mechanisms of HCC metastasis with potential further use as a method for distinguishing between various types of cancer.

Methods: First, we bound various growth factors (GFs) to methacyrlated heparin sulfate (MHS) and encapsulated them in oxidized, methacyralted alginate (OMA). These samples were printed onto 384-Pillar Plates with sidewalls. We tested for stability of GFs for two weeks by quantifying their presence in media using ELISA. After verifying stability of the GFs, we performed a layer-by-layer print where encapsulated GFs were printed on a top layer, and Hep3B cells infected with lentiviruses containing the expression of mCherry were encapsulated and printed on a second layer. We quantified migration over the course of two weeks by taking images at various heights on the pillar and measuring the fluorescence at each position over this time frame.

Results: We found that MHS-bound GFs and GFs without MHS released growth factors for the first 24 hours. However, MHS-bound GFs were released linearly after this time frame, while MHS-free GFs were completely released by the end of the fourth day. Subsequently, all migration experiments were performed using MHS to stabilize GF release. Proliferation seemed to occur in the presence of GFs that would normally promote HCC proliferation in vivo, including hepatocyte GF (HGF) and transforming GF beta 1 (TGFβ1). Migration was promoted in the presence of GFs that are associated with the upregulation of angiogenesis in vivo, including TGFβ1, basic fibroblast GF (bFGF), and vascular endothelial GF (VEGF).

Conclusion: We were successful in the creation of a high-throughput 3D cancer cell migration assay that can be measured in real time. Differences in GF responses can be attributed to the behavior of Hep3B cells compared to other known HCC cell lines. In the future, we hope to incorporate other cell types to create a fuller model of the HCC microenvironment.

Disclosure of Interest: N. Janto Grant / Research support from: NIH-Bridges, A. Roth: None Declared, M.-Y. Lee: None Declared

P202
IL-17A IS ANTI-FIBROTIC IN SYSTEMIC SCLEROSIS SKIN WHERE IT MAY SYNERGIZE OR ANTAGONISE TGF-B
A. M. Dufour1,2, M. Alvarez1,2, S. Lemeille1, M.-E. Truchetet2, N. C. Brembilla1,4, C. Chizzolini1,2
1Immunology and Allergy, University Hospital, Geneva, Switzerland, 2Rheumatology, University Hospital,
Introduction: Systemic sclerosis (SSc) is an autoimmune, inflammatory, connective tissue disease in which skin fibrosis is a major hallmark. While transforming growth factor-beta (TGF-β) is considered a master fibrotic cytokine, the role of interleukin-17A (IL-17A), which levels are increased in SSc skin and other organs, is highly debated [1, 2]. Since epithelial cells are preferential targets of IL-17A, we aimed at investigating the crosstalk of IL-17A and TGF-β in the interactions between epidermis and dermis.

Methods: Primary human keratinocytes were primed with IL-17A and/or TGF-β and conditioned-media were used to stimulate healthy donors (HD) and SSc fibroblasts. Alternatively, organotypic cultures of HD full human skin were challenged with these cytokines. Responses were assessed by quantifying inflammatory mediators and type I collagen (Col-I) levels. The factors produced by keratinocytes were identified by a proteomic approach and their contribution was evaluated by neutralization assays. Signalling transduction pathways were analysed by Western blot. Changes in gene expression in full human skin induced by IL-17A and TGF-β were analysed by high-throughput RNA sequencing (RNAseq).

Results: Unstimulated HD- and SSc-derivated keratinocyte-conditioned media (KCM) promoted collagen production by fibroblasts. Cytokine array analysis and neutralizing assays showed that TGF-β was, at least in part, responsible for the pro-fibrotic effect of KCM. Priming of keratinocytes with IL-17A directly decreased Col-I production and significantly reduced Col-I induced by TGF-β in both, SSc and HD fibroblasts (p=0.005 and p=0.027, respectively). Additionally, in the joined presence of IL-17A and TGF-β, SSc and HD fibroblasts produced at least 6-fold higher levels of IL-6, when compared to cells treated with IL-17A or TGF-β alone. This synergy was, at least in part, p38 MAPK signalling-dependent.

In full human skin, at the protein level IL-17A showed direct anti-fibrotic effects and decreased by 2-fold Col-I production triggered by TGF-β (p=0.016). RNAseq revealed that TGF-β induced the coordinated expression of extracellular matrix (ECM) genes dominated by Wnt signalling and IL-17A strongly promoted the expression of many pro-inflammatory genes including IL-6. The joint presence of IL-17A and TGF-β resulted mostly in the parallel expression of the respectively induced genes, with very little antagonistic effects.

Conclusion: IL-17A is a potent anti-fibrotic factor in the model of keratinocyte – fibroblast interactions, as well as in the full human skin, promoting pro-inflammatory and anti-fibrotic responses. Furthermore, IL-17A acts both as TGF-β antagonist and agonist on Col-I and IL-6 production, respectively. These data may help in directing and interpreting therapeutic approaches in SSc, since IL-17A, TGF-β and IL-6 are target candidates in clinical trials.

Disclosure of Interest: None Declared
Central to the pathogenesis of psoriasis is the IL-23/IL-17 immune axis. Specifically, it has been shown that production of IL-23 by skin-resident myeloid cells drives the expansion of IL-17 secreting gdT cells, which promotes epidermal proliferation and inflammation. However, the initiating factors that activate this inflammatory immune axis are unknown. Prostaglandin E2 (PGE2) is a member of the lipid mediator family that modulates inflammation in a tissue- and cell-specific manner. Although increased PGE2 is observed in the skin of psoriasis patients and has been previously shown to amplify the IL-23/IL-17 axis during neuroinflammatory disease and allergy, its role in cutaneous immunity has not been closely examined. We hypothesized that PGE2 enhances IL-23-driven IL-17 production by skin-resident gd T cells and contributes to pathological skin inflammation.

**Methods:** We used in vitro assays to study the effect of PGE2 on IL-17/IL-22 production by murine gdT cells and to identify the receptors and pathways involved. To understand the in vivo relevance of PGE2 during cutaneous inflammation, we are developing gain and loss of function strategies that specifically target PGE2 or its associated signaling pathway in the context of psoriasis/skin inflammation.

**Results:** Our results indicate that PGE2 increases IL-17 transcription and secretion by murine dermal-resident and circulating gd T cells in a time and dose-dependent manner. In addition, PGE2 activity is mediated through the prostanoit receptors EP2 and EP4 expressed by gdT cells and selectively amplifies IL-17A and F expression without increasing IL-22, a cytokine usually co-produced by IL-17 producing cells.

**Conclusion:** Altogether, our results show a new role for PGE2 in regulating the innate immune IL-23/IL-17 axis. Understanding its pertinence in cutaneous immunity may be relevant for psoriatic and other chronic inflammatory diseases.

**Disclosure of Interest:** None Declared

---

**P205**

**PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF AUTOACTIVE RESIDENT AND RECIRCULATING MEMORY T CELLS IN VITILIGO REVEALS LOCAL IFNG AND CHEMOKINE PRODUCTION IN SKIN ARE USED TO COORDINATE AUTOIMMUNITY**

J. M. Richmond1,2, M. Rashighi1, P. Agarwala2, J. P. Strassner2, M. Garg1, K. I. Essien2, L. S. Pell2, J. E. Harris1

1Dermatology, 2Pathology, UMass Medical School, Worcester, United States

**Introduction:** Vitiligo is an autoimmune skin disease in which CD8+ T cells target and destroy melanocytes, and pathogenesis is dependent upon IFNg and IFNg-dependent chemokines. Tissue resident memory T cells (Trm) that target melanocyte autoantigens form in the skin during vitiligo and persist to maintain disease, as white spots often recur rapidly after discontinuing therapy. Here we show phenotypic and functional analyses of melanocyte-specific Trm and recirculating memory T cell populations.

**Methods:** We used our mouse model of vitiligo and human shave skin biopsies to examine the establishment and clonality of autoreactive Trm in the skin. We also used genetically targeted melanocyte-specific T cells to examine classical surface molecules associated with Trms (CD103, CD44), as well as reporters of T cell cytokine production, activation and signaling (IFNg/GREAT, CXCL9/CXCL10/REX3, and TCR signaling/Nur77-GFP). Last, we treated mice with FTY720 or Thy1.1 depleting antibody to examine the functional contribution of Trm versus recirculating memory T cells in our vitiligo model.

**Results:** Only autoreactive T cells were able to set up residence in the epidermis in our mouse model, and those autoreactive Trm were capable of producing IFNg for long periods of time. While we used a single clone to induce vitiligo in mice, TCR-Vbeta sequencing of human lesional skin revealed a polyclonal T cell response with private specificity. Approximately 80% of lesional epidermal T cells were CD69+CD103+ in both mice and humans, with variable CD44 expression levels. CD103/- and CD44/- T cells revealed that redundant biological pathways are able to generate phenotypically different memory cells that are still capable of mediating disease in mice. The Nur77-GFP and REX3 reporter-expressing mouse T cells demonstrated that both Trm and recirculating memory T cells sense autoantigen in the skin, and secrete IFNg-dependent chemokine alarm signals to recruit other recirculating T cells, respectively. Blockade of recruitment of recirculating memory T cells pools to the skin with FTY720 or depletion with low dose Thy1.1 antibody treatment reversed disease, but not durably, indicating that Trm must work with recirculating T cell populations to maintain disease.

**Conclusion:** Taken together, our data provide phenotypic and functional characterization of skin resident and recirculating memory T cells in vitiligo, and indicate that these cell populations communicate via IFNg-dependent chemokines to coordinate autoimmunity. Targeting the survival or function of these autoreactive T cells may provide novel, durable treatment options for patients.

**Disclosure of Interest:** None Declared

---

**P206**

**ASSOCIATION OF JAK-STAT SIGNALING ACTIVITY WITH IMMUNE CELLS INFILTRATION IN SKIN TISSUE IN PATIENT WITH ATOPIC DERMATITIS**

A. Sekita1,2,*, H. Kawasaki2,3, E. Kawakami2, A. Fukushima2,3, S. Obata3, T. Ebihara3, M. Amagai1,3, H. Koseki1,2

1Center for Integrative Medical Sciences, 2Medical Sciences Innovation Hub Program, RIKEN, Yokohama, 3Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

**Introduction:** Atopic dermatitis (AD) is a chronic, inflammatory skin disease with complex pathogenesis
involving multiple immune pathways such as the Th2 and/or Th17/Th22 axes. Recent studies have revealed the crucial role of the JAK-STAT signaling axis in the pathogenesis of AD. However, the cellular etiology of JAK-STAT pathway-mediated inflammatory responses and skin manifestation remains to be elucidated.

**Methods:** We sampled lesional and non-lesional skin in 64 AD patients as well as normal skin from 26 healthy controls. Whole transcriptome sequencing was performed on extracted RNA. Gene expression patterns were analyzed with a focus on cytokine genes and JAK-STAT regulator genes. We additionally performed immunohistochemical staining on skin samples that are immediately adjacent to the sequencing samples for profiling the infiltration of immune cells (including CD4+ T cells, granulocytes and macrophages) in both the dermis and epidermis.

**Results:** Based on whole genome expression patterns, the mixed population of skin samples was clustered into several groups with only weak correlation to inflammatory status in skin tissues. By instead focusing on the expression patterns of JAK-STAT regulator genes, however, skin samples were clearly clustered into several groups with close link between immune cell infiltration status. Notably, almost one-third of all lesional AD skin samples were clustered into one group, showing a similar pattern to psoriasis vulgaris in terms of expression of JAK-STAT regulator genes. These samples were characterized by severe hyperplasia and intensive accumulation of CD4+ T cells, neutrophils and macrophages in the dermis, which are thought to promote inflammatory responses through JAK-STAT signaling pathway.

**Conclusion:** JAK-STAT-related transcriptome profiling in skin provides novel insights into differential immune regulation and should prove helpful for determining biomarkers of AD and other skin inflammatory disorders.

**Disclosure of Interest:** A. Sekita Grant / Research support from: Japan Agency for Medical Research and Development, H. Koseki Grant / Research support from: Japan Agency for Medical Research and Development, T. Ebihara Grant / Research support from: Japan Agency for Medical Research and Development, A. Fukushima Kawakami Grant / Research support from: Japan Agency for Medical Research and Development, E. narcissus Song * Disclosure of Interest: None Declared

**P207.A**

**A NOVEL, POTENT, ORALLY BIOAVAILABLE ROR-GAMMA-T INVERSE AGONIST INHIBITS IL-17 PRODUCTION AND ATTENUATES DISEASE IN PRECLINICAL INFLAMMATORY DISEASE MODELS.**

A. Symons1, K. Gaida2, R. Ngo3, J. Hirata4, A. Baba4, Y. Enomoto4, E. Kada4, A. Takeuch4, J. Zhang1

1Inflammation & Oncology, Amgen, South San Francisco, 2Inflammation & Oncology, Amgen, Thousand Oaks, 3Therapeutic Discovery, Amgen, South San Francisco, United States, 4Pharmaceutical Discovery, Teijin, Tokyo, Japan

**Introduction:** Pharmacological inhibition of retinoic acid-related orphan receptor C (RORC, RORY, RORyt) has been an active research area for the treatment of human autoimmune diseases. RORyt is the master regulator of pro-inflammatory, interleukin (IL)-17A cytokine expression by CD4+ T lymphocytes (Th17s), γδ T cells and type 3
innate lymphoid cells (ILC3s). Antibodies blocking IL-17A have demonstrated significant benefit in clinical trials and were recently approved for the treatment of moderate-to-severe plaque psoriasis. RORγt is a member of the nuclear hormone receptor family of ligand-regulated transcription factors and as such, is an attractive small molecule drug target for psoriasis, and potentially other autoimmune diseases, via inhibition of IL-17A.

Methods: To identify novel modulators of RORγt, we conducted a high-throughput screen utilizing a ligand displacement assay. Mechanistic assessment and optimization of compound activity was carried out in vitro using coactivator and corepressor peptide recruitment assays, transcription reporter assays, and primary cell IL-17A cytokine production assays. Activity of potent RORγt inverse agonists with good pharmacokinetic properties was tested in vivo in short-term, induced IL-17A production assays and disease efficacy was determined in the mouse experimental autoimmune encephalomyelitis model.

Results: Multiple hits were identified from a high-throughput screen utilizing a RORγt-ligand displacement assay and structure-based optimization of one of these resulted in a lead compound with potent in vitro activity. Biochemical mechanistic assessment demonstrated the compound inhibited NCOA2 coactivator peptide binding and promoted recruitment of an NCOB corepressor peptide to RORγt-ligand binding domain. Basal cellular activity in a RORγt transcription reporter assay was inhibited consistent with action as an inverse agonist. The compound inhibited in vitro differentiation of mouse Th17s but not Th1 or Th2 cells, and TCR-stimulated IL-17A production in human whole blood. Oral administration of the RORγt inhibitor reduced both TCR-induced and IL-18 + IL-23-dependent IL-17A production in mice, with no effect on IFNγ. Furthermore, oral administration of the compound significantly reduced mean disease scores and expression of Th17 signature genes in the mouse experimental autoimmune encephalomyelitis model.

Conclusion: RORγt is a tractable drug target for an orally available therapeutic to treat psoriasis and other inflammatory conditions. We have developed a novel, potent small molecule RORγt inverse agonist for further development that inhibits IL-17A expression, TH17 cell development, and shows robust activity in a rodent model of inflammatory disease.


P207.B

TYPE I INTERFERONS MODULATE ULTRAVIOLET RADIATION INDUCED SUPPRESSION OF IMMUNE RESPONSES

N. Yusuf1*, M. A. Sherwani1, I. Ahmad1, C. Raman2

1Department of Dermatology, 2Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, United States

Introduction: UV (ultraviolet) B induced immune suppression contributes in a major way to the growth and development of UVB-induced skin cancers. Type I interferons (IFNs) are cytokines that play a role in regulation of proliferation, differentiation, and immune function. All characterized type I IFNs transmit their signals through the type I IFN receptor (IFNAR) composed of IFNAR1 and IFNAR2 components.

Methods: IFNAR1 and IFNAR2 exist as heterodimers, and loss of either subunit results in complete abrogation of receptor function. Many human cancers including skin cancer have shown low expression of type I IFNs and their related proteins. Little is known about the role for type I IFNs (IFN-α/β) signaling in UVB induced immune responses.

Results: The purpose of this study was to determine whether type I IFNs contributed to UV-induced immunosuppression. DNA damage responses. IFNAR1 deficient (IFNAR1−/−) on C57BL/6 background and wild type (WT) mice were subjected to a local UVB regimen consisting of 100 mJ/cm2 UVB radiation for 4 days followed by sensitization with the hapten 2, 4-dinitrofluorobenzene (DNFB). Wild type mice exhibited significant suppression of contact hypersensitivity response, whereas IFNAR1−/− mice developed significantly greater suppression.

Conclusion: Our ongoing studies will provide the role of type I IFN in prevention of immunosuppression by solar UV radiation. The information obtained from these studies can be used for development of preventive strategies against UVB induced immune suppression.

Disclosure of Interest: None Declared

P208

RELEASED IFP53 FUNCTIONS AS A CYTOKINE ON VIRUS INFECTION

H.-C. Lee1*, T.-H. Kim1, K. Chathuranga1, J.-S. Lee1

1College of Veterinary Medicine, Chungnam National University, Daejeon, Korea, Republic Of

Introduction: The ARSs are the essential enzymes in translation that catalyze specific amino acid to its cognate tRNA(1). However, some ARS in higher eukaryotes possess non-canonical function to regulate additional cell metabolisms(2). As a part of the non-canonical functions of ARS, here we report the new ability of IFP53 in antiviral innate immune system.

Methods: Human acute monocytic leukemia (THP-1) cell line and human acute myeloid leukemia (U-937) cell line, Mouse leukemic monocyte macrophage (Raw264.7) cell line and human embryonic kidney 293 (HEK293T) cell line were used for in vitro studies. PR8-gfp, VSV-GFP and HSV-gfp were used for cell line infection. VSV-GFP and RSV-gfp were used for in vivo approaches.
**Introduction:** We have established a murine model of chronic pulmonary granulomatous disease elicited by instillation of Multiwall Carbon Nanotubes (MWCNT) with little evidence of fibrosis (AMJRCMB 45:858, 2011). This model exhibits many similarities to human sarcoidosis, a chronic granulomatous disease of unknown etiology characterized by alveolar macrophage deficiency of the nuclear transcription factor, peroxisome proliferator-activated receptor gamma (PPARγ) (Culver et.al 2004 Am J.Respir.Cell. Mol. Bio). More recently we observed that instillation of the mycobacterial antigen, Early Secreted Antigenic Target Protein 6 (ESAT-6), together with MWCNT into wild-type C57Bl/6 mice increased fibrosis as well as granulomatous changes (J Nanomed Nanotechnol 6:340, 2015). Because PPARγ-KO mice exhibit exacerbated granulomatous disease compared to wild type, we hypothesized that PPARγ deficiency might also promote more extensive fibrosis in the MWCNT model.

**Methods:** MWCNT with or without ESAT-6 peptide 14 (NNALQNLARTISEAG) were instilled by oropharyngeal route into macrophage-specific PPARγ-KO or wild-type C57Bl/6 mice. Controls received only vehicle (surfactant-PBS) or ESAT-6 alone. Lung tissues were obtained 20 and 60 days later for: (a) histopathology utilizing Masson’s Trichrome stain for fibrosis detection; and (b) laser capture microdissection (LCM) for qPCR. Bronchoalveolar lavage (BAL) was performed on all groups for gene expression by qPCR and protein evaluation by ELISA.

**Results:** Pulmonary histopathology indicated elevated numbers of granulomas in PPARγ-KO vs wild-type mice as anticipated from previous findings (Huizar et al, Resp. Res., 2013). Modified Ashcroft scoring of Trichrome-stained lung sections detected significantly increased pulmonary fibrosis in mice receiving MWCNT+ESAT-6 compared to MWCNT alone at both 20 and 60 days after instillation; moreover, fibrosis was elevated in PPARγ-KO vs. wild-type. In PPARγ-KO mice, further qPCR studies revealed that MWCNT+ESAT-6 instillation elicited elevated fibronectin expression in granuloma-negative lung tissue, as well as increased Matrix Metalloproteinase (MMP 9) expression in BAL cells compared to MWCNT alone. Evaluation of BAL fluids also indicated significantly elevated quantities of TGFβ protein in PPARγ-KO mice compared to wild-type with highest responses found after MWCNT+ESAT-6 instillation compared to MWCNT alone.

**Conclusion:** Current findings show that in response to combined MWCNT + ESAT-6 instillation, PPARγ-KO mice display elevated pulmonary fibrosis compared to wild-type C57Bl/6 mice, and greater fibrosis than seen with MWCNT instillation alone. Data suggest that (1) PPARγ deficiency exacerbates pulmonary fibrogenic pathways and (2) the murine MWCNT model may offer novel insights into fibrotic transformation and possible therapies.

**Disclosure of Interest:** None Declared

---

**P209**

**EXACERBATED FIBROSIS IN PPAR GAMMA KO MICE INSTILLED WITH MYCOBACTERIAL ANTIGEN TOGETHER WITH MULTIWALL CARBON NANOTUBES (MWCNT) IN A MODEL OF CHRONIC PULMONARY SARCOIDOSIS.**

N. Leffler¹, A. Malur¹, D. E. Vargas¹, B. Barna¹, G. Murray¹, A. Mohan¹, M. J. Thomassen²

¹East Carolina University, Greenville, United States

---

**P211**

**A FUNCTIONAL ROLE FOR CELLULAR HETEROGENEITY IN THE TYPE I INTERFERON RESPONSE TO VIRAL INFECTION**

S. Leviyang¹, I. Griva²

¹Department of Statistics and Mathematics, Georgetown University, Washington, ²Department of Mathematics, George Mason University, Fairfax, United States

**Introduction:** Secretion of type I interferons (IFN) by infected cells mediates protection against many viruses, but prolonged or excessive IFN secretion can lead to immune pathologies. A proper IFN response must therefore maintain a balance between protection and excessive IFN production in the face of a range of viral infections. It has been widely noted that the IFN response of clonal cell lines is often heterogeneous, with only a small fraction of infected cells upregulating IFN expression. Several mechanistic explanations for heterogeneity exist, but no quantitatively supported hypothesis of a functional role for heterogeneity has been advanced.

**Methods:** To investigate the role of heterogeneity in the IFN response, we constructed a mathematical model coupling IFN and viral dynamics. We constructed different models of the IFN circuit, reflecting different forms of the positive feedback loop, different rates of IFN secretion per cell, and different levels of IFN cellular heterogeneity. We also modeled different viral infections by varying infectivity rates and levels of IFN antagonism. We then used our model to investigate characteristics of the IFN response that balance protection against excessive IFN production across a range of viral infections.

**Results:** We find that through cellular heterogeneity, autocrine-mediated IFN signaling can be raised while keeping paracrine-mediated IFN signaling relatively
Introduction: Innate immunity is the first line of defense against virus infections and is marked by production of interferons. A number of cellular double stranded RNA (dsRNA) recognition proteins have been implicated in this interferon (IFN) induced antiviral response. These proteins include the interferon induced, dsRNA dependent protein kinase PKR and DExD/H family helicase, retinoic acid-inducible gene I (RIG-I) like receptors. PACT (PKR Activator) is also a dsRNA binding protein and is a cellular activator of both PKR and RIG-I mediated signaling. Interestingly, PACT is also a binding partner of several viral IFN antagonist, including Ebola virus VP35, influenza A virus (IAV) NS1 where this interaction is shown to impact PACT mediated RIG-I activation of IFN. However, the physiological relevance of PACT participation in an antiviral response and whether viruses have evolved countermeasures to inhibit the activity of PACT remains elusive. In this work, we further explored effect of PACT on these functions in influenza virus.

Methods: We have used co-immunoprecipitation assays for the interaction studies. The virus replication was assessed using plaque assay.

Results: Previously, we characterized a mutually antagonistic relationship between VP35 and PACT where VP35 binding to PACT results in inhibition of PACT stimulated type 1 IFN responses and in turn PACT disables the function of VP35 in viral polymerase complex thereby impeding virus replication. Given the functional similarities of VP35 to IAV NS1, we further examined the NS1-PACT interaction in context of virus infection and host innate immune signaling pathways. Our data suggests that as with VP35, NS1 interaction with PACT disrupts PACT-RIG-I interaction and contributes to inhibition of IFN induction. In addition to NS1 residues R38/K41, which are involved in NS1-dsRNA binding, we found that residues E96/E97 involved in NS1-TRIM25 binding are also important for NS1 inhibition of PACT mediated RIG-I activation. Surprisingly, we also found that PACT overexpression inhibits virus growth. As NS1 is not part of virus polymerase complex, we investigated whether other viral proteins involved in viral RNA synthesis can interact with PACT. Our data show that the PA subunit of the influenza virus polymerase is critical for viral replication, interacts with PACT consistent with recent report. Interestingly, overexpression inhibits virus replication and subsequently decreased wild type viral titers. Furthermore, this also correlated with increase in interferon stimulated genes, specifically in presence of R38A/K41A mutant virus infections. We therefore propose that PACT mediated IFN induction is critical for antiviral effect.

Conclusion: Our data show that PACT interaction with IAV NS1 and polymerase complex proteins, helps in regulate its impact on RIG-I mediated interferon responses and viral RNA synthesis. Our data suggests that PACT plays a central role in maintaining the fine balance innate immune responses and virus replication, thus mimics or upregulators of PACT could serve as novel therapeutics for controlling virus infections.

Disclosure of Interest: None Declared
virus infection. Moreover, we demonstrate that the refractory state is not controlled by transcription, but by epigenetic mechanisms through chromatin rearrangements.

Disclosure of Interest: None Declared

P215
CHARACTERIZATION OF THE GLOBAL CELLULAR TRANSCRIPTOMICS OF TYPE I IFN RESPONSE IN GUINEA PIGS AND INHIBITORY EFFECTS OF KEY GUINEA PIG ISGS TO CYTOMEGALOVIRUS
A. McGregor1, M. Markert1, K. Y. Choi1
1Microbial Path & Immunol, Texas A&M University, College Station, United States

Introduction: Cytomegalovirus (CMV) is a leading cause of congenital disease causing deafness and mental retardation in newborns. The guinea pig has a hemomonochorial placenta similar to humans and is the only small animal model for congenital CMV. Species specificity of human CMV requires the use of guinea pig CMV (GPCMV) in animals. We have utilized this model to study CMV pathogenicity, tropism and the development of intervention strategies. The recent sequencing of the guinea pig genome enabled an evaluation of events associated with virus infection at the cellular level via transcriptomics. We evaluated GPCMV susceptibility to IFN-I, the global guinea pig (GP) cellular transcriptomic response to IFN-I and the inhibitory activity of specific ISGs to GPCMV.

Methods: WT and two mutant GPCMV were evaluated: a truncated IE1 protein mutant (IETPC+) impaired in ability to target ND10 bodies; or a pp65 tegument KO mutant (GP83PC+) unable to target IFI16. Plasmids expressing GP IFN alpha or GP INF beta were transfected onto guinea pig embryo fibroblasts (GEFs) or trophoblasts. Control transfected with mCherry plasmid. At 24h, cells were infected with virus (MOI 0.01 pfu/cell). IFN alpha and beta were equally inhibitory to GPCMV with a 90% knockdown of infection. Next, recombinant IFN alpha was used to treat fibroblast cells (100 U/ml or 1000 U/ml) for 6 hr prior to virus infection. WT GPCMV had a 10% knockdown at 100 U/ml and at 1000 U/ml was 85% inhibited. Both GPCMV mutants were highly inhibited at 100 U/ml: IETPC+ (90%); GP83PC+ (80%). Since recombinant IFN alpha appeared functionally active, the cellular transcriptomics of IFN treated cells was evaluated on GEFs treated with IFN-I (100 U/ml) for 2-24h and compared to equivalent control untreated cells. A guinea pig microarray encoding 21,163 genes was used to evaluate cellular transcriptomics. Additionally, individually upregulated ISGs with potential for antiviral activity were cloned into expression vectors and in transient expression assays evaluated for inhibitory action against GPCMV.

Results: Overall, 170 specific guinea pig ISGs were upregulated greater than 3 fold by IFN-I treatment: 134 genes (2h); 137 (4h); 119 (6h); 79 (24h). String plot and interferome data base analysis indicated high correlation with human IFN-I response (93%). Identified highly upregulated ISGs that were potentially inhibitory to GPCMV were cloned into separate expression vectors. Plasmids were transfected onto fibroblast and trophoblasts and at 24h post transfection cells were infected with WT or mutant GPCMV at MOI=0.1 pfu/cell. ISGs evaluated that were inhibitory to virus included homologs of: RSAD2; Mx; ISG15; IFI16; and IFI27. Both WT and mutant viruses were inhibited to varying levels (ranging 50-98% dependent upon ISG and specific virus) but the IE1 mutant had the highest susceptibility to the various ISGs. The pp65 mutant homolog virus had highest susceptibility to IFI16, especially trophoblast cells.

Conclusion: The guinea pig has a similar IFN-I response to humans. GPCMV is susceptible to IFN-I and specific ISGs can be highly inhibitory when used in transient expression assays. Overall, this approach of evaluating the cellular innate immune response to GPCMV will provide a valuable insight into infection of placental trophoblast and congenital infection in the guinea pig model.

Disclosure of Interest: None Declared

P216
IDENTIFYING KEY HOST FACTORS FOR IMMUNITY BASED ON CROSS-VIRUS COMPARISONS.
1Microbiology & Immunology, University of Texas Medical Branch (UTMB), Galveston, 2Epidemiology, 3University of North Carolina, Chapel Hill, 4Microbiology and Immunology, University of Texas Medical Branch, Galveston, 5University of Wisconsin, Madison, 6Washington University School of Medicine, St. Louis, United States

Introduction: Building upon established systems biology datasets, our approach uses uneven viral antagonism to identify host factors that govern disease following infection. By comparing the host immune response across highly pathogenic coronaviruses and pathogenic influenza virus strains, our results identified differential regulation of a subset of interferon stimulated genes (ISGs) following infection.

Methods: We predicted that modulation of an ISG by one or more viruses indicates a key role in host immunity to virus infection. Utilizing CRISPR-CAS9 generated knockout mice, our targeting screen examined and verified the efficacy of several ISGs against viruses that did not manipulate these genes.

Results: Focusing on differential expression of ISGs during virus infection, we generated knockout mice to evaluate the role of Serping1, Lamp3, and Trim14 on pathogenesis. While absence in vivo had no impact on viral replication, all three knockout mice had changes to in vivo disease following SARS-CoV infection. For both Serping1-/- and Trim14-/- mice, SARS-CoV infection had increased disease which was consistent with in vitro down regulation seen in MERS-CoV and H5N1-VN1203
infection. In contrast, Lamp3 had been down regulated by MERS-CoV, but increased under H5N1-VN1203 infection. Infection of Lamp3/-/- mice attenuated infection with both H5N1-VN1203 and H1N1-2009. However, consistent with targeting by MERS-CoV, Lamp3/-/- had a significant increase in disease and damage following SARS-CoV infection. Lamp3 deficient mice had increased airway debris, expanded inflammation, and reduced lung function. Importantly, TUNEL staining indicated increased apoptosis in the airway of Lamp3/-/- mice relative to control.

Conclusion: Overall, the approach used differences across viral antagonism to identify and validate three novel ISGs that play a role in disease following respiratory virus infection. Together, the results highlight the utility of leveraging systems biology and viral cross comparisons as a means to identify host factors impacting pathogenesis following infection.

Disclosure of Interest: None Declared

P217
ARID5A EXPRESSION IS INDUCED THROUGH MYD88-INDEPENDENT PATHWAY IN RESPONSE TO TLR4 STIMULATION IN IFN-GAMMA-SENSITIZED HUMAN MACROPHAGES
H. Metwally1, T. Kishimoto1
1Immune Regulation, Immunology Frontier Research Center (IFReC), Osaka University, Suita, Japan

Introduction: Sepsis is a life-threatening organ dysfunction resulting from infection, especially gram-negative bacteria. High IL-6 serum level in patients with sepsis is correlated with more severity and higher mortality. However, the exact pathological mechanism resulting in high IL-6 expression and how Arid5a, a post-transcriptional regulator of IL-6, plays a role in sepsis is not fully understood. TLR4 is unique among other TLRs for its ability to signal from the cell surface through TIRAP/MYD88 activating NF-kB pathway that initiates transcription of inflammatory cytokines, then become endocytosed and signal from the endosomal compartment through TRAM/TRIF activating IRAF3/IRF7 pathway that initiates transcription of type I interferons. Moreover, TRIF is not just pivotal for type I interferon expression, but is also important for pro-inflammatory cytokines expression upon TLR4 stimulation, however the exact mechanism is not completely known.

Methods: In this work we used human primary peripheral blood derived macrophages (PBDM) differentiated from peripheral blood mononuclear cells (PBMCs) from healthy donors, as well as macrophages derived from THP-1 human cell line. Molecular biology techniques such as western blot, PCR, ELISA, FACS analysis, luciferase assays, immune-fluorescence, cloning, and DNA/siRNA transfection were used to assess different experimental parameters. Graphs were generated using GraphPad Prism7, and statistical analysis was done using t tests as well as one-way ANOVA.

Results: Arid5a expression upon LPS stimulation depends on TLR4 endocytosis in TRIF-dependent, MYD88-independent signalling. In addition, IFNγ pre-treatment enhances TLR4 endocytosis, hence Arid5a and IL-6 expression in human macrophages. Moreover, Arid5a expression is STAT1 dependent, but is independent on IFNβ or IRF3/IRF7 signalling. Finally, LPS stimulation induces the formation of a novel complex between IKKβ/TBK1 which is required for Arid5a expression.

Conclusion: Our current work sheds the light on a novel TRIF/IKKβ/TBK1/STAT1 signalling axis downstream of TLR4, which is pivotal for Arid5a expression, hence IL-6 mRNA stability. Moreover, our results indicate that TLR4 endocytosis is not only important for IFNβ expression, but also plays important role in IL-6 mRNA stabilization through Arid5a expression. Taken together, our findings add a new piece to the TLR4 signalling puzzle, which will help in better understanding of sepsis pathophysiology, as well as developing better treatment modalities.

Disclosure of Interest: None Declared

P218
N-TERMINAL CYSTEINES OF NLRP3 DETERMINE AGONIST SPECIFICITY
A. Nagar1, T. Rahman2, J. A. Harton2
1Immunology and Microbial Disease, 2Albany Medical College, Albany, United States

Introduction: NLRP-inflammasomes are multi-protein complexes comprised of sensor (NLRPs), adaptor (ASC) and effector (pro-caspase-1). Upon inflammasome assembly, caspase-1 is activated resulting in subsequent proteolytic cleavage of pro-IL-1β to its bioactive form. The NLR containing pyrin domain 3 (NLRP3) inflammasome is the best-studied. The cytokine IL-1β is a master regulator of inflammation placing inflammasomes at the crossroads of innate and adaptive immune response. Consequently, inflammasome dysregulation is a hallmark of various inflammatory diseases. Further, blocking IL-1β is the standard treatment for inflammasomopathies despite the elevated risk of life threatening infections. Thus, in such patients, blocking the sensor NLRP3 will be a safer therapeutic approach. However, the mechanism NLRP3 activation is not understood, making direct targeting NLRP3 difficult. Cysteine within the first 110 residues of NLRP3 have been suggested as a target of activating stimuli. This study investigates the role of C8 and C108 in NLRP3 inflammasome activation.

Methods: C8 and C108f of NLRP3 were mutated to Serine (S) or Alanine (A). The capacity of these mutants to form inflammasomes was evaluated by assessing ASC speck formation (microscopy and TOFIE) and by inflammasome reconstitution assays.

Results: Here, we demonstrate that conserved cysteine 8 and 108 of NLRP3 are important for NLRP3 activation and appear to be important for differentiating between sterile and bacterial stimuli. Further, for sterile agonists, cysteine 8 and 108 may cooperate, consistent with the hypothesis that these cysteines are a site of ROS-
mediated regulation, but inconsistent with the presence of a disulfide bond in NLRP3 1-110 crystal structure as previously suggested.

Conclusion: Our study suggests a function for the N-terminal region of NLRP3 in activation of the NLRP3 inflammasome. However, this cysteine-dependent function may be restricted to sterile agonists. Thus, conserved cysteines 8 and 108 may function as a biochemical node to involved in differentiating between bacterial and sterile agonists.

Disclosure of Interest: None Declared

P219
REACTIVE OXYGEN SPECIES REGULATE CALCINEURIN-TFEB PATHWAY DURING BACTERIAL PHAGOCYTOSIS.

M. Najibi1,*, J. Irazoqui1
1Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, United States

Introduction: We previously discovered an essential role for transcription factor EB (TFEB), the master regulator of autophagy and lysosomal biogenesis, in the activation of host defense genes during phagocytosis of bacterial pathogens by macrophages. We established that TFEB is essential for proper induction of pro-inflammatory cytokines and chemokines in murine macrophages infected with S. aureus or S. enterica. However, the upstream events that controlled activation of TFEB and its close paralog, TFE3 during phagocytosis were not known.

Methods: We used reverse and chemical genetics in Bone Marrow Derived Macrophages (BMDMs) to look at TFEB nuclear localization, lysosomal markers and TFEB target genes expression as readout of TFEB activation in different conditions.

Results: As a measure of biological significance, we performed RNAseq of macrophages that lacked TFEB and TFE3, in response to infection by Salmonella and Staphylococcus. We found drastic defects in gene induction in mutant cells, especially induction of inflammatory cytokines (TNF, IL1, etc) was severely compromised. Overall, the affected genes were functionally classified as IFN response and TNF response, indicating widespread and important impairment of pro-inflammatory cytokine gene programs in TFEB/TFE3 deficient macrophages. Using MyD88/TRIF Double knock out primary Bone Marrow Derived Macrophages (BMDMs), we showed that S. aureus, S. enterica, TLR2 and TLR4 ligands activate TFEB and TFE3 in a TLR independent manner. Then we demonstrated how ROS generated by NADPH oxidase during bacterial phagocytosis trigger a novel signaling cascade involving lysosomal calcium channel, TRPML1, calcineurin, and TFEB/TFE3 to elicit a pro-inflammatory gene expression program in macrophages.

Conclusion: Our findings illuminate a previously unknown feed-forward mechanism that links pathogen phagocytosis with induction of genes that enhance the microbicidal and pro-inflammatory functions of the macrophage.

Disclosure of Interest: None Declared

P220
HEPATOCYTE-INTRINSIC NF-KB SIGNALING IS ESSENTIAL TO CONTROL A SYSTEMIC VIRAL INFECTION

S. Namineni1,2,*, T. O. Connor1, P. Johannsen3, P. Shinde4, K. Borst5, T. Ried6, A. Pandyra6, S. Kurz1, D. Wohleber1, J. Lucifora2, P. Lang3, K. S. Lang3, U. Kalinke2, M. Karin8, P. Knolle1, M. Heikenwaelder1,2
1Technical University Munich, Munich, Germany, 2German Cancer Research Center (DKFZ), Heidelberg, Germany, 3University Hospital Zurich, Zurich, Switzerland, 4Heinrich Heine University, Düsseldorf, 5TWINCORE & Hannover Medical School, Brunswick, 6University of Duisburg, Essen, Germany, 7INSERM, Lyon, France, 8University of California, San Diego, United States

Introduction: The liver is one of the pivotal organs in vertebrate animals, serving a multitude of functions such as metabolism, detoxification and protein synthesis and including a predominant role in innate immunity.

The innate immune mechanisms pertaining to liver in controlling viral infections have largely been attributed to the Kupffer cells, the locally resident macrophages. However all the cells of liver are equipped with innate immune functions including in particular, the hepatocytes. Hence, our aim in this study was to elucidate the innate immune contribution of hepatocytes in viral clearance.

Methods: In this study, we used mice lacking Ikkβ specifically in the hepatocytes, termed IkkβΔHep mice. Blockade of Ikkβ activation in IkkβΔHep mice affects the downstream signaling of canonical NF-κB signaling by preventing the nuclear translocation of NF-κB, an important step required for the initiation of innate immune responses.

Results: Infection of IkkβΔHep mice with lymphocytic choriomeningitis virus (LCMV) led to strongly increased hepatic viral titers – mainly confined in clusters of infected hepatocytes. This was due to reduced interferon-stimulated gene (ISG) expression early during the onset of infection and reduced expression of chemokines attracting CD8+ T-cells during the onset of adaptive T cell response. Decreased ISG production correlated with increased liver LCMV protein and LCMV in isolated hepatocytes from IkkβΔHep mice. A similar phenotype was found in LCMV-infected mice lacking interferon signaling in hepatocytes (IFNARΔHep) suggesting a link between NF-κB and interferon signaling in hepatocytes. We also observed a failure of interferon-mediated inhibition of HBV replication in HepaRG cells treated with NF-κB inhibitors corroborating our initial findings with LCMV infections.

Conclusion: Collectively, The above results clearly highlight a previously unknown and influential role of hepatocytes in the induction of innate immune responses leading to viral clearance during a systemic viral infection with LCMV-WE.
Disclosure of Interest: None Declared

P221 MECHANISTIC EXPLORATION OF IFNL4 FUNCTION USING SITE-DIRECTED MUTAGENESIS
A. Obajemu1,2, O. Onabajo1, J. Vargas1, N. Rao1, B. Muchmore1, N. Earland1, L. Prokunina-Olsson1
1Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, United States
2Department of Genetics, Division of Medical Oncology, National Cancer Institute, Bethesda, United States

Introduction: IFNL4 is a genetically regulated type-III interferon (IFN) that has been associated with clinical outcomes in HCV infection and other disease conditions. Unlike other type-III IFNs, IFNL4 is poorly secreted and accumulates in the cytoplasm, possibly as an evolutionary mechanism to limit its activity. Understanding the mechanisms behind the suppression of IFNL4 secretion may provide a better picture of the role of IFNL4 in clearance of HCV and other infections, and its potential use for the treatment. Here, we used an approach of site-directed mutagenesis to gain further insight into the biology and function of IFNL4.

Methods: We generated 88 mutants by site-directed mutagenesis of the wild-type (WT) IFNL4 expression construct. Mutants were generated to introduce significant qualitative changes (amino acid class switch, helix breakers) in three main categories – 8 mutants of the cysteines, 38 mutants of residues conserved between IFNL4 and IFNL3 (the most similar protein with 30% aa identity) and 38 residues not conserved between IFNL4 and IFNL3. The latter category included 3 natural human polymorphisms - C17Y, R60P and P70S, and 6 residues that differ between the human and chimpanzee IFNL4 proteins – I20V, G129A, S130P, K139R, K154E and V158I. The expression constructs and controls were transiently transfected into HepG2 cells and the conditioned media and cell lysates were analyzed after 48 hours by Western blotting. Conditioned media was also used to treat HepG2-ISRE-Luc cells to evaluate biological activity of the secreted mutant IFNL4 proteins.

Results: We identified 3 mutants (P3A, K154E, and R146K) that significantly (up to 5-fold) increased the secretion of IFN-α4, without compromising its activity. Notably, the less secretable and active version, K154, is present only in humans (including Neandertals), while the E154K version is present in all other species that have IFNL4 and in all other type-III IFNs. All mutations in the cysteine residues disrupted IFNL4 activity except the natural polymorphism C17Y, which is located in the leader peptide, suggesting that it is not involved in a cysteine disulfide bond.

Conclusion: Our site-directed mutagenesis approach provided additional information about functional sites relevant for secretion and biological activity of IFNL4.

Disclosure of Interest: None Declared

P222 DEVELOPMENT OF A HOMOGENEOUS, BIOLUMINESCENT IMMUNOASSAY FOR RELEASED IL-1 BETA
M. O’Brien1,*, J. Cali1, D. Lazar1
1Research & Development, Promega Corporation, Madison, United States

Introduction: Interleukin-1 beta (IL-1β) is an important inflammatory cytokine of the innate immune system, released from monocytes, macrophages, and other cell types in response to a variety of pathogens and other danger signals consequent to inflammasome activation. Our goal was to create a simple, homogeneous immunoassay (no wash steps or required transfers) to directly measure released IL-1β from cells.

Methods: To enable homogeneous IL-1β detection, candidate pairs of anti-human IL-1β antibodies were covalently labeled with NanoBis [NanoLuc® Binary Technology], using complementary Small BiT (SmBiT) and Large BiT (LgBiT) subunits of NanoBiT luciferase. The simple labeling method consists of reacting antibodies with [HaloTag®] succinimidyl ester ligand, removing unreacted ligand, then conjugating the antibodies with recombinant HaloTag-SmBiT or HaloTag-LgBiT protein. Complementary NanoBis-labeled anti-human IL-1β antibody pairs were added directly to treated cells in culture (or optionally to transferred culture medium) then incubated for 1 h. When the labeled antibodies recognize and bind to released IL-1β, the complementary NanoBis are brought into proximity, thereby reconstituting NanoBis luciferase and generating luminescence when the substrate furimazine is subsequently added.

Results: NanoBis-labeled antibody pairs were tested with recombinant IL-1β and the human monocyte THP-1 cell line in 96-well format. A titration of recombinant IL-1β demonstrated a broad dynamic range (3 logs) with a limit of detection of ~4 pg/ml (0.4 pg/well) using a preferred NanoBis-labeled antibody pair. THP-1 cells were differentiated with phorbol myristate acetate and treated with several inflammasome inducers. Numerous toll-like receptor agonists, as well as other inflammasome inducers, triggered release of IL-1β readily detected by the NanoBis-labeled antibodies. Released IL-1β was quantified by direct application of assay reagents to the treated cell well with culture medium present, or to cell culture medium transferred to a separate assay plate. Inflammasome activation was confirmed by measuring caspase-1 activity [Caspase-Glo 1 Inflammasome Assay] from the same wells tested for IL-1β release. Furthermore, we show that the NLRP3 inflammasome inhibitor MCC950 inhibits IL-1β release from treated THP-1 cells as detected with our homogeneous immunoassay. We have compared the NanoBis IL-1β immunoassay results to that obtained with both colorimetric and chemiluminescent IL-1β ELISAs. We are also in the process of testing PBMCs for IL-1β release with this novel assay format.

Conclusion: A simple, homogeneous assay has been developed for measuring released IL-1β. This "add and
**Cytokines 2018 – Abstracts**

**P223**
**NRF2 IS A NEGATIVE REGULATOR OF STING DURING METABOLIC REPROGRAMMING**

D. Olagnier,1, *A. M. Brandtloff,1 C. Gunderstoffer,1 N. V. Villadsen,1 C. Krapp,1 A. L. Thielke,1 A. Laustsen,1 S. Per,2 A.-L. Hansen,1 L. Bonefeld,1 J. Thyristed,1 V. Bruun,1 M. B. Iversen,1 V. M. Artegaolita,2 R. Lin,4 S. Balachandran,2 Y. Luo,1 M. Nyegaard,1 B. Marrero,3 R. Goldbach-Mansky,6 K. Fitzgerald,6 L. O’Neill,6 F. V. de Paoli,6 H. C. Bertram,1 M. R. Jakobsen1, T. B. Poulsen1, C. K. Holm1

1 Aarhus University, Aarhus C., Denmark, 2 Fox Chase Cancer Center, Philadelphia, United States, 3 Aarhus University, Aarslev, Denmark, 4 Lady Davis Institute, McGill University, Montreal, Canada, 5 NIAID, NIH, Bethesda, 6 University of Massachusetts Medical School, Worcester, United States, 7 Trinity College, Dublin, Ireland

**Introduction:** Metabolic reprogramming of innate immune cells has been reported as a critical process regulating the release of pro-inflammatory cytokines upon TLR stimulation. Conversely, Nrf2 transcription was recently described as a suppressor of inflammation in a context of metabolic reprogramming. STING (STimulator of Interferon Genes) is a central endoplasmic reticulum adaptor protein that stimulates the induction of various genes to suppress pathogen proliferation and drive adaptive immunity in response to microbial or self-DNA. Besides from regulating protective immune responses to pathogens, STING also contributes to autoimmune and inflammatory syndromes. It is currently unknown if and how metabolic reprogramming and/or Nrf2 regulates antiviral immune responses.

**Methods:** RNAseq analysis was used to study the gene expression profile of human cells lacking Nrf2 following siRNA silencing. Manipulation of the Nrf2 pathway using genetic inhibition strategies allowed to investigate STING protein and mRNA levels by immunoblotting and qPCR, respectively. The plasmacytoid dendritic cell line PMDC05 was used to study the molecular details of the repression of STING expression in a context of metabolic reprogramming following TLR engagement. Finally, the relevance of our data was highlighted by the pharmacological modulation of the Nrf2 pathway in fibroblasts isolated from SAVI patients.

**Results:** Comparison of gene expression profile by RNAseq analysis of siRNA control and siRNA Nrf2-treated human epithelial cells showed an increase in the interferon-stimulated gene response. Specifically, silencing of Nrf2 increased STING mRNA and protein levels. Enhanced Nrf2 signaling abolished STING-dependent antiviral signaling hence facilitating replication of DNA viruses. Mechanistically, Nrf2 regulated STING expression post-transcriptionally by increasing STING mRNA stability. Interestingly, treatment of the human pDC cell line PMDC05 with LPS or the TLR7 agonist gardiquimod led to a time-dependent repression of STING expression in metabolically reprogrammed cells. Impairment of STING expression was sufficient to significantly inhibit its functionality as pre-treatment with LPS abolished the release of type I IFNs in response to cGAMP. Mechanistically, the accumulation of the metabolite itaconate activated Nrf2 to repress STING expression. Lastly, treatment with itaconate or with the chemical Nrf2 inducer sulforaphane repressed STING expression and the release of type I IFNs in cells from patients with the STING dependent interferonopathy SAVI.

**Conclusion:** These data identify Nrf2 as a novel regulator of the antiviral response through the modulation of STING expression during metabolic reprogramming and establish the molecular basis for Nrf2-mediated anti-inflammatory therapeutic approaches.

**Disclosure of Interest:** None Declared

**P224**
**EBOLAVIRUS GLYCOPROTEIN-MEDIATED INFLAMMATORY RESPONSES**

J. Olejnik,1,2,* D. Cantoni3, J. S. Rossman3, E. Muhlberger1,2

1 Microbiology, 2 National Emerging Infectious Diseases Laboratories, Boston, United States, 3 School of Biosciences, University of Kent, Canterbury, United Kingdom

**Introduction:** The Ebolavirus genus is subdivided into 5 distinct species differing in pathogenicity. The highly pathogenic Zaire ebolavirus (EBOV) causes a hemorrhagic disease in humans with case fatality rates up to 90%. Fatal EBOV infection is associated with an uncontrolled inflammatory response. Macrophages are primary target cells of EBOV, and a profound proinflammatory response is observed in these cells during EBOV infection. This response is triggered by the EBOV glycoprotein (GP) via activation of Toll-like receptor 4 (TLR4) and can be inhibited by TLR4 antagonists. Notably, treatment with TLR4 antagonists protected mice against lethal EBOV infection (Younan et al. 2017). In contrast to EBOV, Reston virus (RESTV), a member of the ebolavirus genus that is thought to be nonpathogenic in...
humans, fails to stimulate a strong host response in human macrophages due to the inability of RESTV GP to stimulate TLR4 (Olejnik et al. 2017).

**Methods:** We employed virus-like particles (VLPs) containing either wildtype or mutant versions of EBOV and RESTV GP to treat human macrophages and analyzed the resulting proinflammatory responses, including NFκB activation, cytokine expression, and cytokine release. To determine if EBOV GP triggers TLR4 activation in a species-specific manner, we analyzed EBOV GP-mediated NFκB activation in human versus mouse macrophages. In addition, we have tested various anti-inflammatory compounds for their ability to suppress NFκB activation triggered by EBOV GP as a first step for future in vivo studies.

**Results:** We will present data defining the regions in EBOV GP required for TLR4 activation and provide insight into the ability of EBOV GP to activate TLR4 in different species. We will also present our results on the inhibition of EBOV GP-mediated immune signaling using anti-inflammatory treatment.

**Conclusion:** This work will provide the basis for future in vivo studies targeting the EBOV GP-induced immune response that seems to be detrimental to the infected host during severe EBOV disease.

**Disclosure of Interest:** None Declared

**P225**

**AN ANTIVIRAL BRANCH OF THE IL-1 SIGNALING PATHWAY RESTRICTS IMMUNE-EVASIVE VIRUS REPLICATION**

M. H. Orzalli1,*, A. Smith2, K. A. Jurado3, A. Iwasaki3, J. Garlick2, J. Kagan1

1Division of Gastroenterology, Boston Children's Hospital, 2Department of Diagnostic Sciences, Tufts University School of Dental Medicine, Boston, 3Department of Immunobiology, Yale University, New Haven, United States

**Introduction:** Virulent pathogens often cause the release of host-derived damage-associated molecular patterns (DAMPs) from infected cells. During encounters with immune-evasive viruses that block inflammatory gene expression, preformed DAMPs provide important backup inflammatory signals that ensure protective immunity. Whether DAMPs exhibit additional backup defense activities is unclear.

**Methods:** To determine whether host cells contain backup antiviral defenses, we examined antiviral responses elicited from immune-evasive virus infected keratinocytes in conventional 2D tissue culture and 3D models of human skin.

**Results:** Herein, we report that viral infection of barrier epithelial cells elicits the release of preformed interleukin-1 (IL-1) family cytokines, including the DAMP IL-1α. In organotypic, 3D tissue models of human skin, keratinocyte-derived IL-1 acts on the underlying fibroblasts to induce an interferon(IFN)-like state that is necessary to restrict viral replication. Mechanistic analysis revealed a novel branch in the IL-1 signaling pathway that induces IFN-stimulated gene expression in infected cells. This activity is most important to control immune-evasive virus replication in fibroblasts and other barrier cell types.

**Conclusion:** Together, these results highlight IL-1 as an important backup antiviral system to ensure barrier defense.

**Disclosure of Interest:** None Declared

**P226**

**ASIAN AND AFRICAN LINEAGE ZIKA VIRUSES SHOW DIFFERENTIAL ABILITY TO REPLICATE AND REGULATE INNATE IMMUNITY IN HUMAN MONOCYTE-DERIVED DENDRITIC CELLS AND MACROPHAGES**

P. Osterlund1,*, M. Jiang1, V. Westenius1, S. Kuivanen2, R. Järvi1, L. Kakkola3, R. Lundberg2, K. Melen1, M. Korva4, T. Avsic5, O. Vapalahti2, I. Julkunen3

1National Institute for Health and Welfare (THL), 2University of Helsinki, Helsinki, 3University of Turku, Turku, Finland, 4University of Ljubljana, Ljubljana, Slovenia

**Introduction:** Zika virus (ZIKV) belongs to Flaviviridae and it is transmitted by Aedes species mosquitoes in tropical and subtropical areas. In general, ZIKV infection in humans is mild or subclinical, but during the recent epidemic in the Pacific Islands and the Americas, it is evidenced to cause a congenital infection with fetal brain abnormalities, including microcephaly. More detailed understanding of ZIKV-host cell interactions and regulation of innate immune responses during the infection, especially in the human system, is required.

**Methods:** In the present study, we show viral growth differences of different ZIKV lineages, including recent epidemic and a historical ZIKV isolates, in human blood monocyte-derived dendritic cells (DCs) and macrophages, and we describe the innate immune responses in these primary human cell models induced by the ZIKV infection.

**Results:** Recent Asian lineage Zika viruses, both the isolates of fetal brain and adult blood, readily replicated in human monocyte-derived DCs and the infection led to the production of infectious viruses. In DCs, ZIKV infection triggered the induction of cytokine genes, such as IFN-λ1, IFN-beta, CXCL10 as well as IFN-inducible MxA gene. In macrophages, instead, Asian lineage ZIKV showed poor replication and weak induction of cytokine genes. Interestingly, both cell types were highly permissive to the historical 1961 African lineage, low passage virus isolate and strong virus replication led to significant innate immune responses as noted by a strong IFN-λ1 and CXCL10 gene expression. Still, the 2016 Asian lineage ZIKV isolate was very sensitive to the antiviral actions of IFN-beta. This virus also replicated efficiently in mouse fibroblasts lacking a functional IFNAR1 receptor indicating an important role for type I IFNs in restricting the replication of ZIKV.

**Conclusion:** In summary, our data indicates that ZIKV evolution, from the ancestral African strains to recent Asian-American strains has led to significant phenotypic differences in the replication characteristics and regulation
of host innate immune responses in primary human macrophages and DCs. While the African ZIKV strain readily replicates and induces strong cytokine responses in human macrophages, the more recent Asian-American lineage viruses fail to do so. In conclusion, our findings show that the recent epidemic ZIKV strains have evolved towards more immunosuppressive phenotype, which could contribute to the increased pathogenicity of the recent ZIKV strains in humans.

Disclosure of Interest: None Declared

P227
GEF-H1 REGULATES TYPE I AND TYPE III INTERFERON EXPRESSIONS FOR ANTIVIRAL HOST DEFENSES IN THE INTESTINE
Y.-C. Peng1, Y.-C. Chen1, H.-S. Chiang1,2
1Department of Life Science, 2Genome and Systems Biology Program, National Taiwan University, Taipei, Taiwan, Province of China

Introduction:
Enteric viruses, such as rotavirus and noroviruses, are the most common causes of gastroenteritis in infants and young children. Growing evidences have established that type III interferon (IFN) is the major component of antiviral immune responses at mucosal barriers. However, the primary regulator of type III IFN expression in the gut during enteric virus infection is still elusive. Here we showed that a microtubule-associated protein: guanine nucleotide exchange factor H1 (GEF-H1), which has been identified as the essential molecule for RIG-I-like receptor (RLR)-mediated type I IFN responses, is critical for antiviral immunity in the gut.

Methods:
Wild-type (WT) and GEF-H1-deficient C57BL/6 mice were inoculated with reovirus T3D orally or administrated with poly(I:C) HMW intraperitoneally. The small intestines of WT and GEF-H1-deficient mice were isolated to perform plaque assays, immunofluorescence staining, and ELISA. Intestinal epithelial cells and laminar propria cells of WT and GEF-H1-deficient mice were purified for quantitative RT-PCR. The molecular mechanisms of GEF-H1 in the induction of Ifnb1 or IFNL1 promoter activation were validated by luciferase reporter assays in HEK293T cells.

Results:
GEF-H1 protected mice against reovirus infection as the viral replication was enhanced in the small intestine of GEF-H1-deficient mice. The increased viral replication was due to the insufficient production of both type I and type III IFNs in the intestine. Furthermore, the expressions of Ifnb and Ifnl were profoundly reduced in the intestinal lamina propria cells and epithelial cells isolated from poly(I:C)-administrated GEF-H1-deficient mice respectively. Expression of GEF-H1 significantly amplified MAVS-induced activation of Ifnb1 or IFNL1 promoters. Moreover, the enhanced MAVS-mediated Ifnb1 or IFNL1 promoter activation was dependent on the nucleotide change activity of GEF-H1.

Conclusion:
In summary, our results revealed that GEF-H1 is the critical factor that control both type I and III IFN expressions for the enteric virus restriction in the intestine.

Disclosure of Interest: None Declared

P228
AUTOCRINE PROSTAGLANDIN E2 FEEDBACK THROUGH THE EP4 RECEPTOR RESTRICTS ENDOosomal TLR4/TRIF SIGNALING AND TYPE I INTERFERON PRODUCTION
D. Perkins1,*, K. Richard1, A.-M. Hansen1, W. Lai1, S. Nallar1, B. Koller2, S. vogel1
1University of Maryland Baltimore, Baltimore, 2University of North Carolina, Chapel Hill, United States

Introduction:
Toll like receptor 4 (TLR4) dependent inflammatory responses to Gram negative LPS are initiated from both the cell surface through MyD88, and concomitantly from intracellular endosomes through the adaptor TRIF. Feedback mechanisms limiting TLR4/TRIF complex assembly and type I interferon (IFN) production from endosomes are not known.

Methods:
An initial bio-informatics based screen was used to identify a prostaglandin receptor as a regulator of the TLR4/TRIF complex during Gram-negative bacterial infection of macrophages. In vitro LPS stimulation of primary WT and TRIF−/− murine macrophages and human cell lines as well as in vitro and in vivo infection by Gram negative pathogens E. coli and Salmonella typhimurium in the presence or absence of distinct prostaglandin inhibitors or genetic knockouts in PGE2 sensing was used to test this bio-informatic prediction, and define the molecular mechanism.

Results:
The bioactive lipid prostaglandin PGE2 is rapidly produced and secreted by macrophages within minutes of TLR4 activation. Pharmacologic or genetic inhibition of PGE2 production or sensing via the high affinity EP4 receptor, significantly and specifically enhances the amplitude and duration of TLR4/TRIF dependent IRF3 and Caspase-8 activation as well as increasing interferon-beta transcription independent of MyD88 dependent signaling. Mechanistically, PGE2 feedback was found to restrict early CD41 dependent events in the TLR4/TRIF signaling cascade, by means of EP4 dependent effectors

Conclusion:
This work argues that rapid inducible PGE2 production is a means to restrict TLR4 signals arising from an intracellular location and to thereby limit the amount of IFN produced, and potentially cell death, during Gram negative bacterial infections.

Disclosure of Interest: None Declared

P229
TYPE I INTERFERON IS IMPORTANT FOR ACINETOBACTER BAUMANNII RESOLUTION IN A PNEUMONIA MODEL
S. Pires1,*, D. Parker1

1University of Maryland Baltimore, Baltimore, United States
Introduction: Acinetobacter baumannii is an opportunistic pathogen that has emerged as a global threat in recent years in healthcare settings and the community, due to high morbidity and mortality rates and its expanding antibiotic resistance. We have been investigating the contribution of interferon (IFN) signaling to bacterial infections and sought to determine the role of type I signaling in A. baumannii infection.

Methods: Type I interferon signaling was neutralized in C57BL/6J mice with anti IFNAR or IgG control antibodies prior to intranasal infection with $10^{7}$ cfu of A. baumannii AB5075. The type I interferon response to A. baumannii infection was also assessed in vitro using a collection of knockout immortalized BMMs. IFN-β levels were quantified by ELISA and real-time PCR. Phagocytosis was inhibited using the inhibitor of actin polymerization cytochalasinD. TLR4 signalling was inhibited in vitro using LPS from Rhodobacter sphaeroides.

Results: Anti-IFNAR treated mice had a 5-fold increase of bacteria in the airway as detected in bronchoalveolar lavage fluid (BALF; P<0.01), a 78-fold increase in bacteria in the lung tissue (P<0.01) and a 26-fold increase in bacteria detected in the spleen (P<0.05) compared to WT infected mice. This type I IFN signaling was dependent upon the TLR4-TRIF pathway, given that the absence of TLR4 or TRIF the response was reduced by over 99% (P<0.01). The ability to induce Ifnb also required the uptake of A. baumannii as induction of both live and heat killed bacterial preparations were inhibited by 87% (P<0.05) and 91% (P<0.01) respectively, when phagocytosis was inhibited. It has been recently described that A. baumannii undergoes phase variation, switching between two colony types (opaque and transparent). This is more apparent in particular mutations of A. baumannii. Through screening of a transposon mutant library of A. baumannii we observed this phase variation and observed that the in the tested strains translucent versions had a three-fold increase in Ifnb induction (P<0.001). Also, by inhibiting TLR4 we confirmed that Ifnb induction is TLR4 dependent in a phase variant independent way. Furthermore, killing assays with BMDCs showed that the transparent colony versions had a 98% reduction in survival (P<0.0001), albeit while inducing a greater type I IFN response.

Conclusion: Our data points to an important role for type I IFN signaling in controlling A. baumannii infection as well as identifying a bacterial alteration that alters the magnitude of the type I IFN response in a phase variable dependent way.

Disclosure of Interest: None Declared

P230

INFLAMMASOME-INDEPENDENT ACTIVATION OF IL-1β IN THE LUNG IN RESPONSE TO STAPHYLOCOCCUS AUREUS INFECTION

Introduction: Staphylococcus aureus is an important human pathogen, in particular the methicillin resistant S. aureus (MRSA) strain USA300 that is epidemic in the United States. S. aureus is able to activate type III interferon (IFN-λ) pathway that signals through IFN-λ receptor (IFNLR), which is primarily located on epithelial cells and neutrophils. Our goal was to understand the mechanisms behind the improved clearance and reduced pathology in Ifnlr1-/- mice in response to S. aureus infection in our acute pneumonia model.

Methods: A model of S. aureus acute pneumonia using the isolate USA300 was used with wild-type, Ifnlr1-/-; Nlrp3-/- and Casp1-/- mice in 4 h and 24 h infections. Multiplex ELISA quantitated cytokine levels. Neutrophils were purified from bone marrow of naïve WT and Ifnlr1-/- mice and from lung of WT and Ifnlr1-/- mice following a 4 h infection. Neutrophil elastase (NE) and active caspase-1 expression levels were determined by western blot. The NE inhibitor sivelestat was given to mice the day prior and on the day of infection.

Results: Ifnlr1-/- mice exhibited significantly improved bacterial clearance (>90%) from the airway and lung tissue (P<0.001), following a 24 h infection. Ifnlr1-/- infected mice showed a broad reduction in cytokine production assessed in bronchoalveolar lavage fluid (BALF). One cytokine, IL-1β, displayed a 97% drop in Ifnlr1-/- infected mice (P<0.001). In a 4 h infection model, when inflammatory cytokines such as TNF and IL-6 were not reduced, a 75% decrease in IL-1β was observed (P<0.0001). Purified neutrophils from bone marrow of Ifnlr1-/- mice following S. aureus infection showed a slight decrease (10%) in IL-1β production when compared to the WT. However, neutrophils purified from lungs of Ifnlr1-/- mice following a 4 h infection displayed a 50% decrease (P<0.05) in IL-1β levels compared to neutrophils isolated from WT mice. Nlrp3-/- and Casp1-/- mice intranasally infected with S. aureus displayed significantly less IL-1β relative to WT mice (77%; P<0.05 and 79%; P<0.001, respectively) after a 4 h infection. Remarkably no significant differences were observed in IL-1β levels in both KO mice following a 24 h infection. It has been reported that the neutrophil serine protease neutrophil elastase (NE) can also process IL-1β. The level of caspase-1 p20 in the lung was lower in Ifnlr1-/- mice at both 4 h and 24 h of infection (41%, P=0.0518 and 66%, P= 0.0725, respectively) when compared to WT. Interestingly a 52% decrease in NE protein levels was also observed at 24 h infection. NE inhibitor sivelestat was given to mice the day prior and on the day of infection. Both Nlrp3-/- mice infected with S. aureus and treated with sivelestat showed a 39%
to mediate dsRNA’s protective effect. Thus, the Ifits, out of hundreds of ISGs, were identified as the anti-colitis ISGs. Moreover, poly(I:C) mediated protection was completely abolished in intestinal epithelial cells (IEC)-specific Ifit2/-/- mice, demonstrating that Ifit2 expression in these cells is essential for protection. Our studies employing the Citrobacter induced model of infectious colitis further confirmed the critical role of the Ifits in protecting against acute intestinal inflammation. Finally, pre-infection of WT mice with enteroviruses induced the Ifit genes in their colons and decreased their susceptibility to DSS-induced acute colitis, thereby establishing that IFN responses triggered by viruses can protect against bacterial inflammation.

Conclusion: Our study suggests that Ifits are the downstream effector proteins that prevent intestinal inflammation. It also links a specific class of ISGs to IBDs for the very first time. Since viral infections can induce the Ifit genes by triggering IFN signaling, such infections have the potential of safeguarding against acute inflammation caused by resident bacteria. Detailed mechanistic insight into the pathway involved in Ifits mediated protection may identify novel targets for therapeutic interventions.

Disclosure of Interest: None Declared

P232
INNATE IMMUNE SIGNALING DRIVES PATHOGENIC EVENTS LEADING TO AUTOIMMUNE DIABETES
N. Qaisar1, B. Satish1, A. Kucukural2, R. Racicot1, G. Ryan1, M. Garber2, J. Mordes3, J. Wang1
1Medicine, 2Bioinformatics Core, University of Massachusetts Medical School, Worcester, United States

Introduction: Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the immune-mediated destruction of insulin-producing β cells of pancreatic islets. Genetic and environmental factors orchestrate an immune-mediated functional loss of β cell mass, leading to the clinical disease. Viral infections may act as initiating triggers for T1D. Importantly, virus-induced innate immune responses, particularly type I interferon (IFN-I), have been implicated in the initiation of islet autoimmunity. Our focus is to define the role of the IFN-I signaling pathway in T1D development by using LEW.1WR1 rats, in which viral challenge triggers insulitis and diabetes.

Methods: We generated an IFN-receptor subunit 1 (IFNAR1)-deficient LEW.1WR1 rat using CRISPR-Cas9 genome editing and compared wild-type (WT) and IFNAR1-deficient rats for the incidence of diabetes following viral challenge. We performed flow cytometric analysis of T cells in spleen preceding insulits in both WT and IFNAR1-deficient rats after viral infection. Furthermore, we compared gene expression changes in spleens from virus-infected WT and IFNAR1-deficient rats using bulk RNA-seq at the prediabetic stage.

Results: The functional disruption of IFNAR1 significantly delays the onset and frequency of diabetes following viral challenge. Examination of splenic T cells before the onset
of insulinitis and diabetes reveals a significant decrease in CD8+ T cells in IFNAR1-deficient compared to wild-type (WT) rats. In addition, splenic regulatory T cells are diminished in WT but not IFNAR1-deficient rats. RNA-seq reveals diminished interferon-stimulated genes and inflammatory gene expression in spleens from IFNAR1-deficient rats relative to WT rats.

**Conclusion:** These results establish a direct link between IFN-I, inflammatory responses, and T cell responses in the pathogenesis of autoimmune diabetes.

**Disclosure of Interest:** None Declared

**P233**

**EVOLUTIONARY CONSERVATION OF ISG15’S ABILITY TO NEGATIVELY REGULATE IFN SIGNALING**

X. Qiu1*, D. Bogunovic1

1Microbiology, Icahn School Of Medicine At Mount Sinai, New York, United States

**Introduction:** Interferon-stimulated gene 15 (ISG15), which encodes a ubiquitin-like protein, was conventionally considered as an antiviral effector due to its type-I-interferon (IFN-I)-inducible nature. Recent human data suggest that it primarily serves to downregulate IFN signaling via stabilization of ubiquitin specific peptidase 18 (USP18), a negative regulator of the IFN pathway. Since murine Isg15 is not capable of IFN-I regulation, we aim to establish an evolutionary perspective of when ISG15 evolved as a negative regulator of IFN signaling and identify a model organism for the development of antiviral drugs targeting either ISG15 or USP18.

**Methods:** In order to find the species in which ISG15 negatively regulates the IFN pathway as human ISG15 does, we are conducting an evolutionary screening for the stabilization effect by overexpressing 3xFLAG-ISG15 and USP18-V5 constructs of a variety of species including rodents, primates, and a few placental mammals in HEK293T cells. We analyzed protein expression level using western blotting.

**Results:** Previous data of the lab showed that free intracellular ISG15 sustains USP18 levels in humans but not in mice. Fibroblasts derived from ISG15-deficient individuals not only exhibited low levels of USP18 and a persistence increase in IFN-mediated ISG expression, but also an enhanced resistance to a wide range of viruses, all of which were not observed in Isg15−/− murine cells.1 Our current results demonstrate that ISG15 sustains USP18 levels in humans and rhesus macaques, but not in mice, ferrets, and guinea pigs.

**Conclusion:** Our data suggest that the ability of ISG15 to stabilize USP18, a function crucial for IFN regulation, is acquired in primates. This acquired function may serve an evolutionary advantage for species with longer lifespans, during which repeated IFN-mediated inflammation is advantageous but must be tightly regulated.

**Disclosure of Interest:** None Declared

**P234**

**IL-17B USES IL-17RA AND IL-17RB TO INDUCE TYPE-2 INFLAMMATION FROM HUMAN IMMUNE CELLS**

V. Ramirez-Carrozzi1*, R. Pappu2

1Immunology, Genentech, South San Francisco, 2Immunology, Genentech, South San Francisco, United States

**Introduction:** The IL-17 cytokines comprise six family members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E/IL-25 and IL-17F. Cytokine signals propagate via binding to heterodimeric complexes composed of the IL-17 receptor family (IL-17R) subunits. Although five members of this family have been identified, IL-17RA-IL-17RE, structure-function studies indicate IL-17RA is a common subunit shared by IL-17A, IL-17F, IL-17C and IL-25, with specificity dictated by a high affinity interaction between the cytokine and a second receptor subunit. Functionally, IL-17A, IL-17F and IL-17C contribute to neutrophil-mediated responses to control bacterial and fungal infections, and dysregulated expression is associated with autoimmunity and cancers. However, IL-25 is important for anti-parasitic immunity, and is implicated in allergy and atopy. In contrast, little is known about the related cytokine IL-17B. Biochemical studies reveal that like IL-25, IL-17B can bind to IL-17RB in vitro. Ectopic expression of IL-17B has been shown to promote inflammatory arthritis in mice, and in the oncogenic setting IL-17B supports epithelial cell tumorigenesis in an autocrine manner. However, recent reports implicate IL-17B as having a protective role in the colon through inhibition of IL-25 binding to IL-17RA-IL-17RB. These studies all focus on analyzing an immune response stemming from over-expression of the protein in the mouse, with epithelial cell activation being the primary readout, and do not address whether IL-17B has a role in human immune cells.

**Methods:** PBMC stimulation

Human PBMCs were cultured in the presence of IL-2 and TSLP stimulated for 72h with the indicated amounts of IL-17B or IL-25. For antibody blocking experiments, PBMCs were pre-incubated for 30 minutes with anti-IL-17RA, anti-IL-17RB or isotype control antibodies, and stimulated with IL-17B or IL-25 in the presence of IL-2 and TSLP. For IL-17R-Fc blocking experiments, cytokines at concentrations described above, were pre-incubated with soluble human IL-17RA-Flag, soluble human IL-17RB-Fc or control-Fc before addition to the PBMC cultures. After 3 days of culture, supernatants were harvested and assessed for IL-5 and IL-13 production by ELISA.

**Results:** Using quantitative RT-PCR we found that IL-17B shares overlapping expression with IL-25 in human tissues. We utilized a direct ELISA to measure IL-17B interaction with IL-17RB and observed a dose-dependent interaction between IL-17B with IL-17RB. Stimulation of human PBMCs with IL-17B leads to a dose dependent induction of IL-5 and IL-13. Both IL-17RA and IL-17RB are required for IL-17B activity. We found that IL-17B stimulates Type-2 responses from human NKT, CD4+ CRTH2+ Th2 and ILC2s in an IL-17RA-IL-17RB
Introduction: The paramyxoviruses are a group of enveloped viruses. These include measles virus (MeV) as well as emerging viruses, such as Nipah and Hendra viruses (NiV and HeV). NiV, is a bat-borne zoonotic virus that has caused outbreaks in Malaysia and Bangladesh associated with mortality rates in humans ranging from 40 to 90%. This, along with the lack of therapeutics or a vaccine, has resulted in a biosafety level 4 classification for NiV.

The immune response to paramyxoviruses is first launched through activation of the Type I interferon (IFN) response. Numerous previous reports discovered the antagonism effect of the V protein in the Pathogen Recognition Receptor (PRR) MDA5 activation, but it has been unclear whether paramyxoviruses have a mechanism for inhibiting other PRRs such as RIG-I.

Methods: We analyzed the V proteins of different paramyxovirus genera: Henipavirus, Morbillivirus, Respirovirus and Rubulavirus.

Results: Here, we will present a generalized mechanism by which the V protein of NiV prevent the activation of RIG-I. Our findings indicate that NiV V and other paramyxovirus V proteins interact with TRIM25 and RIG-I to prevent ubiquitination of RIG-I, thereby inhibiting IFN induction.

Conclusion: This mechanism of RIG-I inhibition by paramyxovirus V proteins is distinct from the reported LGP2-dependent mechanism, and from the manner in which V proteins inhibit MDA5. In addition to being multifunctional proteins capable of acting on multiple aspects of innate signaling, the paramyxovirus V proteins can also act at multiple steps in the same pathway.

Disclosure of Interest: None Declared
types to identify IFNλ-producing cell populations in the lungs of IAV-infected mice.

**Results:** While epithelial cells of the respiratory tract represent the primary target of IAV infection, we found that they did not significantly contribute to IFNλ production, a result supported by our finding that IFNλ protein levels are unaffected in the absence of MAVS, which is thought to mediate IFNλ induction in this cell type. Instead we found pDCs, a cell type known for robust IFNα production via TLR/MyD88 signaling, to be the major producers of IFNλ during IAV infection with pDC depletion during influenza infection resulting in significantly reduced levels of both IFNα and IFNλ. In addition, we were able to demonstrate that pDCs rely on Type I IFN responses for optimal IFNλ production, as pDCs from IFNAR-/- mice are unable to synthesize IFN protein in response to influenza virus infection. IRF7, a transcription factor critical for the activation of various IFN genes, was expressed at significantly lower levels in IFNAR-/- pDCs, an observation which may explain their inability to upregulate IFNαs during IAV infections.

**Conclusion:** pDCs are the predominant IFNα-producing cell population in response to IAV, unlike other virus infections in which epithelial cells are the major source. Thus, the in vivo producers of Type III IFNs in response to respiratory virus infection are pathogen dependent, a finding which may explain the varying levels of cytokine production induced by different viral pathogens.

**Disclosure of Interest:** None Declared

**P238**

**IFN-LAMBDA RECEPTOR EXPRESSION AND DOWNSTREAM SIGNALING IS CELL TYPE SPECIFIC IN PERIPHERAL BLOOD IMMUNE CELLS**


University Of Alberta, Edmonton, Canada

**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. We previously found that IFN-λ3 regulates human H1N1 influenza vaccine antibody responses, but the mechanism of inhibition is unknown. Since there are conflicting reports regarding direct IFN-λ response and expression of IFN-λR1 on human immune cells, we characterized the IFN-λ3 binding pattern and cellular responses of multiple immune cell subsets.

**Methods:** Since available anti-IFN-λR1 antibodies for flow cytometry are suboptimal, we utilized our novel flow cytometry method to quantify IFN-λ3 binding to IFN-λR1/IL-10R2 on the surface of each major immune cell subset within healthy human donor peripheral blood mononuclear cells *ex vivo* (n=20) or after 1-3 days of *in vitro* stimulation (n=8). Changes in gene expression were quantified by RT-qPCR and RNA sequencing of total PBMCs, or magnetically isolated monocytes or B cells (>97% pure).

**Results:** We found that IFN-λ3 binding varies in a cell-type specific manner and that only certain activation signals (eg. TLR7/8 activation) increase IFN-λ3 cell-surface binding. We could not detect significant IFN-λ3 binding to natural killer cells or T cells, however, IFN-λ3 bound to varying proportions of monocytes, plasmacytoid dendritic cells, B cells and myeloid dendritic cells. Surprisingly, the transcriptional response also varied between cell types. IFN-λ3 stimulation significantly upregulated antiviral IFN stimulated gene (ISG) expression (eg. OAS1, IFIT1) in B cells, but not monocytes. RNA sequencing analysis of primary monocytes stimulated with IFN-λ3 confirmed the lack of antiviral ISGs. Unlike in epithelial cells where type I and type III IFNs stimulate very similar gene expression patterns, only ~25% of genes deregulated were shared between IFN-α and IFN-λ3 stimulation of primary monocytes.

**Conclusion:** IFN-λ3 directly binds subsets of human peripheral blood immune cells, but the response varies depending on the cell type. Knowing which immune cell subsets express the IFN-λ receptor and respond to IFN-λ will be crucial for determining how IFN-λs modulate the adaptive immune response to a pathogen, allergen or vaccine.

**Disclosure of Interest:** None Declared

**P239**

**FUNCTIONAL ANALYSIS OF IRF9 POST-TRANSLATIONAL MODIFICATIONS**

K. S. Schulz, K. L. Mossman

1Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, Canada

**Introduction:** The immune system, consisting of the innate and the adaptive immune systems, protects the organism against infections and the damage they cause. One of the central reactions of the innate immune system in response to a virus infection is the production of type I Interferon (IFN). The IFN is then secreted by the cell and signals in an autocrine and paracrine manner through binding to its receptor. This results in the phosphorylation of Stat1 and Stat2, which then form the ISGF3 (IFN-stimulated gene factor-3) complex together with IRF9. The complex translocates into the nucleus, binds to the IFN-stimulated response element and induces the expression of IFN stimulated genes (ISGs), which results in the formation of an antiviral state within the cells. When performing 2D-Gels of IRF9, we noticed that the nuclear form of IRF9 is modified on multiple sites. Modification of IRF9 has been suggested in past studies, but never been analyzed in detail. Here we present data on how IRF9 is post-translationally modified and how these modifications influence the formation of an antiviral response within the cell.

**Methods:** To analyze IRF9 modifications within the context of the antiviral response, IRF9 point mutations
were created based on modification sites identified by proteomic discovery mass spectrometry. Using a lentiviral system, the mutated and the wild type IRF9 proteins were expressed in human cells (A549) deficient in IRF9.

**Results:** When analyzing the ability of the cells to induce an antiviral state in response to treatment with IFN, we found that A549-IRF9ko cells expressing the wild type IRF9 were able to induce an antiviral state similar to wild type cells, whereas cells expressing a mutant IRF9 protein were not.

**Conclusion:** Further analysis will focus on determining how IRF9 is modified and which function the modifications have in the formation of an antiviral response.

**Disclosure of Interest:** None Declared

**P240**

**ZINC-FINGER ANTIVIRAL PROTEIN ISOFORMS ORCHESTRATE VIRUS RESTRICTION AND INTERFERON RESOLUTION ACTIVITIES**

J. Schwerk1,2, F. Soveg3, A. Ryan4, K. Thomas1, L. Aarreberg1, S. Ozarkan1, A. Forero1, A. Kel1, J. Roby1, L. So1, M. Gale Jr.1, M. Daugherty2, R. Savani1

1UNIVERSITY OF WASHINGTON, Seattle, 2UNIVERSITY OF CALIFORNIA SAN DIEGO, La Jolla, United States

**Introduction:** Resolution of antiviral immune responses and return to homeostasis upon viral clearance is crucial to prevent a chronic IFN signature and immunopathology. Post-transcriptional regulation of mRNA is a major regulatory mechanism controlling expression of immune gene proteins. In this study, we have identified zinc-finger antiviral protein (ZAP, PARP13, ZC3HAV1) as an RNA-binding protein that acts in a distinct, isoform-specific manner as either a viral restriction or an IFN resolution factor. The long isoform of ZAP (ZAP-L) is expressed at basal levels and has previously been characterized as a direct viral restriction factor through binding of viral RNA and its recruitment to the exosome machinery. The short isoform of ZAP (ZAP-S) however is induced upon IFN stimulation through alternative splicing. While both isoforms contain identical RNA-binding domains, their individual roles during antiviral immune responses are not well characterized.

**Methods:** We performed proteomic screens and RNA immunoprecipitation experiments, CRISPR- and siRNA-mediated gene silencing, as well as confocal laser scanning microscopy and subcellular fractionation approaches to identify differential functions of ZAP isoforms during the antiviral immune response.

**Results:** We show that ZAP-S binds and negatively regulates IFN mRNA, thus serving as a negative feedback regulator of the IFN response. ZAP-deficient hepatocytes show elevated IFN expression upon sensing of viral nucleic acids or viral infection, while allowing for increased viral replication at the same time. Distinct subcellular localization of ZAP-S and ZAP-L conferred by the presence of a C-terminal prenylation motif in ZAP-L mediates isoform-specific mRNA targeting: While ZAP-S is diffusely cytoplasmic, ZAP-L forms perinuclear foci and co-localizes with vesicles that stain positive for viral RNA and markers of endolysosomes. Differences in intracellular localization alter ZAP isoform specificity for host and viral RNA, resulting in opposing effects on viral replication during the innate immune response.

**Conclusion:** Distinct localization and expression kinetics of ZAP isoforms confer differential target RNA specificity and opposing functions during viral infection: ZAP-L is a direct antiviral effector through binding of viral RNAs, whereas ZAP-S can serve as a IFN resolution factor by dampening the immune response through host mRNA binding. Collectively, we show that the host utilizes alternative splicing to diversify the function of an RNA-binding protein that, through differential subcellular targeting, performs isoform-specific functions in viral restriction and IFN resolution.

**Disclosure of Interest:** None Declared

**P241**

**HARNESSING CELLULAR HETEROGENEITY TO IDENTIFY NOVEL REGULATORY MODULES CONTROLLING CYTOSOLIC SENSING OF NUCLEIC ACIDS**

S. Shapira1,2, J. Kim1, S. Mayer1, E. Winkelmann1, H. Ding1, O. Rokach1, A. Calilano1

1Systems Biology, 2MicroBiology and Immunology, Columbia University, New York, United States

**Introduction:** In the event of viral infection, mammalian cells possess a number of antiviral programs that are part of the innate immune response. Detection of viral nucleic acids by host sensors, such as RIG-I (retinoic acid-inducible gene I) and cGAS/STING (cyclic GMP-AMP synthase/stimulator of interferon genes), is a critical initiation step leading to the activation of interferon regulatory factors (IRFs), such as IRF3, and induction of antiviral programs that include production of type I interferons. While these and other players have been implicated in controlling cytosolic responses to nucleic acids, precise quantitative relationships between regulatory modules remains unknown.

**Methods:** Here, we apply a multiplexed single-cell RNA-sequencing approach together with computational methods to define transcriptional responses mediated by these pathways. We harness intrinsic cellular heterogeneity to infer regulator-target relationships from the correlation and mutual information in gene expression data.

**Results:** Individual cell states and iterative monitoring of perturbation responses in normal and knockout human cells enable identification of major regulon components that are central to modulating cytosolic sensing of nucleic acids. Indeed, we rediscover known sensors, kinases, transcription factors, and targets, as well as identify novel components of this pathway.

**Conclusion:** This expanded model of nucleic acid sensing further elucidates early events in the antiviral response and extends the list of positive and negative regulators of this central component of immunity.
No conflicts of interest to declare
Disclosure of Interest: None Declared

P242
SCREENING AND IDENTIFICATION OF BINDING DOMAIN OF RHVD VP60 TO HISTOBLOOD GROUP ANTIGENS
Y. Song1,*, Y. Zuo1, F. Wang1, B. Hu1, Z. Fan1, M. Chen1
1, Jiangsu Academy of Agricultural Sciences, Nanjing, China

Introduction: Rabbit Hemorrhagic Disease Virus (RHDV) belongs to the family Caliciviridae and is the etiological agent of the Rabbit Hemorrhagic Disease (RHD) which also known as rabbit plague. RHDV was shown to bind to the HBGA type 2, A type 2 and B type 2 oligosaccharides. These structures were shown to be present on the surface of the epithelial cells of the upper respiratory and digestive tracts that the virus firstly encounters when infecting the host. Subsequent studies revealed that several caliciviruses use the carbohydrate moiety of host-cell histo-blood group antigens (HBGA) for attachment (e.g. ABH/O and Lewisantigens) initiating their replication cycle.

Methods: We constructed target peptide random library of VP60 protein. The binding domains of RHDV VP60 to HBGA were screened by the VP60 gene-specific phage display peptide library of rabbit hemorrhagic disease virus with good specificity and richness. The binding domains were further identified by prokaryotic expression and synthetic H type 2 blood group oligosaccharides-VLP sloopbing assay.

Results: The titer of peptide library was about 4.3×10^12 PFU/mL, and the size and position of the inserted fragments had a good randomness and diversity. Results indicated that the richness and specificity of the library were well. We finally found that two binding sites RFADIDHR (291-298) and VLQFWY(312-317) of RHDV VP60 to HBGAs.

Conclusion: The specific phage display peptide library of Rabbit Hemorrhagic Disease Virus VP60 gene has been established successfully. Binding domains of VP60 to HBGAs were identified employing the specific phage display peptide library.

Disclosure of Interest: None Declared

P243
ACTIONS OF INTERFERONS AT THE MATERNAL-FETAL INTERFACE
1School of Graduate Studies, Health Campus of Newark, Rutgers, Newark, New Jersey, USA, Rutgers New Jersey Medical School, 2Departments of Microbiology, Biochemistry, and Molecular Genetics, 3Pathology and Laboratory Medicine, New Jersey Medical School, Cancer Institute of New Jersey at University Hospital, New Jersey Medical School, Newark, United States

Introduction: The placenta is a short-lived organ that forms during pregnancy and plays an essential role in the development of the fetus. While most pathogens cannot cross the placenta, a subset of pathogens including Zika virus have this capability and may cause severe birth defects. Mouse models of Zika virus infection have shown the importance of interferons (IFNs) in restricting viral transmission to the fetus. However, the precise mechanisms and cell types within the placenta that mediate IFN-induced antiviral protection are presently uncharacterized. Two types of IFNs (IFN-α/β and IFN-λ) are produced as a part of the innate response to viral pathogens, and these in turn trigger expression of the numerous proteins which mediate host anti-viral defenses. While there is published evidence suggesting the existence of a heightened antiviral state in the placenta, the role of IFNs has not been established.

Methods: Pregnant female C57BL/6 mice were injected with 2μg of either mIFN-α2 or mIFN-α2 intravenously at E12.5. The mice were then harvested 40 min post injection and the fetuses along with the placenta were isolated. Tissues were formalin fixed and paraffin embedded, and stained for immunohistochemistry and immunofluorescence staining with appropriate antibodies. Trophoblast-derived cell lines BeWo, HTR-8/SVneo and JAR were maintained in appropriate medium. The cells were treated with hIFNα or hIFN-λ1 and cell lysates obtained and analyzed for pSTAT1 by immunoblotting.

Results: Using the presence of nuclear pSTAT1 as an indicator of IFN responsiveness, we surveyed placental tissues from pregnant female mice (E12.5) injected with either type I or type III interferons. IFN-responsive cells were also positive for Cytokeratin 8 (CK8), a marker of trophoblast lineage. The majority of the cells responding to type III interferons in the placenta are the syncytiotrophoblasts, and more specifically syncytiotrophoblasts-I (SynT-I) as they were immunopositive both for pSTAT1 and monocarboxylate transporter 1 (Mct-1). The human placenta treated with hIFN-α, but not hIFN-λ3, stained positive for nuclear pSTAT1 by Immunohistochemistry (IHC). The human trophoblast cells lines BeWo, JAR and HTR-8/SVneo responded to both type I and type III interferons as determined by immunoblotting.

Conclusion: Our studies show that all murine trophoblasts are responsive to type I IFNs, but only in the outmost layer of the mouse placenta, SynT-I, are cells responsive to both type I and type III IFNs at E12.5 developmental stage. This does not reflect our results using human full-term placental tissue where trophoblasts respond only to type I IFN treatment. As we have observed in other systems, in vivo and in vitro results are inconsistent as human trophoblast cell lines phosphorylate STAT1 in response to either cytokine. These data underscore the importance of IFNs at the maternal-fetal interface.
but also suggest fundamental differences in innate immune mechanisms in mouse and human placentae.

Disclosure of Interest: None Declared

P244
IN VIVO IDENTIFICATION OF CELL TYPE-DEPENDENT DISTINCT AND REDUNDANT FUNCTIONS OF INTERFERONS IN PROTECTION AGAINST INFLUENZA

S. Stifter1,2,*, A. Sawyer1, N. Bhattacharyya1,2, W. J. Britton2,3, A. Sher4, C. G. Feng1,2
1Infectious Diseases and Immunology, The University of Sydney, 2Tuberculosis Research Program, The Centenary Institute, 3School of Medicine, Sydney Medical School, The University of Sydney, Camperdown, Australia, 4Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, United States

Introduction: Interferons (IFNs) regulate immunity by controlling the recruitment and activation of cells, and by inducing direct anti-viral effector mechanisms via expression of hundreds of IFN regulated genes. Although these effector mechanisms have been studied extensively in the context of genetically deficient mice, gene expression analysis and direct anti-viral activity in vitro, we still have an incomplete understanding of how these pleiotropic cytokines execute their host protective functions in a whole animal in vivo.

Methods: To better describe the spatio-temporal functions of IFNs, we developed a novel reporter mouse strain capable of identifying individual IFN-responsive cells in vivo. The M1Red transgenic mice express the fluorescent protein DsRed driven by the IFN-inducible Irgm1 promoter. Using multi-parameter flow cytometry, high resolution microscopy and gene analysis, we characterised IFN-responsive cell populations in mice following influenza virus infection.

Results: We show that during a respiratory infection with influenza virus, IFN signalling is activated in a subset of cells in the lungs and distal tissues such as the bone marrow. Interestingly, we found that the cellular response to IFN signalling is controlled at multiple levels, namely by anatomic site, cell lineage and cellular activation status. Using reporter mice deficient in IFN receptor components, we delineated the synergistic versus redundant roles of IFN signalling in leukocyte and tissue cell subsets.

Conclusion: IFNs are pleiotropic cytokines with a complex host of functions. The analysis of tissue wide responses, although informative, do not provide the necessary detail to accurately dissect cell-type dependent roles of IFNs. Our data provide novel insight into the complexity of IFN signalling and suggest a key role for cellular diversity in shaping the host IFN response in vivo.

Disclosure of Interest: None Declared

P245
AGE-RELATED LOSS OF MIGRATORY DENDRITIC CELLS IMPAIRS THE EARLY INNATE ANTIVIRAL RESPONSE

C. Stotesbury1,*, E. Wong1, L. Sigal1
1Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, United States

Introduction: Elderly individuals are more susceptible to infection due to alterations in the innate and adaptive immune responses. We have previously shown that aged C57B/L6 (B6) mice have impaired maturation and recruitment of natural killer (NK) cells to the draining lymph node (dLN) following ectromelia virus (ECTV) infection, resulting in mortality in normally resistant B6 mice. We have also recently identified the mechanisms whereby NK cells are recruited to the dLN of young B6 mice in order to aid in controlling the replication and dissemination of ECTV. We have found that in young B6 mice, NK cells are recruited to the dLN via an intricate interplay between CD11c+MHC-II+ skin-migratory dendritic cells (skin-mDCs), early interferon-gamma (IFNγ) producing-group 1 Innate Lymphoid Cells (ILCs), and CXCL9+ producing-inflammatory monocytes (iMOs). Soon after infection of young B6 mice, skin-mDCs traffic to the dLN to recruit iMOs via production of CXCL9. In addition, the dLN of young B6 mice show decreased production of IFN-γ, which is necessary for the optimal recruitment of NK cells into the dLN. In turn, the NK cells restrict the systemic spread of the virus. Whether these mechanisms remain intact in aged mice is not known.

Methods: Through the use of in vivo mouse models, reporter viruses, flow cytometry, and QPCR we sought to explore if the above mechanisms are retained during the course of aging.

Results: Here we show that in aged B6 mice infected with ECTV, the number of skin-mDCs in the dLN is reduced compared to young B6 mice. Furthermore, aged B6 mice show decreased production of IFN-γ by group 1 ILCs, have decreased recruitment of iMOs into the dLN, which have a significant increase in their infection rate and decreased production of CXCL9. In addition, the dLN of aged mice endure increased viral loads.

Conclusion: Together, our data suggests that the reduced trafficking of skin-mDCs to the dLN results in decreased iMO recruitment to the dLN, poor IFN-γ production by group 1 ILC, and reduced CXCL9 production by uninfected iMO. These defects could be partly responsible for the previously observed impaired recruitment of NK cells to the dLN and their inability to control the virus in aged mice.

Disclosure of Interest: None Declared
AN INTEGRATIVE ANALYSIS OF POST-TRANSCRIPTIONAL REGULATION OF TYPE-I INTERFERON SIGNALING
S. Straub1*, S. C. Forster1, L. J. Gearing1, N. P. Croft2, T. H. Beilharz2*, K. L. Jeffrey2, P. J. Hertzog1
1Hudson Institute of Medical Research, 2Biomedicine Discovery Institute, Monash University, Clayton, Australia, 3Massachusetts General Hospital, Harvard Medical School, Boston, United States

Introduction: The innate immune system is the primary defense mechanism of the host and is induced by inflammatory stimuli, which are produced by a variety of pathogens. The innate immune response, especially interferon signaling, needs to be tightly regulated to fight infections but avoid toxicity at the same time. MicroRNAs (miRNAs) are a central component of post-transcriptional regulation. Most miRNAs bind in the 3’ untranslated region (UTR) of transcripts, the region between the stop codon and the poly-A tail. The length of the 3’UTR is determined by cleavage and polyadenylation of the naïve transcript in response to binding of different factors to polyadenylation signals. In the last few years it has been shown that polyadenylation sites within the same UTR are used differentially in response to different stimuli. They therefore present a potential regulatory element, due to gain or loss of miRNA and protein binding sites.

Alternative polyadenylation is important for a number of different biological processes, like cell proliferation and differentiation, embryonic development or cancer. A study on alternative polyadenylation in macrophages in response to vesicular stomatitis virus infection, which leads to IFN-beta production, also revealed its importance in the antiviral immune response.

Methods: The Hertzog lab performed RNA sequencing on primary mouse bone marrow macrophages (BMMs) and human induced pluripotent stem cell (iPSC) derived macrophages that had been treated with type-I interferon (IFN). To examine the small RNA response to interferon, we also performed high throughput sequencing of Ago2 bound RNA after a crosslinking IP (HITS-CLIP). To determine the influence of IFN-beta on 3’UTR dynamics in BMMs we performed poly-A-tail sequencing (PAT-seq) after 3h and 12h of treatment and correlated the results with our RNA-seq analysis.

We established the effect of IFN beta treatment on protein levels in BMMs by quantitative mass spectrometry. Further investigation in iPSC derived macrophages or human blood monocyte derived macrophages will follow. To better understand the functional changes induced by type-I interferons we are also investigating changes in the metabolome in BMMs and human iPSC derived macrophages through quantitative metabolomics approaches using mass spectrometry.

Results: We investigated the induction of genes following stimulation with different interferons and looked for a common gene signature. Our experiments revealed more than 100 miRNAs were induced or repressed upon IFN beta treatment of mouse BMMs, and that a large number of these miRNAs were novel. These miRNAs are predicted to target major components of innate immune response, like Tlr4, Ifnar1, Stat1, Jak1 or P2rx7.

The RNA-seq results were correlated with our proteomics studies and will be put in context of changes in the metabolome upon interferon stimulation.

Conclusion: Together, these experiments provide the basis for constructing a global regulatory network of the interferon response.

Disclosure of Interest: None Declared

P247 SIRNAS CONTAINING A UNIQUE 5-NUCLEOTIDE MOTIF ACT AS A QUENCHER OF IFI16-MEDIATED DNA SENSING IN INNATE IMMUNE RESPONSE
H. Sui1*, J. Yang1, X. Hu1, Q. Chen1, B. T. Sherman1, H. C. Lane2, T. Imamichi1
1Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, 2National Institute of Allergy and Infectious Diseases, Bethesda, United States

Introduction: We have previously reported an off-target effect by small interfering RNA (siRNA) that synergistically enhances DNA-mediated IFN-lambda 1 induction in HeLa cells and human primary dendritic cells (DCs) (1). In this induction, RIG-I and IFI16 form a complex through siRNA. The dissociation of IFI16 from this complex in the presence of DNA activates the STING-TBK1-IRF3 signalling pathway. In the present study, we provide evidence for an additional new role for siRNA.

Methods: Total 48 chemically-synthesized siRNAs with various target and sequences were screened to evaluate the impact of siRNA on DNA-mediated innate immune response. IFNs or inflammatory cytokines production were measured using real time RT-PCR with specific probes. And THP1-Lucia™ ISG cells were used in the study to evaluate various DNA agonists-mediated innate immune response, indicated by Lucia luciferase activity. A pull-down assay using biotinylated-DNA and an AlphaScreen proximity analysis were designed to discover the interactions or binding affinity among siRNA, DNA and DAN sensors, IFI16 (2), Ku70(3,4) or cGAS (5).

Results: We found that siRNA containing a unique 5-nucleotide (nt) motif (motif siRNA) suppressed the siRNA-enhanced DNA-mediated induction of IFNs and inflammatory cytokines (CXCL10, TNFα and RANTES) up to 95%. Using a series of variants of the motif siRNA, we determined that the inhibition capability was dependent on the location of the motif. The motif induced suppression only if it was located at the 3’ or 5’ terminus of the siRNA sense strand. siRNA containing the motif suppressed plasmid DNA, Poly(A:T), VACV-60 or HSV-70-mediated
innate immune responses in a dose-dependent fashion. Motif siRNA at a concentration of 50 nM suppressed those DNA agonist-mediated signaling by at least 50% but had no effect on 2’3’-cGAMP-induced signaling. Additionally, we revealed that the motif siRNA inhibited DNA but not RNA virus-mediated IFN induction, indicating that the motif RNA regulated most DNA-sensing pathways except cGAS-mediated downstream signaling. A pull-down assay using biotinylated-DNA illustrated that the motif siRNA interrupted the binding of DNA to IFI16. An AlphaScreen proximity analysis further clarified that the motif siRNA had 2.5-fold higher affinity to IFI16 than its counterpart, a siRNA without the 5-nt motif. Furthermore, we confirmed that the motif siRNA abolished phosphorylation of STING, the downstream signaling pathway related to DNA-mediated IFI16 activation.

**Conclusion:** Collectively, these findings shed light on a novel function of siRNA with a specific 5-nt motif to quench innate immunity.

**Disclosure of Interest:** None Declared

**P248**

**ROLE OF INTERLEUKIN-27 IN THE REGULATION OF IMMUNE RESPONSES AGAINST CHRONIC MALARIA INFECTION**

O. Sukhbaatar1, D. Kimura 1, M. Miyakoda1, S. Nakamae1, K. Kimura 1, H. Yoshida2, K. Yui1

1Molecular Microbiology and Immunology , Nagasaki University, Japan, Nagasaki, 2Biomolecular Sciences, Saga Medical School, Saga, Japan

**Introduction:** IL-27 is a negative regulator of T cell function during malaria infections. We previously reported IL-27-producing malaria-specific CD4+ T cells, Tr27 cells, and their regulatory roles in the immune responses during infection with *Plasmodium berghei ANKA* (PbA)1. Tr27 cells were also induced during the infection with *Plasmodium chabaudi chabaudi* AS (Pcc), a chronic model of malaria infection.

**Methods:** In this study, we evaluated the role of IL-27 in the regulation of the immune responses using a model of chronic malaria infection and IL-27Rα knock-out mice (IL-27R KO).

**Results:** The levels of parasitemia were not significantly different during the early phase of Pcc infection between C57BL/6 (WT) and IL-27R KO mice. However, during the chronic phase, IL-27R KO mice exhibited reduced parasitemia and their CD4+ T cells produced IFN-γ in response to Pcc antigens at levels higher than those from WT mice. When the mice were cured by anti-malaria drug, IFN-γ production by CD4+ T cells remained high in IL-27R KO mice in response to Pcc antigens than those from WT mice. These Pcc-primed mice were challenged with heterologous malaria parasites, PbA, Pcc-primed IL-27R KO mice displayed higher proportions of antigen-specific activated (CD11a<sup>high</sup>CD49d<sup>high</sup>) CD4+ T cells when compared with Pcc-primed WT mice, and enhanced parasite control. CD4+ T cells from IL-27R KO mice produced IFN-γ at levels higher than those from WT mice in response to PbA antigens, while these cells produced IL-10 at levels lower than those from WT mice.

**Conclusion:** These studies suggest that IL-27 signaling promote IL-10 production, and inhibit protective immune responses during the chronic phase of Pcc infection.

**Disclosure of Interest:** None Declared

**P249**

**INHIBITION OF DENDRITIC CELLS IL-12 PRODUCTION BY PARACOCCIDIODES BRASILIENSIS**

A. H. Tavares1,*, G. S. Silva1, D. Silva1

1University of Brasilia, Brasilia, Brazil

**Introduction:** A key component of the innate immune response is dendritic cells (DCs), which are able to recognize, via pattern recognition receptors (PRR), pathogen associated molecules and activate adaptive T-helper (Th) cell responses. In particular, a Th1-based response is considered protective against *Paracoccidioides brasiliensis* (Pb), the causative agent of Paracoccidioidomycosis. The development of Th1 cells relies on the production of IL-12p70 by DCs and we show here that Pb inhibits the production of this cytokine.

**Methods:** Throughout the work bone marrow derived DCs from C57BL/6 mice were infected with virulent yeast cells of Pb isolate 18. When indicated, infected DCs were treated with PRRs ligands PAM3CSK4 (TLR-2) or zymosan (Dectin-1 and TLR-2) and interaction conducted for 24h for cytokine quantification. We also evaluated DCs maturation using flow cytometry and DCs-induced T cell activation of Th responses by Th1, Th2, and Th3 characteristic cytokines production analysis. A paired two-tailed Student’s t-test was used, and a p value ≤ 0.05 was considered statistically significant. In addition, multiple group comparisons were conducted using one-way ANOVA, followed by Bonferroni tests as appropriate.

**Results:** IL-1β, TNF-α and IL-6 were produced in response to the fungal infection and an amplified secretion was observed when agonists were added. In contrast, IL-12p70 were not significantly produced by both infected and infected plus agonist treated-DCs, despite the fact that agonists alone induced significantly IL-12 secretion. Furthermore, Pb drastically reduced the production of IL-12p70 induced by LPS. Interestingly, fungal induced inhibition was not observed for the p40 subunit of IL-12, suggesting that Pb inhibits the specific p35 IL-12 subunit. In fact, mRNA IL-12p35 expression was severely inhibited by Pb in LPS-treated cells. Also, using transwell system we found that direct contact of DCs and Pb is necessary for the inhibition of IL-12p70. Considering this result and the fact that fungal pathogens were shown to suppress IL-12p35 via PRR (mincle) engagement, we used DCs treated with blocking antibody against Mincle or DCs from mice lacking dectin-1, dectin-2, dectin-3 or TLR2 to assess IL-12p70 production. None of these approaches was able to recover IL-12 production. Regarding maturation, the treatment of DCs with the PRRs ligands induced significant maturation as assessed by the expression of CD80 and...
CD86. Finally, It has been shown that DCs infected with Pb fail to activate a Th1 response and our results support this data, as shown by the significant production of IL-13 (Th2) when compared with the IFN-γ (Th1) by T cells co-cultured with infected DCs. Only with the addition of PRR ligands Th cells polarization is reversed despite the fact of impaired IL-12p70 production, the major inducer of Th1 differentiation. This fact may be explained by the increase of IL-18 and IL-27 production by DCs treated with the PRR ligands. These cytokines are related with Th1 differentiation induction and Th2 inhibition.

**Conclusion:** Our preliminary results show that Pb inhibits IL-12p35 production by DCs and the treatment with PRRs ligands enhances DCs activities and may serve to induce a potential protective response.

**Disclosure of Interest:** None Declared

**P250**

**INTRANASAL DELIVERY OF THE TLR7 AGONIST, IMIQUIMOD, PROTECTS AGAINST INFEOEINES A VIRUS-INDUCED MORBIDITY IN MICE**

E. E. To1,2, J. R. Erlich1, R. Luong1,2, F. Liong1, S. Liong1, K. S. Hendricks2, S. Bozinovski2, H. J. Seow1, J. J. O'Leary3,4, D. A. Brooks5, R. Vlahos1, S. Selemidis1

1Biomedical and Health Sciences, Royal Melbourne Institute of Technology, 2Pharmacology, Monash University, Melbourne, Australia, 3Medicine, Trinity College, 4Sir Patrick Dun’s Laboratory, St James’ s Hospital, Dublin, Ireland, 5Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia

**Introduction:** Influenza A virus (IAV) infections constitute a global health and economic burden that continues to cause widespread epidemics annually, which can become life-threatening. The development of anti-viral drug resistance and the lack of effective vaccines for newly emerging viral strains highlight the urgent need for novel pharmacological strategies that prevent viral pathology, irrespective of the infecting strain. Toll-like receptor 7 (TLR7) is a pattern recognition receptor, which drives a powerful anti-viral response, characterized by the production of Type I interferons and pro-inflammatory cytokines that aid in viral clearance (1). Therefore, our aim was to determine the effect of the TLR7 agonist imiquimod on morbidity, lung inflammation, lung function and antibody production caused by IAV infection in mice.

**Methods:** Saline or imiquimod (50µg/mouse) was delivered intranasally once daily to anaesthetised male C57BL/6J mice one-day prior to infection with a low (103 PFU/mouse) or high dose (106 PFU/mouse) of the mouse-adapted Hong Kong X31 IAV strain until mice were culled at day 3 (d3) or 7 (d7) post-infection for analysis. Bronchoalveolar lavage (BAL) extraction was performed to assess airway inflammation, where BAL was differenti a ted into macrophages, neutrophils and lymphocytes. Pulmonary inflammation was examined using H&E staining and pro-inflammatory cytokines and viral mRNA expression was quantified using qPCR. Lung function measurements were carried out using a low-frequency forced oscillation technique and a small-animal ventilator (flexiVent; Scireq). Antibody isotypes in the BAL fluid were measured using the Mouse Isotyping 7-plex ELISA Kit.

**Results:** Imiquimod significantly suppressed body weight loss caused by IAV infection with a maximum reduction of ~60% starting from day 4 (d4) (103 PFU/mouse, n=7-13, p<0.001). At d3 post-infection, imiquimod treatment caused a marked reduction (~50%) in airway and peribronchial inflammation and BAL neutrophil cell counts (105 PFU/mouse, n=8-15, p<0.01) but did not alter macrophage and lymphocyte populations. Imiquimod treatment also resulted in a significant reduction in neutrophil chemoattractants (CCL3, IL-17A and CXCL2), pro-inflammatory cytokines (TNF-α and IL-6) and viral mRNA expression. Furthermore, imiquimod treatment prevented the increase in viral-induced total respiratory system resistance. At d7 post-infection (103 PFU/mouse, n=7-13), there was a modest but significant increase in various antibody isotypes (IgE, IgM, IgG1, and IgG2a)(p<0.05) in BALF following imiquimod treatment compared to the virus control cohort.

**Conclusion:** Our findings highlight the potential of imiquimod as a therapeutic option for the treatment of influenza disease.

**Disclosure of Interest:** None Declared

**P251**

**REGULATION OF THE INTERFERON RESPONSE BY CIS AND TRANS-ACTING LONG NON-CODING RNAS**

S. Valadkhan1, L. Plasek1

1Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, United States

**Introduction:** Transcriptome-wide analyses have pointed to a key role for long non-coding RNAs (lncRNAs) in regulation of diverse aspects of the immune response, including the interferon (IFN) arm of the innate immune response. We have previously shown that upon stimulation of primary human cells with IFN alpha, a large number of lncRNAs show dramatic changes in expression level. In-depth studies on two of the most differentially expressed lncRNAs, NRIR and BISPR, showed that both of them were involved in regulation of the expression of protein-coding interferon-stimulated genes (ISGs). Our studies showed that NRIR negatively regulated the IFN-induced expression of several ISGs, including CMPK2, RSAD2/Viperin, ISG15 and CXCL10 (Kambara et al., 2014). On the other hand, BISPR, which shares the same promoter with BST2/Tetherin, positively regulated the expression of this gene (Kambara et al., 2015). To gain further insight into the role of lncRNAs in the IFN response, we performed a comprehensive analysis of the expression pattern of IncRNAs following IFN stimulation and the potential IncRNA-mediated cis- and trans-regulatory networks during the IFN response.

**Methods:** We analyzed the response of both protein-coding genes and lncRNAs to stimulation by IFN alpha, beta and gamma in both immune system and non-immune
cells through an in-depth analysis of over thirty RNA-seq assays. In addition to defining the differentially expressed genes in each category and the tissue-specificity of the IFN response, we also identified the potential regulatory networks involving regulation of neighboring or remote loci by IncRNAs.

**Results:** Our comprehensive analysis of the IFN response indicates the presence of a number of genes from both protein-coding and non-coding categories which are induced or repressed in response to IFN stimulation in a non-cell-type-specific manner, along with a much larger number of cell type-specific differentially expressed genes. Interestingly, we could identify a large number of loci where IncRNAs showed a reproducible coordinated expression pattern relative to their neighboring protein-coding genes. Preliminary studies in cell lines have confirmed a subset of the identified coordinated expression patterns, pointing to the presence of a widespread IncRNA-mediated regulatory network during the IFN response.

**Conclusion:** Our analyses point to a widespread regulatory role for IncRNAs in regulation of expression of ISGs following the induction of IFN response. Further analysis of the identified IncRNA-mediated potential regulatory networks will not only provide a more accurate picture of the regulation of the IFN response, but also may identify new therapeutic targets and clinical markers which can be used to manipulate/predict the magnitude and character of the innate immune response.

**Disclosure of Interest:** None Declared

**P252**

**IRA1 CONTAINING SUPRAMOLECULAR ORGANIZING CENTERS NON-TRANSCRIPTIONALLY PRIME INFLAMMASOMES IN RESPONSE TO DUAL-TLR STIMULATION**


1LISB, 2RTB, 3LPD, 4NIAID, 5CCR, NCI, Bethesda, United States, 6Institute of Innate Immunity, Bonn, Germany, 7Xencor, Inc., Monrovia, United States

**Introduction:** Macrophages encounter various PAMPs and a coherent immune response involves crosstalk among multiple host PRR pathways that are often organized through higher-order signaling complexes called supramolecular organizing centers (SMOCs). Here, we identify an Interleukin-1 receptor-associated kinase 1 (IRAK1) containing SMOC formed on co-stimulation of TLR4 and TLR1/2, or on bacterial infection, that is distinct from the myddosome and trifosome and links multi-TLR signaling to inflammasome activation.

**Methods:** Both primary and immortalized murine macrophages were treated with single vs dual TLR ligands and assayed through a combination of immunofluorescence, confocal microscopy, high content imaging, colocalization analysis, proximity ligation assays and independent component analysis. Cytokine mRNA and protein were measured by qPCR and ELISA/cytokine bead array respectively. *Yersinia pseudotuberculosis* infections were done in WT and Irak1−/− mice and serum cytokines were assayed using cytokine bead array.

**Results:** We find that IRAK1 SMOCs are formed specifically on dual-TLR stimulation and are enriched for downstream TLR signaling components including TFK1, TRAF6, TAK1 and TAB2. These SMOCs were also enriched for MAPKs like ERK5 and JNK and MAPKKs like MAPK7. The IRAK1 SMOCs did not associate with TLR2, TLR4, MyD88, IRAK2, IRAK3 and TICAM2, and were not associated with any membrane bound organelles. Additionally, the IRAK1 SMOCs did not colocalize with the proteasome or known E3 ligases in TLR signaling like βTrCP1 and Pellino 1/2. Further analysis of IRAK1 SMOCs showed an enrichment of inflammasome components such as ASC. BMDMs primed with dual TLR ligands secreted more IL1α/β than when primed with single TLR ligands, and these IL1 secretion levels were attenuated in Irak1−/− macrophages. The mRNA and protein levels of inflammasome regulators were comparable in WT and Irak1−/− macrophages. This suggests a hitherto unrecognized non-transcriptional role for IRAK1 in coordinating inflammasome activation in response to the more physiological priming input of combined PRR ligands. In a *Yersinia pseudotuberculosis* infection model, we observed increased susceptibility in Irak1−/− mice, suggesting a critical role for IRAK1 SMOCs in shaping the immune response to a multi-PAMP pathogen through multiple host PRR pathways.

**Conclusion:** We have identified a critical role for murine IRAK1-containing SMOCs in diversification of immune pathway activation downstream of combined TLR stimuli, and we demonstrate that the loss of this IRAK1 function leads to a previously unappreciated susceptibility of Irak1−/− mice to Yersinia infection.

This work was supported by the Intramural Research Program of NIAID, NIH.

**Disclosure of Interest:** None Declared

**P253**

**TLR9-SIGNALING REQUIRES LIGAND-INDUCED PHOSPHORYLATION OF TWO SPECIFIC TYROSIINE RESIDUES BY EGFR AND SYK**

M. M. Veleeparambil, D. Poddar, G. C. Sen

1Department of Inflammation and Immunity, Lerner Research Institute Cleveland Clinic, Cleveland, United States

**Introduction:** Signaling by Toll-like receptor 9 (TLR9) requires its ligand-induced Tyr-phosphorylation. We have reported that the Tyr kinase activity of EGFR is essential for TLR9 signaling both in vitro and in vivo. In the current study, we further investigated the nature of involvement of EGFR and another Tyr kinase, Syk, in TLR9 signaling.

161
**Method**: Mouse myeloid cells and human 293XL-TLR9 cells were used for our studies. Immuno-precipitation followed by Western blotting and mass spectrometry was used to analyze Tyr-phosphorylation of TLR9. RT-qPCR and ELISA were used for measuring gene induction. EGFR and Syk kinase inhibitors or ablation of protein expression by shRNAs were used to eliminate the two enzymes; their association with TLR9 was monitored by co-immunoprecipitation. The p values were calculated using a two-tailed unpaired Student t test.

**Results**: Kinase inhibitors and gene knock-down experiments demonstrated that gene induction by TLR9 signaling required the kinase activities of both EGFR and Syk, and physical interaction of TLR9 with EGFR and Syk was needed for its ability to signal. Moreover, ligand-dependent Tyr phosphorylation of TLR9 required both EGFR and Syk kinase activities. The two specific Tyr residues in the cytoplasmic domain of TLR9, which were selectively phosphorylated by EGFR and Syk, were identified by mass spectrometric analyses. Our results demonstrated that Syk was activated by Lyn, in a ligand-dependent manner, and activated Syk could bind to TLR9 and phosphorylate its target Tyr residue. EGFR, on the other hand, was constitutively bound to TLR9; but it could phosphorylate its target Tyr residue only after Syk had phosphorylated its own target.

**Conclusion**: Our results indicate that signaling and induction of all genes by TLR9 are dependent on phosphorylation of two specific Tyr residues present in its cytoplasmic domain. EGFR and Syk are the Tyr kinases that phosphorylate them and hence, required for TLR9 actions.

**Disclosure of Interest**: None Declared

---

**P254**

CHARACTERIZATION OF IFNE AND IFNK RECEPTOR BINDING AND NEUTRALIZATION BY THE POXVIRUS ANTAGONIST B18R

B. Harris1, J. Schreiter2, M. Chevrier2, J. Jordan2, M. R. Walter1

1Microbiology, UAB, Birmingham, 2Janssen Research and Development, LLC, Spring House, United States

**Introduction**: IFNe and IFNk induce microbial immunity at mucosal surfaces and in the skin. They are members of the type-I IFN family, which consists of 16 different IFNs, that all signal through the common IFNAR1/IFNAR2 receptor complex. To date, a biochemical analysis of human IFNε and IFNk binding to the IFNARs has not been performed. To fill this knowledge gap, the receptor binding properties of IFNe and IFNk have been determined and compared to IFNα2 and IFNω. As part of our analysis, we observed that the poxvirus antagonist B18R exhibited poor neutralization of IFNε and IFNk, relative to IFNα2 and IFNω. Thus, we characterized B18R binding to all 16 IFNs. These studies have implications for understanding the unique functions and evolution of the type-I IFN family.

**Methods**: All IFNs were expressed in E.coli. as insoluble inclusion bodies. The IFNs were refolded from solubilized inclusion bodies and purified by affinity chromatography and anion exchange chromatography. The proteins were characterized by mass spectrometry and then subjected to luciferase-based gene expression assays. IFNAR and B18R binding was performed using surface plasmon resonance (SPR) techniques. Models of the proposed interactions were created using Pymol.

**Results**: IFNe and IFNk protein preparations were ~1000-fold less active than IFNα2 in cellular assays. Interestingly, IFNe and IFNk both exhibited disrupted IFNAR2 binding affinity, while their IFNAR1 affinities were similar to IFNα2 and IFNω. As observed for the cellular IFNAR2 receptor, B18R exhibited weaker binding to IFNe and IFNk, relative to IFNα2 and IFNω. The weaker affinity of IFNe and IFNk for IFNAR2 and B18R was due to reduced association rate constants for IFNAR2 and B18R. The binding data was used to build structural models of the IFNε/α-IFNAR and IFNω/κ-B18R complexes, which have been used to identify putative residues responsible for the unique binding phenotypes of IFNe and IFNk.

**Conclusion**: IFNe and IFNk have disrupted IFNAR2 binding properties. Our study suggests IFNe and IFNk may have reduced their IFNAR2 affinities to escape neutralization by virus, yet retain sufficient cellular activities to promote immunity at barrier surfaces.


---

**P255**

LOW-DOSAGE PVSRIPO STIMULATES AND SUBVERTS IFN RESPONSES IN CANCER AND DENDRITIC CELLS

R. Walton1, M. C. Brown2, E. K. Holl3, D. Boczkowski3, V. Chandramohan2, S. Nair3, M. Gromeier2

1Molecular Genetics and Microbiology, 2Neurosurgery, 3Surgery, Duke University, Durham, United States

**Introduction**: Oncolytic viruses are meeting their promise as cancer treatments by focusing on promotion of anti-tumor immunity over direct cancer cell killing. However, many mechanisms which promote anti-tumor immunity also limit viral replication, and replication of oncolytic viruses is a key part of their efficacy. Virus:host innate immune relationships in cancer is thus an area of extreme interest. Our lab developed an oncolytic human rhinovirus:poliovirus chimera, PVSRIPO, which is in clinical trial against glioblastoma, an aggressive form for brain cancer. Using PVSRIPO, and the related picornavirus encephalomyocarditis virus (EMCV), we investigated MDA5 and IFNs in infection of cancer cells. MDA5 is a pattern recognition receptor, targeted to viral double-stranded RNA produced by picornaviruses. We
also investigated PVSRIP0’s role in anti-tumor immune responses after treatment of cancer cells via activation of human dendritic cells.

**Methods:** DM440, a human melanoma cell line, was engineered for stable MDA5 depletion by lentivirus transduction containing MDA5 shRNA. PVSRIP0 or EMCV was added at a multiplicity of infection (MOI) of 0.1 or 0.01 and samples collected for immunoblot, ELISA, or viral titer. Cells were also incubated with IFN-α2 for 24 hours, infected, and assayed as above. Human dendritic cells (DCs) were infected at 1, 10, 50, or 100 MOI and collected as above. DCs were also treated with lysate from PVSRIP0-treated SUM149 human breast cancer cells, non-infectious lysate, or virus-filtered lysate. DCs were analyzed by flow cytometry or used for a cytotoxic HLA-matched T-cell assay using europium-labeled target cells, including cancer cells, tumor antigen transfected DCs, and control antigen expressing DCs.

**Results:** DM440 cells produce IFNs in an MDA5-dependent manner after PVSRIP0 and EMCV infection, and mount intact antiviral immune responses to IFNs. MDA5 activation and IFN treatment inhibit EMCV significantly more than PVSRIP0. Human DCs infected by PVSRIP0 are and activated without significant cytotoxicity. Lysate from cancer cells treated with PVSRIP0 activates human DCs in a virus dependent manner, and DCs activated in this way promote cytotoxic T-cells targeted to tumor-specific antigen.

**Conclusion:** PVSRIP0 is an IFN-resistant oncolytic virus that provokes MDA5 activation of innate immune responses in cancer cells, but is not significantly inhibited by those immune responses. Further, DCs are activated post PVSRIP0 infection, which can lead to tumor-specific cytotoxic T-cell responses, and may lead to antigen spreading. These two phenotypes, DC activation and IFN resistance, are likely related.

**Disclosure of Interest:** R. Walton: None Declared, M. Brown: None Declared, E. Holl: None Declared, D. Boczkowski: None Declared, V. Chandramohan: None Declared, S. Nair Grant / Research support from: Department of Defense breast cancer research program (BCRP) award W81XWH-16-1-0354, M. Gromeier Shareholder of: Istari Oncology, Inc.

**P256**

**IFN-λ ENHANCES TLR3 SIGNALING OF HUMAN INTESTINAL EPITHELIAL CELLS MEDIATED ANTI-HIV ACTIVITY**

X. Wang1, X. Guo1, R. Zhou1, H. Liu1, B. Zhang1, W. Ho1

1Pathology and Laboratory Medicine, Temple University, PHILADELPHIA, United States, 2School of Basic Medical Sciences, Wuhan University, Wuhan, China

**Introduction:** IFN-λ has been shown to have antiviral activity against a broad spectrum of viruses, including HIV. We previously revealed that human intestinal epithelial cells (IECs) express functional toll-like receptor 3 (TLR3), the activation of which releases antiviral factors that inhibit HIV infection of macrophages.

**Methods:** In this study, we examined the effect of IFN-λ on TLR3 signaling of IECs in the context of induction of the anti-HIV interferon-stimulated genes (ISGs) with RT-qPCR, western blot, and Flow cytometry methods.

**Results:** We demonstrated that IFN-λ was able to upregulate the expression of TLR3 and activate TLR3 signaling by Poly I: C, producing a number of antiviral ISGs (ISG15, ISG56, GBP5, and Viperin). In addition, we found that exosomes released from IFN-λ-sensitized and Poly I: C-stimulated IECs contained the antiviral ISGs and the HIV restriction microRNAs (miRNA-28 and miRNA-29a, b, c). The exosomes with the antiviral factors could be taken up by macrophages, resulting in HIV inhibition.

**Conclusion:** These findings indicate that IFN-λ enhances TLR3 signaling of IECs-mediated the antiviral immune response, which may have a key role in the gastrointestinal (GI) innate immunity against HIV infection.

**Disclosure of Interest:** None Declared

**P257**

**EVOLUTIONARY CONSERVED FLAVIN-CONTAINING MONOOXYGENASE FUNCTIONS IN HOST DEFENSE IN C. ELEGANS**

K. Wani1, S. Taubert2, J. E. Irazoqui1

1Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, United States, 2University of British Columbia, Vancouver, Canada

**Introduction:** The innate immune response functions as a first line of host defense against pathogenic microorganisms in all multicellular organisms. _C. elegans_ mounts an innate immune response against multiple pathogenic microorganisms. In response to pathogenic bacteria _S. aureus_, _C. elegans_ rapidly induces the expression of multiple host defense genes. Furthermore, the expression of a majority of these defense genes is under control of HLH-30, the _C. elegans_ homolog of TFEB (transcription factor EB) that also functions in the innate immune response in mammals. In addition to its role in host defense against _S. aureus_, HLH-30 acts as a nutritionally controlled stress-response factor. Thus, we sought to deconvolve the response to the nutritional challenge from the anti-bacterial response against _S. aureus_.

**Methods:** We performed RNAseq profiling in wildtype and _hlh-30_ mutant animals that were either infected with _S. aureus_ or were starved.

**Results:** Our RNAseq analysis shows that there are multiple genes that are specifically induced upon _S. aureus_ infection, and not under starvation. Furthermore, such genes are induced in an HLH-30-dependent or -independent manner. One of the genes that is strongly induced by infection and not starvation is _fmo-2_, which
encodes a member of the flavin-containing monooxygenase family in C. elegans. fmo-2 transcriptional reporters showed much higher upregulation of GFP during S. aureus infection than during starvation. Further, animals lacking fmo-2 succumbed to S. aureus infection faster than wildtype animals. To interrogate the mechanisms that regulate FMO-2 during S. aureus infection, we analyzed C. elegansmodENcode data and found that two transcription factors, HLH-30 and NHR-49 (ortholog of mammalian HNF4a/PPARα), bind to fmo-2 regulatory sequences. To understand whether FMO-2 is regulated by HLH-30 and/or NHR-49 during S. aureus infection, we measured fmo-2 expression in hlh-30 and nhr-49 mutant animals. We found that fmo-2 mRNA induction is greatly impaired in animals lacking hlh-30 and completely eliminated in nhr-49 mutant animals during infection. Similar results were obtained using fmo-2 promoter-driven transcriptional reporter lines. These results suggested that similar to HLH-30, NHR-49 functions in C. elegans host defense during S. aureus infection. To test this hypothesis, we infected nhr-49 mutants with S. aureus and found that they exhibited defective survival of infection. In contrast, animals with a gain-of-function mutation in nhr-49 survived better during infection and had higher levels of fmo-2 mRNA compared to wildtype. Cell-specific rescue experiments showed that nhr-49 works in the intestine to induce fmo-2 during infection.

Conclusion: Our results suggest a model in which transcription factors HLH-30 and NHR-49 bind to the fmo-2 promoter to affect its upregulation specifically in the intestine.

Disclosure of Interest: None Declared

P258
THE NOTCH SIGNALING PATHWAY CONTROLS BASOPHIL RESPONSES DURING HELMINTH-INDUCED TYPE 2 INFLAMMATION
L. Webb1*, O. Oyesola1, S. Frueh1, S. Peng1, R. Cubitt1, J. Grenier1, C. Danko1, E. Tait Wojno1
1Baker Institute for Animal Health and College of Veterinary Medicine, 2Cornell University, Ithaca, United States

Introduction: Type 2 inflammation is characterized by production of the cytokines IL-4, IL-5 and IL-13 and is required for clearance of gastrointestinal helminth parasites, which infect over 2 billion people worldwide. Basophils are innate immune cells that promote expulsion of the helminth Trichuris muris in mice. Key cytokines, such as IL-3, IL-33 and TSLP, that can influence basophil function have been identified. However, the molecular mechanisms that control basophil function and gene expression during helminth-induced type 2 inflammation remain unclear.

Methods: We utilized immunofluorescence and flow cytometry to characterize the basophil population in different tissues during T. muris infection. Basophils sorted directly ex vivo from mouse spleen were used alongside cytokine stimulation and Notch inhibitors to investigate the impact of the Notch pathway on basophil function in vitro. A mouse model that specifically lacks functional Notch signaling only in basophils was used to assess the transcriptional profile of immune cells from naive and infected mice. These mice were then used to analyze the influence of Notch signaling on basophil function in helminth-induced inflammation and the impact of curtailment of this pathway on the outcome of infection.

Results: We show that during T. muris infection, basophils localized to the intestine and upregulated components of the Notch signaling pathway, which regulates inflammatory gene expression programs. In vitro, Notch inhibition abrogated IL-3- and IL-33-elicited IL-4 and IL-6 production from basophils by directly targeting Il4 and Il6. Transcriptional profiling of Notch-deficient basophils revealed that Notch directs basophil responsiveness to inflammatory cues and effector gene expression. Mice lacking basophil-intrinsic Notch signaling had impaired worm clearance and decreased intestinal type 2 effector mechanisms following infection.

Conclusion: These findings demonstrate that Notch regulates basophil gene expression and effector function during helminth-induced type 2 inflammation. We are now actively investigating how basophils interact with other cells in the intestine to orchestrate optimal type 2 immune activation, and the role Notch plays in this process. We are also investigating how Notch signaling is integrated with the key cytokine signals responsible for their activation, IL-3 and IL-33. Our current findings increase our understanding of the molecular signals that control innate immunity during type 2 inflammation and may have far-reaching impact on the development of therapeutics aimed at modulating type 2 immune responses.

Disclosure of Interest: None Declared

P259
THE ROLE OF INTERLEUKIN-10 IN REGULATING NEUROINFLAMMATION RELEVANT TO TAUOPATHIES
L. Weston1, S. Jiang1, D. Chisholm2, K. Bhaskar1
1Molecular Genetics Microbiology, University of New Mexico, Albuquerque, United States

Introduction: Tauopathies include a number of neurodegenerative diseases associated with pathological formations of microtubule-associated protein tau (MAPT or tau) in the brain. Neuroinflammation is a common feature of tauopathies and data shows that microglia-mediated inflammation promotes tau phosphorylation, a signature of tau pathology. However, the role of the anti-inflammatory cytokine, interleukin-10, in regulating tauopathies remains unclear. The aim of this study is to determine if IL-10 has a role in regulating neuroinflammation that promotes tau pathology.

Methods: First, we compared the effects of LPS-induced inflammation on tau pathology in Il10-deficient versus wildtype C57BL/6 control mice. Next, to examine the effects of Il10 deficiency in our human tau (hTau)
transgenic mouse model, we bred Il10-deficient mice to our hTau mice. Brains were perfused and microdissected to determine neuroinflammation and tau phosphorylation using qPCR, MSD, Western blot and IHC.

**Results:** First, Il10-deficient mice injected with LPS showed significantly enhanced tau phosphorylation within 24 h in the hippocampus compared to LPS injected wildtype mice. Interestingly, levels of total tau were significantly reduced in LPS-injected Il10-deficient mice compared to vehicle injected Il10-deficient and wildtype control mice (with or without LPS). LPS injected Il10-deficient mice had significantly elevated levels of activated p38 mitogen activated protein kinase (p38 MAPK) compared to LPS injected wildtype mice. Next, preliminary examination of just two of our Il10-deficient hTau mice showed no significant differences in tau pathology in Il10-deficient hTau versus control hTau mice at an early time point.

**Conclusion:** Our results suggest IL-10 plays a role in limiting inflammation-induced tau pathology in C57BL/6 mice. Preliminary data on Il10-deficient hTau mice are currently insufficient to determine if Il10 deficiency exacerbates tau pathology. However, several more target mice are currently under investigation.

**Disclosure of Interest:** None Declared

---

**P260**

**SANDFLY FEVER SICILIAN VIRUS NSS DIRECTLY TARGETS PROMOTER-BINDING OF IRF3 TO INHIBIT TYPE I INTERFERON INDUCTION**

J. D. Wuerth1, M. Habjan2, A. Pichlmair2, G. Superti- Furga3, F. Weber1

1Institute for Virology, Justus-Liebig University Giessen, Giessen, 2Max Planck Institute of Biochemistry, Martinsried, 3Technical University of Munich, Munich, Germany, 4Center for Molecular Medicine, Vienna, Austria

**Introduction:** Phleboviruses are emerging viruses with a wide spectrum of virulence: for example, Rift Valley fever virus (RVFV) is highly pathogenic, whereas widely distributed Sandfly fever Sicilian virus (SFSV) causes intermediate pathogenicity with mild to incapacitating fevers.

Phleboviruses activate RIG-I signaling and interferon (IFN) induction via a tri-phosphorylated dsRNA-forming promoter structure, the "panhandle". This, however, is counteracted by the non-structural protein NSs, the major virulence factor of phleboviruses. While the NSs protein of highly pathogenic RVFV is known to entirely shut off cellular transcription by targeted sequestration and destruction of host factors, the role of the NSs protein of less pathogenic SFSV has remained elusive.

**Methods:** Tandem affinity purification and mass spectrometry, co-immunoprecipitation, luciferase reporter assay, RT-qPCR, confocal microscopy, domain mapping, promoter binding assay

**Results:** Here, we show that both parental SFSV and its NSs protein inhibit the transcriptional induction of type I IFN. To elucidate the underlying molecular mechanism, we applied tandem affinity purification and mass spectrometry (Pichlmair et al. Nature 2012), and identified interferon regulatory factor 3 (IRF3) as host interactor. Subsequent co-immunoprecipitation and reporter assays confirmed IRF3 as specific interactor and functional target of SFSV NSs: SFSV NSs alone was sufficient to abrogate IRF3-dependent promoter activity induced via MAVS, TRIF, TBK1, or constitutively active IRF3. However, phosphorylation, dimerization and nuclear accumulation of IRF3 were not affected. Instead, SFSV NSs was found to act by masking the DNA-binding domain of IRF3 and thereby hindering promoter transactivation.

**Conclusion:** SFSV NSs is thus surprisingly different from the NSs of highly pathogenic RVFV, as it does not induce a general host transcription block but instead specifically targets IFR3-driven gene expression. Hence, although these and other phleboviruses are highly related, their NSs proteins display remarkably diverse strategies of counteracting the IFN system, probably correlating with their respective virulence levels. To our knowledge, this is not only a new IFN-antagonistic strategy among phleboviruses, but also the first report of a virulence factor of an RNA virus that directly disrupts the binding of activated IRF3 to the IFN promoter in this manner.

**Disclosure of Interest:** None Declared

---

**P261**

**ELEVATED NUCLEAR LAMIN A IS PERMISSIVE FOR NEUTROPHIL TRANSENDOTHELIAL MIGRATION BUT NOT FOR MOTILITY THROUGH DENSE COLLAGEN I BARRIERS**

S. K. Yadav1, S. W. Feigelson1, F. Roncato1, M. Antman-Passig2, O. Sheif2, J. Lammerding3, R. Alon1

1Department of Immunology, Weizmann Institute Of Science, Rehovot, 2Bar Ilan Institute of Nanotechnologies and Advanced Materials, Bar Ilan University, Ramat Gan, Israel, 3Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, New York, United States

**Introduction:** Nuclear deformation is a rate limiting step for migratory cells to pass through narrow spaces. Mechanical stability of the nucleus is dictated by lamins, which form a structural network underlying the inner nuclear membrane. Lamin A is a key intermediate filament component of the nuclear lamina which is downregulated during granulopoiesis. When elevated, lamin A restricts nuclear squeezing through rigid confinements. The nuclei of circulating T cells and neutrophils express low levels of lamin A/C. To address the role of nuclear deformability and mechanical stiffness in leukocyte migration through endothelial barriers and collagenous matrices, we used a model system based on DMSO differentiated HL-60 (dHL-60) cells overexpressing CXCR2 which upon in vitro differentiation give rise to neutrophil-like leukocytes which demonstrate robust ability to cross inflamed endothelial cells under shear flow [1]. We then tested how upregulation of lamin A in these dHL-60 cells affects their...
ability to transit through rigid micron-scale constrictions, endothelial monolayers and collagen I barriers.

**Methods:** Stably modified lamin A overexpressing (Lamin A-OE) variant of CXCR2-HL-60 cell line was generated by retroviral transduction with pRetroX-PrelaminA-IRESS-ZsGreen1 vector [2].

Chemokinesis across 3 or 5 μm rigid pores was measured following standard boyden chamber assay. Leukocyte migration across inflamed HDMVECs was analyzed as described [3].

3D cell motility assays: Neutrophil-like dHL-60 cells were resuspended in cold collagen I solution and injected into the CXCL1 coated Ibidi chamber. Cells were incubated at 37 °C to allow collagen polymerization. Images were acquired using Olympus IX83.

**Results:** Lamin A overexpression in neutrophil-like dHL-60 cells does not affect their GPCR and integrin levels or CXCR2 activity but, restricts their ability to squeeze through small rigid pores. LaminA-OE neutrophil-like cells cross inflamed endothelial barriers through paracellular gaps with partially delayed kinetics. LaminA-OE cells exhibit slower nuclear squeezing and generate larger endothelial gaps during paracellular TEM under shear flow. Most interestingly, lamin A overexpression in neutrophil-like cells restricts nuclear squeezing and chemokine-driven elastase-mediated motility through dense collagen I barriers.

**Conclusion:** Increased nuclear stiffness does not affect chemokinesis but restricts migration through small, rigid pores. Stiffer nucleus delays nuclear squeezing through endothelial junctions and increases the gap size but overall it is permissive for leukocyte TEM. Increased nuclear stiffness restricts elastase dependent crossing of dense collagen I barriers.

**Disclosure of Interest:** None Declared

**P262**

**SYSTEMIC MATERNAL ANTI-VIRAL RESPONSES LEAD TO ABNORMAL FETAL NEURAL TUBE DEVELOPMENT**

L. Yockey1,*, D. Musaev1, C. Qi1, N. Sestan1, A. Pyle2,3, A. Iwasaki1,3

1Yale School of Medicine, 2Yale University, New Haven, 3Howard Hughes Medical Institute, Chevy Chase, United States

**Introduction:** Congenital infections can lead to severe fetal defects, including microcephaly, growth restriction, and fetal demise. The mechanisms by which distinct but diverse pathogens interfere with fetal development are not well understood.

**Methods:** Recently, we showed that the type I interferon responsiveness of the placenta and fetus mediate fetal demise using a mouse model of Zika virus infection1. In order to examine the direct effect of an antiviral response on fetal development, we challenged dams with intraperitoneal or intravascular injections, respectively, of the viral-mimic double-stranded RNA ligands, Poly (I:C) and a selective retinoic acid inducible gene I (RIG-I) ligand, stem loop RNA (SLR)2. We examined the mediators of this response using genetic knockout mice of different components of the antiviral pathway, including MAVS, the adaptor downstream of RIG-I, and the type I IFN receptor (IFNAR). We also determined the effects on the fetus throughout development using morphologic analysis and immunofluorescence staining for developmental markers.

**Results:** Challenge of pregnant mice with Poly (I:C), led to rapid fetal resorption in WT mice, as has been shown previously3. However, when the dam lacked IFNAR, the fetuses were protected from demise but went on to develop anterior neural tube defects, including exencephaly. Challenge of mice with a selective RIG-I ligand, SLR, which induces a strong IFN response, led to fetuses with exencephaly and anophthalmia. Unlike with the Poly (I:C) challenge, these fetuses remained viable to term. These effects were dependent MAVS. The SLR challenge led to a deficiency in the dorsal neuroprogenitors (Pax6+) in the anterior neural tube.

**Conclusion:** A systemic maternal antiviral immune response is sufficient to induce abnormal neural tube development in an interferon-independent manner. Similar neural tube defects have previously been shown using models of the bacterial pathogen associated molecular pattern (PAMP), lipopolysaccharides (LPS)4. This model provides an opportunity to further investigate the mechanisms by which a systemic maternal immune activation (MIA) can impact fetal neural tube development. Our study has the potential to explain some of the early events leading to neural tube defects, many of which have no known genetic or environmental cause.

**Disclosure of Interest:** None Declared

**P263**

**PATHOGEN-ASSOCIATED MOLECULAR PATTERNS INDUCED ADP FACILITATES MONOCYTE RECRUITMENT THROUGH CCL-2 PRODUCTION IN BACTERIAL INFECTION**

X. Zhang1,*, M. Qian1

1East China Normal University, Shanghai, China

**Introduction:** Innate immunity is the first line for the host to protect from invading pathogens and the interface of the interactions between the host and the microbiota. Several types of extracellular molecules called danger-associate molecular patterns (DAMPs) were identified as endogenous danger signals that alert the innate immune system to abnormal cell death, to stress, even to microbial invasion. Extracellular nucleotides including adenosine 5’-triphosphate (ATP), adenosine 5’-diphosphate (ADP) and uridine-5’-diphosphate (UDP) involve in inflammatory response.

**Methods:** Among them, ADP has been shown to play a significant role during inflammation, but research on function and signaling induced by extracellular ADP via specific P2Y receptors has not been presented in regulating innate immune response, like host defense against bacterial infections, and it’s vague for us to
explorer the molecular mechanism of extracellular nucleotides.

**Results:** Here, we demonstrate that excess ADP is released from immune cells during bacterial infection. And, extracellular ADP enhances MCP-1 mediated monocytes/macrophages recruitment *in vitro* and *in vivo*, so that resulted in a more efficacious clearance of invaded *E.coli* 0111:B4 and lower mortality in peritonitis mouse model through activation of Gαs-coupled P2Y receptors. Furthermore, we also provide evidence that increased intracellular concentration of cAMP (inhibition of Gαs-coupled P2Y receptors signaling) blocked the ADP-dependent activation of ERK MAPK pathway via PKA phosphorylation, which in turn associated with MCP-1 production as well as migration in macrophages.

**Conclusion:** Taken together, our findings uncover extracellular ADP can be as a kind of endogenous immunoregulatory modulator in the up-regulation of host innate immune response against bacterial infections through a certain unknown mechanism. Overall, our study reveals a relationship between danger signals and innate immune responses, which suggests the potential therapeutic significance of ADP-mediated purinergic signaling in infectious diseases.

**Disclosure of Interest:** None Declared

---

**P264**

THE MOLECULAR BASIS OF HEPATITIS B VACCINE NON-RESPONSIVENESS

A. Ziegler1,2, M. Ulthe1, V. von Bülow2, M. Verboom3, M. Hallensleben3, S. Zimmermann-Schriek2, C. Davenport4, L. Wiehlmann4, C. Falk5, U. Kalinke1

1Experimental Infection Research, TWINCORE GMBH, 2Occupational Health Service, 3Department of Transfusion Medicine, 4Core Unit ‘Next Generation Sequencing’, 5Institute of Transplant Immunology, Hanover Medical School, Hannover, Germany

**Introduction:** After a complete cycle of three vaccinations against the hepatitis B virus (HBV), most vaccinated individuals show protective anti-HBs antibody titers of more than 100 IU/L that are long-lasting. However, approximately 5% of all vaccinated people do not mount protective antibody responses and are so-called non-responders. The titer of non-responders stays permanently lower than 10 IU/L, which can constitute a significant problem for health-care workers, who are at high risk to get in contact with contaminated blood. In the case of influenza vaccination, the phenomenon of non-responsiveness increases with advanced age. In contrast, the group of HBV non-responders already includes young and otherwise healthy people. Although the phenomenon is known for many years, the molecular basis of HBV vaccine non-responsiveness is still not well understood.

**Methods:** We initiated an observational study to monitor the immune reactions after a HBV vaccination to compare the immune responses of non-responders with those of responders. This immunomonitoring includes a cytometer-based analysis of blood samples to analyze the distribution of various immune cell subsets as well as their activation status. Additionally, the cytokine responses of responders and non-responders will be analyzed. The second arm of the project includes a whole genome sequencing, which allows the search for genetic markers that are associated with vaccine responsiveness.

**Results:** We adapted a bioinformatics approach aiming for cluster analyses of flow cytometry data. First results will be presented during the conference.

**Conclusion:** Based on the results, we hope to identify biomarkers, which would allow the prediction of HBV vaccination non-responsiveness. This knowledge will be the basis to develop improved vaccination strategies that induce protective antibody responses also in non-responders.

**Disclosure of Interest:** None Declared

---

**P265**

CYTOKINES ARE MODULATE BY EICOSANOIDS IN CELLS STIMULATED WITH BOTHROPS SNAKE VENOM

K. F. Zoccal1,2, G. Z. Ferreira1, M. K. B. Prado1, L. G. Gardinassi1, S. V. Sampaio1, L. H. Faccioli1

1Departamento de Análises Clínicas, Toxicológicas e Bromatológicas. , Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (FCFRP-USP), 2Centro Universitário Barão de Mauá, Ribeirão Preto, Brazil

**Introduction:** Envenomation by Bothrops sp. promotes the production of inflammatory mediators that are responsible for local and/or systemic effects. Interaction between venom components and host cells induces chemokine, cytokine and eicosanoid storms. However, little is known about the production of inflammatory mediators, and their regulation by bioactive lipids, in response to *B. jararaca* or *B. jararacussu* venoms.

**Methods:** Therefore, this study focused on the following aspects: 1) the production of cytokines and chemokines in response to *B. jararaca* or *B. jararacussu* venoms by immortalized cell lineages of human monocytes and mouse alveolar macrophages; 2) the effects of exogenous PGE2 or LTB4 on the production of cytokines and chemokines by cell lineages stimulated with Bothrops venoms; 3) the influence of NF-κB activation on this process.

**Results:** In this study, we demonstrate that *B. jararaca* or *B. jararacussu* venoms induce macrophage inflammatory protein 1-alpha (MIP-1α) and IL-1β production by THP-1 (cell line of human peripheral blood monocytes) and AMJ2-C11 (cell line of mouse alveolar macrophage). We also uncovered that venoms, from both Bothrops species, induce NF-κB activation by THP-1 cells. Furthermore, we observed that exogenous PGE2 reduces MIP-1α production, while increasing IL-1β production in cells stimulated by *B. jararaca* or *B. jararacussu* venoms. Interestingly, exogenous LTB4 had the opposite effect by reducing IL-1β and increasing MIP-1α released by THP-1 and AMJ2-C11 cell lines.

**Conclusion:** Our results suggest that, differential eicosanoid metabolism in myeloid cells is tightly
associated with the production of inflammatory mediators after stimulation B. jaranaca or B. jararacussu venom.

Disclosure of Interest: None Declared

P265.A
TARGETING STING WITH COVALENT SMALL-MOLECULE INHIBITORS
A. Ablasser1,*
1Global Health Institute, EPFL, Lausanne, Switzerland

Introduction: Senescence, a cellular program triggered by various distinct stresses, has emerged as an important contributor to aging-associated diseases. One critical feature, which underlies some of the maladaptive effects of senescent cells, is their inflammatory secretome, collectively referred to as the senescence-associated secretory phenotype (SASP). Recently, we have defined a critical role for the innate DNA sensing pathway comprising cyclic GMP-AMP synthase (cGAS) and Stimulator of interferon genes (STING) in the regulation of the SASP. Briefly, we found that cGAS recognizes aberrant cytosolic chromatin fragments (CCFs) in senescent cells and, in turn, triggers the production of SASP factors through STING. Our finding of aberrant activation of innate immune signalling in senescence raises the possibility that targeting this pathway may provide beneficial effects in senescence-associated pathologies. However, the development of pharmacological inhibitors that specifically act on molecules of the innate DNA sensing pathway has remained a major challenge.

Methods: Here we use distinct triggers of senescence and evaluate the effect of pharmacological inhibition of STING during senescence in cells in vitro and in a disease model of senescence in vivo.

Results: We report the discovery of highly potent and selective small molecule antagonists of stimulator of interferon genes (STING). In depth characterisation of the compounds uncovered an entirely unexpected mechanism to pharmacologically antagonise STING signalling. We show that the discovered compounds reduce STING-mediated inflammatory cytokine production in various contexts in vitro and, moreover, we demonstrate their therapeutic utility in autoinflammatory disease in mice. Finally, we demonstrate the effect of acute inhibition of STING in contexts of cellular senescence.

Conclusion: In sum, our work describes the efficacy of STING antagonists and provide a proof-of-concept of the realization of anti-STING therapies. We propose targeting STING with small molecules may be beneficial for diseases caused by chronic inflammation, potentially also diseases driven by the SASP.

Disclosure of Interest: A. Ablasser Consultant for: IFM Therapeutics

P265.B
ANTIMICROBIAL EFFECT OF IL-26 AGAINST BORRELIA BURGDORFERI, THE LYME DISEASE SPIROCHETE
A. Shah1,*, A. Marin1, J. Cervantes1
1Texas Tech University Health Science Center - El Paso, El Paso, United States

Introduction: Lyme disease is the most common arthropod borne infection in the United States. The disease mainly originates from the inflammatory response of the human host to Borrelia burgdorferi (Bb). A fraction of infected individuals will suffer from inflammatory complications despite the use of antibiotics. Alternative forms of treatment are necessary.

We here tested the antimicrobial activity of IL-26, a recently categorized as an antimicrobial peptide (AMP) against Bb in vitro.

Methods: We utilized temperature-shifted Bb to mimic changes in the bacterium when transmitted from the tick to the mammalian host. To test the effect of IL-26 in the growth of Bb, we utilized a colorimetric viability assay. This method can evaluate multiple concentrations of IL-26 in a 96 well plate format and allows quantification of growth through colorimetric detection.

Results: We determined a MIC value of 162 ug/ml for IL-26 monomer and 107ug/ml for IL-26 dimer. Inhibition of Bb growth was observed as early as 12 hours, and persisted for up to 48 hours.

Conclusion: As inflammatory sequelae of Bb infection appears to be refractory to antibiotics in many cases, novel antimicrobial agents are crucial. Our results showed that IL-26 (monomer and dimer) has the potential to control Bb growth in vitro. Further research aiming to characterize the role of IL-26 in controlling other aspects of the inflammatory response against Bb are currently in pursuit.

Disclosure of Interest: None Declared

P265.C
REPLICATION HETEROGENEITY DRIVES DISTINCT CELLULAR RESPONSES TO INFLUENZA A INFECTION IN VIVO
E. Fay1,*, L. Sjaastad2, J. Fiege2, M. Macchietto2, I. Stone2, M. Markman2, S. Shen2, R. Langlois2
1Microbiology & Immunology, 2University of Minnesota, Minneapolis, United States

Introduction: Influenza A virus (IAV) infects a broad range of cell types within the respiratory tract (1). The cells initially targeted by the virus in a naïve host are the site of primary replication and virus spread. These cells are difficult to detect using replication competent IAV as the virus rapidly spreads to secondary cells. To overcome this obstacle, we utilize a fluorescence expressing single cycle IAV (sciAV) to identify and characterize the cells initially infected in the mouse lung.

Methods: Three fluorescent sciAVs were generated as previously described (2): sciAV-mCherry, sciAV-GFP, and sciAV-destabilized GFP (destGFP), which expresses a GFP protein with a short half-life (3). Mice were infected with a fluorescent reporter sciAV and lungs harvested for analysis by flow cytometry or epifluorescent microscopy at 12-48 hours post infection (hpi). For sequential infections,
mice were first infected with sclAV-GFP, then sclAV-mCherry at 24 hpi, and harvested at 48 hpi. Populations of infected and uninfected epithelial cells were sorted out for RNA-seq at 24 hpi. 

**Results:** Following infection with sclAV-mCherry, we observed two distinct populations of infected lung epithelial cells: cells with high virus replication and cells with low virus replication. This suggests that some cells within the lung are intrinsically permissive to virus replication while others are able to blunt replication. Using RNA-seq, we found distinct cellular pathways that are significantly impacted in each population of infected cells compared to uninfected cells. Additionally, there are subsets of interferon-stimulated genes that are specifically upregulated in each population. These results were corroborated with sclAV-destGFP, which identifies cells with active virus replication. We have also used sequential infection with sclAVs to analyze how tropism changes during virus spread. We found that ciliated cells are better protected from secondary infection compared to other cell types, and this is at least partially mediated by type I and type II interferons.

**Conclusion:** We demonstrated heterogeneity in the levels of virus replication in vivo. Using two different virus reporters we revealed that distinct sets of antiviral genes are expressed in cells harboring low and high levels of virus replication. Through a sequential infection strategy, we found that virus tropism is altered during virus spread where ciliated epithelial cells have augmented protection from infection. These results demonstrate the dynamic environment within tissues that is driven by both virus replication levels and the cell type infected.

**Disclosure of Interest:** None Declared

---

**P265.D**

**KINETICS OF DOWNREGULATION OF TRANSCRIPTION FACTOR E2-2 BY TLR7, TLR9, AND TLR4 AGONISTS IN PRIMARY HUMAN PLASMACYTOID DENDRITIC CELLS**

H. Dewald1,*, P. Fitzgerald-Bocarsly1,2

1Rutgers University School of Graduate Studies, 2New Jersey Medical School, Newark, United States

**Introduction:** Plasmacytoid dendritic cells (pDCs) are the main producers of type I interferons (IFN-α) in the body. pDCs are transcriptionally controlled by the basic loop-helix-loop transcription factor E2-2 (TCF4). E2-2 governs pDC development, function, and phenotype. Stimulation of pDCs leads to decreased E2-2 levels, possibly contributing to a change in phenotype and function and allowing pDCs to become more adept antigen presenting cells. However, the mechanisms that regulate E2-2 are not fully understood. It remains unknown what signals induce downregulation in human pDCs and the kinetics of E2-2 regulation. Therefore, we investigated what ligands contribute to regulation of E2-2 and how stimulation impacts E2-2 expression over time.

**Methods:** Peripheral blood mononuclear cells (PBMCs) were treated with TLR 7 ligand R848 for 6 hours, and TLR 7 agonist HSV-1, TLR 9 agonists influenza A, CpGA, and CpGB, and recombinant IFN-a2b, and TLR agonist lipopolysaccharide (LPS) for up to 24 hours. E2-2 expression was analyzed via flow cytometry using methanol permeabilization. PBMCs were pre-treated with IFN-a2b for 1 hour followed by a 6-hour R848 stimulation to determine if IFN-a2b could prevent E2-2 downregulation.

**Results:** R848 induced downregulation of E2-2 by 6 hours; there was no significant downregulation at earlier time points. Stimulation with viruses and CpGs did not significantly decrease E2-2 expression at 6 hours; however, it was significantly downregulated by 18 hours. Treating cells with LPS induced the greatest decrease in E2-2 expression of the ligands tested at 18 and 24 hours. Treating cells with recombinant IFN-a2b did not induce downregulation of E2-2 at the protein level and pre-treating cells with recombinant IFN-a2b for 1 hour before R848 stimulation did not prevent downregulation.

**Conclusion:** We have established the kinetics of E2-2 downregulation during in vitro stimulation of human pDCs with TLR7, 9, and 4 ligands and determined IFN-adoes not cause diminished E2-2 expression. We found that significant E2-2 downregulation does not occur until after maximal IFN-a-production when pDCs are stimulated with virus or R848. Interestingly, LPS, which does not induce IFN production, caused a more profound downregulation of E2-2 than TLR7 or 9 agonists. Since previous mouse models have indicated that IFNAR signaling is required for E2-2 downregulation, we treated PBMCs with IFN-a and found that it alone is not sufficient to induce downregulation of E2-2 and pre-treatment with IFN-a does not prevent downregulation. ELucidating the regulation and kinetics of E2-2 expression will further our understanding of the role E2-2 plays in the transition from interferon producing cells to antigen presenting cells and how this is impacted during disease.

**Disclosure of Interest:** None Declared

---

**P265.E**

**G(ALPHA)I2 SIGNALING REGULATES INFLAMMASONOME ACTIVITY AND CYTOKINE PRODUCTION BY BIASING MACROPHAGE PHENOTYPE DETERMINATION**

N. R. Nabari1,2,*, A. Vural1, I.-Y. Hwang1, S. Sohn2, C. Park1, M. C. Karlsson2, J. Blumer3, J. H. Kehrl1

1Laboratory of Immunoregulation, NIAID, NIH, Bethesda, United States, 2Department of Microbiology, Tumor, and Cell Biology, Karolinska Institutet, Stockholm, Sweden, 3Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, United States

**Introduction:** Macrophage polarization is driven by signaling from the tissue microenvironment. G-protein coupled receptors (GPCRs) are essential in transducing cell surface signals, and heterotrimeric Gα signaling links these receptors to downstream effectors to shape biological responses. Several Gα coupled GPCRs have...
been implicated in driving both M1 and M2 polarization in a ligand specific manner, presumably due to biased agonism by different ligands. Here, we use genetically modified mice to investigate the physiological role of Gα2 in macrophage polarization.

**Methods:** We generated bone marrow derived macrophages (BMDMs) from mice deficient in Gα2 (Gna2-/-) and knock-in (KI) mice with a point mutation resulting in excess Gα2 signaling (Gna2G184S/G184S, G184S KI). We monitored IL-1β secretion after activation of the NLRRP3, AIM2, and NLRC4 inflammasomes, and cytokine release (TNF-α, IL-6, and IL-10) after stimulation with LPS. We used RT-PCR to quantify mRNA of typical M1 genes (TNF-α, iNOS, and IL-12p40) after LPS stimulation and typical M2 genes (Ym1, Fizz1, and Arg1) after IL-4 stimulation. Finally, we looked at the ability of Gα2 deficient BMDMs to suppress T cell proliferation in co-culture.

**Results:** Gα2 deficient BMDMs released significantly less IL-1β than wild type (WT) controls for all inflammasome assayed, while G184S KI BMDMs release more IL-1β. We determined these differences are due to differences in the LPS priming phase of inflammasome activation, as Gα2 deficient BMDMs showed less TNF-α, IL-6, and pro-IL-1β after LPS stimulation while G184S KI BMDMs showed increases in each of those cytokines. Of note, Gα2 deficient macrophages had higher levels of IL-10 secretion after LPS stimulation while BMDMs with excess Gα2 signaling had lower levels of IL-10 secretion, indicating Gα2 may play a role in macrophage polarization. LPS stimulated Gα2 deficient BMDMs showed decreased TNF-α, IL-12p40, and iNOS mRNA and increased Ym1 and Fizz1 mRNA after IL-4 stimulation. On the other hand, G184S KI BMDMs had higher levels of pro-inflammatory markers (TNF-α, IL-12p40 and iNOS) after LPS and lower levels of anti-inflammatory markers (Arg1, Ym1, Fizz1) after IL-4 stimulation. Finally, co-culture of Gα2 deficient BMDMs with CD3/CD28 stimulated T cells showed that both M1 and M2 polarized Gα2 deficient BMDMs suppressed T cell proliferation to a greater extent than the WT controls.

**Conclusion:** Our results indicate that excess Gα2 signaling drives M1 macrophage polarization, while a deficiency in Gα2 signaling drives M2 macrophage polarization. These differences in macrophage phenotype determination affect cytokine secretion after a variety of stimuli. These results suggest that stimulation or inhibition of macrophage specific Gα2-coupled GPCRs could lay the foundation for the treatment of many inflammatory diseases via macrophage reprogramming.

**Disclosure of Interest:** None Declared

---

**P265.G**

**TYPE I IFNS DRIVE HEMATOPOIETIC PROGENITOR CELL LOSS VIA RIPK3-DEPENDENT IL-18 PRODUCTION AND RIPK1-DEPENDENT CELL DEATH**

K. C. Macnamara¹, J. Smith¹, J. Howard¹

¹Immunology and Microbial Diseases, Albany Medical College, Albany, United States

**Introduction:** Type I interferons (IFNα/β) modulate immune responses to most pathogens and are thought to suppress hematopoietic function, yet their impact on hematopoietic stem and progenitor cells (HSC/HSPCs) during infection remains unclear. Type I IFNs are elicited by many infectious organisms, and while necessary for protection against many viral infections, they can also induce pathology in a number of bacterial infections. As HSC/HSPCs support production of all immune cells, their function is vital to appropriate host defense responses.

**Methods:** Thus, we used gene-targeted mice and a murine model of tick-borne infection to investigate the
release in a caspase-11 dependent manner following Gram-negative bacterial infection.

Results: We have characterized the molecular basis of secretion of a candidate alarmin in vitro and in vivo as well as its function in murine model of sepsis.

Conclusion: Overall, these findings provide new insights into potential biomarkers and therapeutic targets for blocking the lethal inflammation in sepsis.

Disclosure of Interest: None Declared

P266
VIRAL INFECTION DRIVES PLASTICITY IN COMMENSAL-INDUCED GAMMA DELTA T CELLS AT THE OCULAR SURFACE

D. M. Previte1,2, B. R. Treat1, A. J. St. Leger1,2
1Ophthalmology, 2Immunology, University of Pittsburgh, Pittsburgh, United States

Introduction: Ocular colonization with the commensal Corynebacterium mastitidis (C. mast) induces the recruitment of interleukin (IL)-17 producing γδ T cells to the conjunctiva, which protect the ocular surface from bacterial or fungal infections. Here, we show that the presence of C. mast provides a similar γδ T cell-mediated protection against corneal infection with Herpes Simplex Virus type 1 (HSV-1), which is a leading cause of blindness in developed countries.

Methods: The eyes of three-week-old C57Bl/6 mice were inoculated with 5x10^5 colony forming units (cfu) C. mast every two to three days for a total of three inoculations. Two weeks later, C. mast colonization was verified by conjunctival swabs on TSA blood agar plates. C. mast- or non-colonized animals were then infected via the cornea with a pathogenic (RE Lausch) or non-pathogenic (KOS) strain of HSV-1 at a dose of 1x10^5 plaque forming units (pfu) per eye. Pathology was measured by using fluorescein staining to assess corneal epithelial lesions at 2 days post-infection (dpi) and by measuring corneal opacity at 14 dpi. Animals were sacrificed at various times post-infection and eye draining lymph nodes, conjunctivae, corneas and trigeminal ganglia were taken for analysis by multi-parameter flow cytometry. Naive and C. mast-inoculated only animals served as controls.

Results: Our results reveal that colonization of the conjunctiva with C. mast reduces pathology associated with corneal HSV-1 infection compared to C. mast negative controls. Differences in disease progression were observed as early as 3 dpi, with sustained protection seen in C. mast+ animals at 14 dpi (peak disease). Specifically, mice colonized with C. mast had fewer macrophages and neutrophils in the cornea at 3 dpi, indicative of more efficient viral clearance. Concurrently, conjunctival γδ T cells, which only produced IL-17 in response to C. mast, acquired an ability to produce IFNγ simultaneously with IL-17. After acute infection and the initial inflammatory response subsided, γδ T cells reverted back to only producing IL-17. Upon infection with a non-pathogenic strain of HSV-1 (KOS), this dual production of IL-17 and IFNγ was not observed.
Conclusion: Our data indicate that in addition to protecting the ocular surface from bacterial and fungal infections, conjunctival colonization with *C. mast* also reduces pathology associated with blinding viral infection. This is likely due to the *C. mast*-dependent recruitment of γδ T cells to the conjunctiva, which we show exhibit a degree of plasticity that allows them to produce both IL-17 and IFNγ. As this phenotype is only demonstrated upon infection with a pathogenic strain of HSV-1 (RE Lausch) and not with a non-pathogenic strain of HSV-1 (KOS), we conclude that the degree of inflammation induced by pathogens drives γδ T cell plasticity and the downstream anti-viral response(s). Future studies include elucidating the mechanism that drives dual cytokine production in these γδ T cells and resolving the influence of this subset on anti-viral adaptive immune responses and viral latency.

**Disclosure of Interest:** None Declared

---

**P267**

BACTERIA IN HUMAN INTESTINE PROMOTE MUCOSAL IMMUNE DEVELOPMENT IN UTERO


1Medicine, 2Pediatrics, UCSF, 3Infectious Disease Initiative, Chan Zuckerberg BIOHUB, San Francisco, United States

Introduction: Mucosal immunity influences host-microbial interactions and is evident in the human fetal intestine by 11-14 weeks of gestation1; the developing intestine is populated by memory T cells2 and dendritic cells capable of responding to microbial stimuli2. Recent evidence for bacterial presence in utero comes from DNA-based, culture-independent studies of the placenta3 and neonatal meconium4. However, whether intestinal microbial encounters occur in utero and shape immune maturation has not been investigated.

Methods: We established a bank of 50 fetal meconium samples collected, which underwent 16S V4 rRNA gene profiling to identify a putative microbiome and in parallel, the adjacent lamina propria was digested and T cells were profiled by flow cytometry.

Results: We identified subsets of fetal meconium that were relatively enriched in *Lactobacillus* or *Micrococcus*, which related to divergent proportions of lamina propria innate-like PLZF-CD161+ CD4+ T cells in paired intestinal samples. Mimicking conditions in the fetal intestine enhanced isolation of viable *Lactobacillus* and *Micrococcus* strains from fetal meconium. In contrast with phylogenetically related reference strains, fetal isolates utilized placental hormones, remained viable within macrophages, and exhibited genus-specific capacity to promote immune tolerance. *Lactobacillus* isolate inhibited NLRP3 activity for inflammation in the dextran sodium sulfate (DSS)-induced murine model of colitis. Body weight was monitored, tissue pathology was assessed histologically, and cytokine production was measured in colon explants and in splenic macrophages and neutrophils. Additionally, the bacterial content in stool was analyzed by quantitative PCR.

Results: PKR reduced DSS-induced weight loss and repressed inflammasome activity. Correspondingly, inhibiting the NLRP3-inflammasome restored control of weight in the absence of PKR expression. PKR promoted modest tissue injury at the early onset of colitis, but reduced more severe pathology at later time points and changed gut physiology, as evidenced by altered goblet cells and changed abundance of *Bacteroides* species in the stool. These different functions of PKR showed variable dependence for substrate phosphorylation.

Conclusion: PKR maintains gut homeostasis via control of inflammasome activity.

**Disclosure of Interest:** None Declared
P269
MULTI-TARGETING STRATEGIES AGAINST INFLAMMATORY DISEASES
U. Saqibi1,2
1Indian Institute of Technology, Indore, India
Introduction: Myeloid differentiation primary response protein 88 (MyD88) has long been termed as a central player in the inflammatory pathway. More recent studies clearly suggest that it is an important therapeutic target in inflammation. On the other hand, arecent study about orphan nuclear receptor (Nur77)and p38 interaction leading to increased Lipopolysaccharide (LPS)-induced hyperinflammatory response, suggests this binary complex as a therapeutic target. In the current study, we have designed inhibitors that can inhibit both MyD88 and Nur77 at the same time. Since both MyD88 and Nur77 are an integral part of the pathways involving LPS-induced activation of NF-kB mediated inflammation, we tried to target both proteins with the same library in order to retrieve compounds having dual inhibitory properties.
Methods: To perform this, we developed a homodimeric model of MyD88 and along with the crystal structure of Nur77, screened a virtual library of compounds from the Traditional Chinese medicine (TCM) database containing about 61,000 compounds.
Results: The resulting hits were analyzed for their efficacy for dual binding and were probed for developing a common pharmacophore model which could be used as a prototype to screen compound libraries as well as to guide combinatorial library design to search for ideal dual-target inhibitors.
Conclusion: Thus, our study delineates the identification of novel leads having dual inhibiting effects due to binding to both MyD88 and Nur77 targets.
Disclosure of Interest: None Declared

P270
PYRIN INFLAMMASOME REGULATES MUCOSAL IMMUNITY THROUGH DISTINCT MECHANISMS
D. Sharma1,2; A. Malik1; C. Guy3; P. Vogel1; T.-D. kanneganti1
1Immunology, St. Jude Children’s Research Hospital, 2Immunology, St Jude Children’s Research Hospital, Memphis, United States
Introduction: Mutations in the Mediterranean fever gene (MEFV or pyrin) are associated with hereditary autoinflammatory disease and severe IBD. Inflammatory bowel diseases (IBD) increase the risk for colorectal cancer. Expression of MEFV, a sensor protein that the initiates assembly of the inflammasome complex, is increased in colon biopsies from patients with colorectal cancer. We investigated the role of pyrin in intestinal homeostasis in mice.
Methods: Mefv−/−and C57/BL6 mice were subjected to azoxymethane - dextran sodium sulfate (AOM-DSS) model of colitis and colon cancer, irradiation-induced injury, and CD40 induced colitis. An organoid culture was used to assess the proliferative potential of stem cells. Colon tissues were collected at different time points during colitis development and analyzed by histology, immunohistochemistry, immunoblotting, or ELISAs (to measure cytokines). Spleen and mesenteric lymph node were collected, processed, and analyzed by flow cytometry. Colon epithelial permeability was measured in mice with colitis by gavage of fluorescent dextran and quantification of serum levels.
Results: Pyrin was expressed in colons of control mice and expression increased during chronic and acute inflammation. Mefv−/−mice developed more severe colitis than control mice, with a greater extent of epithelial hyperplasia and a larger tumor burden. Mefv−/−mice had increased epithelial permeability early during DSS administration and increased levels of inflammatory cytokines and chemokines during acute colitis. On the other hand, IL18, which depends on the inflammasome for maturation and release, was significantly lower in colons of Mefv−/−mice. Further, the inflammatory milieu in Mefv−/−mice was pro-tumorigenic with increased STAT3 activation, and stem cell activity in the colon and reduced cytotoxic T cell activation in draining lymph nodes and spleen. Stem cell proliferative potential was not affected by pyrin; thus, the increased tumorigenesis was associated with the inflammatory status in the colon. Administration of rIL18 to Mefv−/−mice reduced epithelial permeability, intestinal inflammation, the severity of colitis, and colon tumorigenesis. The protective role of pyrin in restricting mucosal damage did not extend to other models of inflammation including irradiation-induced injury and colitis induced by anti-CD40 antibody. In fact, overt activation of pyrin leads to IL-18 mediated colitis in a mouse model of pyrin auto activation.
Conclusion: These data demonstrate that Pyrin activation plays a dichotomous role in mediating mucosal immunity. While pyrin activation promotes restorative response during epithelial damage, unregulated pyrin activation within the immune cells promotes inflammation and colitis.
Disclosure of Interest: None Declared

P271
LONG NON-CODING RNAS (lncRNAs) AS REGULATORS OF INTESTINAL HOMEOSTASIS AND INFLAMMATION
L. Shmuel Galia1,2; F. Humphries1, K. A. Fitzgerald1
1Department of infectious Disease, UMASS medical school, Worcester, United States
Introduction: LncRNAs are the most abundant non-coding RNAs in the mammalian genome. We have recently identified long intergenic non-coding RNA-EPS (lincRNA-EPS) that controls the basal expression of immune genes in macrophages. LincRNA-EPS deficient mice exhibit hyper-susceptibility to inflammatory challenge and lethality following endotoxin induced septic shock.
Methods: RNA sequencing, NanoString, in vivo DSS-induce colitis mice model, ELISA, histology, qPCR

Results: Interestingly, lincRNA-EPs is highly abundant in intestinal tissues, with highest expression observed in colon tissue. RNA-seq analysis on purified colonic monocytes and macrophages from mice exposed to DSS revealed downregulation of lincRNA-EPs. Indeed, RNAseq analysis also identified other novel, differentially expressed IncRNAs in the gut. To this end, we validated the transcript levels of these genes using custom made Nanostring codesets. Nanostring analysis revealed changes to transcript levels of novel IncRNAs that may contribute to the disease pathology. Based on the previously reported role of IncRNA-EPs as a repressor of inflammatory responses and its downregulation in experimental colitis we hypothesized that lincRNA-Eps may suppress inflammatory responses to maintain intestinal homeostasis. Thus, lincRNA-EPs deficient mice and WT littermates were administered DSS and assessed for intestinal inflammation. LncRNA-EPs-deficient mice were more susceptible to colitis when compared to WT littermates, as indicated by increased clinical signs of colitis and elevated cytokines in colonic monocytes and epithelial cells.

Conclusion: Overall, this data indicates that lincRNA-Eps expression in the colon prevents inflammatory responses to maintain intestinal homeostasis. Taken together, this study emphasizes the contribution of IncRNAs as immune-regulators in colitis and may lead to the discovery of novel mechanisms of disease pathogenesis and new targeted therapies.

Disclosure of Interest: None Declared

P272
DIFFERENTIAL INDUCTION OF INTERFERON STIMULATED GENES BETWEEN TYPE I AND TYPE III INTERFERONS IS INDEPENDENT OF INTERFERON RECEPTOR ABUNDANCE

M. Stanifer1*, K. Pervolaraki1, S. Rastgou Talem2, F. Bormann2, T. Hoefer2, S. Boulant1,2
1University Hospital Heidelberg, 2DKFZ, Heidelberg, Germany

Introduction: It is currently believed that type I and III interferons (IFNs) have redundant functions. However, the preferential distribution of type III IFN receptor on epithelial cells suggests functional differences at epithelial surfaces.

Methods: Here, using human intestinal epithelial cells and human mini-gut organoids we could show that although both type I and type III IFNs confer an antiviral state to the cells, they do so with distinct kinetics. Using a combination of data-driven mathematical modeling and experimental validation, we addressed the molecular reason for this differential kinetic of ISG expression.

Results: Type I IFN signaling is characterized by an acute strong induction of interferon stimulated genes (ISGs) and confers fast antiviral protection. On the contrary, the slow acting type III IFN mediated antiviral protection is characterized by a weaker induction of ISGs in a delayed manner compared to type I IFN. Moreover, while transcript profiling revealed that both IFNs induced a similar set of ISGs, their temporal expression strictly depended on the IFNs, thereby leading to unique antiviral environments. We could demonstrate that these kinetic differences are intrinsic to each signaling pathway and not due to different expression levels of the corresponding IFN receptors. We report that type III IFN is specifically tailored to act in specific cell types not only due to the restriction of its receptor but also by providing target cells with a distinct antiviral environment compared to type I IFN.

Conclusion: We propose that this specific environment is key at surfaces that are often challenged with the extracellular environment.

Disclosure of Interest: None Declared

P273
SUPPRESSION OF IL-17F, BUT NOT OF IL-17A, PROVIDES PROTECTION AGAINST COLITIS BY INDUCING TREG CELLS THROUGH MODIFICATION OF THE INTESTINAL MICROBIOTA
C. Tang1*, Y. Iwakura1
1RESEARCH INSTITUTE FOR BIOMEDICAL SCIENCES, TOKYO UNIVERSITY OF SCIENCE, Noda, Japan

Introduction: The cytokines IL-17A and IL-17F have 50% amino-acid identity and bind the same receptor. Although IL-17A is reported to protect host from colitis by maintaining intestinal tight-junction, the potential function of IL-17F in intestinal immunity have remained obscure.

Methods: Here, by administrating IL-17A, IL-17F and IL-17A&F deficient (Il17a−/−, Il17f−/−, Il17a−/−Il17f−/−) mice with DSS, or by transferring CD25 CD45RB+CD4+ T cells from these mice into lymphocyte-deficient Rag2−/− mice, we investigated the influence of these IL-17 family members on the development of intestinal inflammation. By sequencing analysis of ribosomal RNA in 16S region, we identified the modification of fecal microbiota from these mutant mice.

Results: We found that Il17f−/− mice resisted chemically induced colitis, but Il17a−/− mice did not, and that Il17f−/− CD45RB+CD4+ T cells induced milder colitis in lymphocyte-deficient Rag2−/− mice, accompanied by an increase in intestinal regulatory T cells (Treg cells). Clostridium cluster XIVa in colonic microbiota capable of inducing Treg cells was increased in both Il17f−/− mice and mice given transfer Il17f−/− T cells, due to decreased expression of a group of antimicrobial proteins. There was substantial production of IL-17F, but not of IL-17A, not only by naive T cells but also by various colon-resident cells under physiological conditions. Furthermore, antibody to IL-17F suppressed the development of colitis, but antibody to IL-17A did not.

Conclusion: These observations show the immune function of IL-17F is different from that of IL-17A in the intestine, and suggest that IL-17F is an effective target for the treatment of colitis.
Disclosure of Interest: None Declared

P274
VERSATILE EFFECTS OF IL-6 ON NITRIC OXIDE PRODUCTION DURING ULCERATIVE COLITIS

R. Toumi1,2, I. souffli1, H. rafa1, C. touil3
1Cellular and molecular Biology, University of Sciences and Technology Houari Boumedienne, Algiers, Algeria

Introduction: Ulcerative colitis (UC) belongs to a group of chronic inflammatory bowel diseases (IBD), they are chronic conditions that involve inflammation of the gastrointestinal tract. They occur due to an exacerbated immune response to the intestinal microbiota (Caro 2012). These disorders of multifactorial etiology are characterized by a dysfunction of the immune system. The balance between pro-inflammatory cytokines, anti-inflammatory cytokines, and immunoregulatory cytokines is disturbed. Moreover, evidence from animal and clinical studies demonstrate a positive correlation between an increased concentration of nitric oxide (NO) and the severity of the disease. Interestingly, proinflammatory cytokines are involved in the up-regulation of inducible oxide synthase (iNOS) expression in IBD (Van Deventer, 1997). However, anti-inflammatory and immunoregulatory cytokines are responsible for the negative regulation of iNOS. A positive correlation between NO production and increased pro-inflammatory cytokine levels were reported in patients with IBD. High levels of cytokines, especially IL-6, sIL-6R and free radicals, including nitric oxide, are involved in their physiopathological mechanisms. However, several data attribute an important role of IL-6 in the maintenance of intestinal homeostasis (Mitsuyama et al., 2006).

Methods: In our study, we were interested in evaluating the effect of IL-6 on nitric oxide production by peritoneal macrophages in an experimental model of acute colitis induced by DSS and by colonic biopsies of patients with active UC. To induce the experimental acute colitis, mice received 2.5% of DSS in drinking water for 7 days.

Results: Our results showed high levels of NO production and an important cellular proliferation in colonic biopsies of UC patients treated with IL-6. Our results suggest that the effects of IL-6 in maintaining chronic state in IBD including induction of iNOS expression in colonic mucosa. We suggest that IL-6 would up regulate iNOS activation through activation of the NF-kB transcription factor and induction of pro-inflammatory cytokines expression. However, our results showed that stimulation of peritoneal macrophages with IL-6 decreased the level of nitric oxide production. Concomitantly, a change in the morphology of macrophages was observed. These data suggest an anti-inflammatory effect of IL-6. It is likely that high rate of endogenous IL-6 reported in DSS induced acute colitis serves as a negative feedback mechanism that limits inflammatory reactions. In conclusion, our study highlights the dual role of IL-6.

Conclusion: On the one hand, IL-6 contributes to the resolution of the acute inflammatory phase but promotes the exacerbation of the inflammatory response during the chronic phase.

Disclosure of Interest: None Declared

P275
LOSS OF INTEGRIN AVB8-MEDIATED TGFβ ACTIVATION BY CD4\(^+\) EFFECTOR MEMORY T CELLS ENHANCES IMMUNITY AND PATHOGEN CLEARANCE TO SECONDARY VIRAL INFECTION.

S. Houston1, C. McEntee2, J. Casulli1, C. Smedley1, M. Fife2, T. Griffith2, M. Pepper2, T. Hussell1, M. Travis1,2
1University of Manchester, Manchester, United Kingdom, 2University of Minnesota, Minneapolis, 3University of Washington, Seattle, United States

Introduction: The adaptive immune response is characterised by the ability of lymphocytes to respond rapidly and effectively to previously encountered antigen. Key to immunological memory are memory T cell populations, which include effector memory T cells (T EM), central memory T cells (T CM), and resident memory T cells (T RM). Understanding how memory T cell responses are regulated to promote immunological memory, especially at mucosal sites where infection risk is high, is crucial in identifying novel strategies to boost secondary immune responses.

A critical cytokine that regulates a broad range of T cell functions at mucosal barriers, including memory responses, is TGFβ. Many cells make TGFβ, but always as a latent complex that requires activation to function. How TGFβ responses are controlled to regulate memory T cell responses is unknown.

Methods: We used conditional knockout murine models of influenza infection, combined with ex vivo analysis of cells using tetramers and flow cytometry, to identify pathways that regulate TGFβ function during memory T cell responses.

Results: Here we show that a population of CD4\(^+\) T EM are capable of activating latent forms of TGFβ. Mechanistically, this ability to activate TGFβ is dependent on expression of the integrin, αvβ8. This pathway is functionally important, as lack of expression of the integrin on T EM develop an expansion of pulmonary antigen-specific effector memory CD8\(^+\) T cells after secondary infection with influenza. This expansion of effector memory CD8\(^+\) T cells is accompanied by an increase in granzyme B production and enhanced viral clearance.

Conclusion: Thus, CD4\(^+\) Tem-mediated TGFβ activation via integrin αvβ8 appears to limit the expansion and function of antigen-specific CD8\(^+\) T cells during a memory response. Such work has important implications in potential therapeutic strategies aimed at boosting secondary immune responses to infection.

Disclosure of Interest: None Declared

P276
SYSTEMIC IL-18 AND INTESTINAL MHC-II ARE REGULATED DISTINCTLY BY MICROBIAL DYSBIOSIS AND INFLAMMASOME ACTIVATION.
Introduction: IL-18 is an inflammasome-activated cytokine best known for its synergistic effects on cytotoxicity and on production of IFNgamma. Its role in human disease is unknown, but it is elevated in peripheral blood in nearly all inflammatory diseases. Most peripheral IL-18 is bound by its endogenous inhibitor, IL-18 Binding Protein (IL-18BP). Macrophage Activation Syndrome (MAS) is a life-threatening cytokine storm disorder often occurring in patients with rheumatic diseases. MAS has been associated with both hyperactivity of the NLRC4 (but not NLRP3 or MEFV) inflammasome and elevated IL-18. Excess IL-18 exacerbates various murine models of MAS, and IL-18 blockade may be an effective treatment for human MAS.

Methods: Cytokine measurements were performed by bead-based immunoassay. MHC-II expression was evaluated by RNA-seq, qPCR, and flow cytometry. Primary intestinal organoids were cultured with or without recombinant cytokines for 72 hours.

Results: In contrast with modest serum IL-18 elevation in most auto- and hyperinflammatory diseases measured, IL-18 levels in MAS were dramatically elevated, resulting in measurable free IL-18. Notably, some MAS patients’ IL-18 remains highly elevated for years regardless of disease activity, suggesting it may be a chronic factor increasing susceptibility for MAS. Mice carrying an NLRC4 gain-of-function mutation matching that of a human NLRC4-MAS patient (NLRC4GOF mice) had elevated serum IL-18 derived entirely from Intestinal Epithelial Cells (IECs). The most dramatic transcriptional difference between WT and NLRC4GOF intestinal epithelium was upregulation of genes associated with MHC-II, and NLRC4GOF IECs showed increased homeostatic proliferation. MHC-II upregulation in NLRC4GOF IECs was dependent on IEC production of IL18. NLRC4GOF mice showed serum IL-18 elevation from birth, but did not upregulate MHC-II until after weaning. Mice lacking II18bp also showed spontaneously increased IEC MHC-II. Whereas only small intestinal IECs upregulated MHC-II in NLRC4GOF mice, Rag1-/- mice upregulated MHC-II in both colonic and small intestinal IECs. Likewise, elevated serum IL-18 in NLRC4GOF mice occurred despite broad spectrum-antibiotics, whereas antibiotics in Rag1-/- mice normalized serum IL-18. Rag1 deficiency synergized with NLRC4GOF to drive highly elevated serum IL-18. Despite reports of IECs responding to IL-18, we found only IFNgamma was capable of upregulating MHC-II on WT intestinal organoids. In vivo, upregulation of IEC MHC-II upon TLR9 stimulation (an IFNgamma stimulus) did not occur until 4-7 days after stimulation.

Conclusion: These data suggest systemic IL-18 occurs due to dysregulation of intestinal immunity. NLRC4 hyperactivity and Rag1 deficiency likely drive systemic IL-18 through complementary mechanisms: substrate cleavage and dysbiosis-induced II18 transcription, respectively. These mechanisms may synergize in patients at risk for MAS, illustrating how intestinal dysregulation may drive susceptibility to life-threatening systemic inflammation.

Disclosure of Interest: None Declared

P277 NEW LESSONS IN ANTIFUNGAL IMMUNITY: THE ORAL EPITHELIUM EXPLOITS A FUNGAL VIRULENCE FACTOR TO DRIVE INNATE IL-17 RESPONSES DURING INFECTION

A. Verma1, J. Richardson2, D. Moyes3, B. Hube1, J. Naglik1, S. Gaafen2
1Rheumatology and Clinical Immunology, University of Pittsburgh, Pittsburgh, United States, 2Mucosal and Salivary Biology Division, 3Centre for Host-Microbiome Interactions, King’s College London, London, United Kingdom, 4Dept of Microbial Pathogenicity Mechanisms, Hans Knöll Institute, Jena, Germany, 5Mucosal and Salivary Biology Division, King's College London, London, United Kingdom, 6Division of Rheumatology and Clinical Immunology, University of Pittsburgh, Pittsburgh, United States

Introduction: IL-17-driven immunity is a crucial component of innate host defenses against oral Candida albicans infections. In this study, we determined the molecular mechanism that instigates the innate IL-17 machinery in the oral mucosa during C. albicans infection.

Methods: Mice were orally challenged with C. albicans and immune parameters including T cell proliferation, proinflammatory gene induction, and fungal clearance were analyzed in tongue tissues between days 1-5 post infection. Nur77-eGFP reporter mice were used as a tool to analyze TCR activation in oral TCRαβ+ cells during infection. To assess the involvement of the hyphal-derived fungal toxin, Candidalysin in triggering innate IL-17 responses, mice were orally infected with a strain of C. albicans deficient in Candidalysin expression (Ece1Δ/Δ strain).

Results: During the innate response, IL-17 is produced by gamma-delta T cells and a poorly understood population of innate-acting CD4+ alpha-beta T cells (TCRαβ+ cells), but only the TCRαβ+ cells proliferate during acute infection. Confirming the innate nature of these cells, their TCR was not detectably activated during the primary response, as evidenced by Nur77-eGFP mice that report TCR signaling. Rather, the proliferative expansion of innate TCRαβ+ cells was driven by both intrinsic and extrinsic IL-1 signals. Unexpectedly, there was no requirement for CCR6/CCL20-dependent recruitment or prototypical fungal pattern recognition receptors. However, C. albicans mutants that cannot switch from yeast to hyphae showed impaired TCRαβ+ cell proliferation and IL-
17 expression. This prompted us to assess the role of Candidalysin, a hyphal-associated pore-forming toxin that damages oral epithelial cells and triggers production of inflammatory cytokines including IL-1 and IL-36. Candidalysin-deficient strains failed to up-regulate IL-17 or drive the proliferation of innate TCRαβ+ cells. In sharp contrast to IL-1 driving Type-17 responses, IL-36 induced IL-23 but did not impact IL-17 expression in the tongue during infection, indicating an alternate pathway of protection against pathogenic fungi. Finally, Candidalysin signaled in cooperation with IL-17 to further augment the proinflammatory cytokine response in the oral epithelium, thus serving as a feed-forward activation loop to amplify antifungal host defenses.

**Conclusion:** Our findings uncover a previously unrecognized role for a hyphal-derived pore-forming fungal toxin in instigating innate IL-17 responses during oral candidiasis. These discoveries reveal fundamental differences between activation of innate versus adaptive Type-17 immunity in the oral mucosa and why IL-17 is triggered only when the host senses pathogenic forms of *C. albicans.*

**Disclosure of Interest:** None Declared

**P278**

**IFN-LAMBDAA ENHANCES INFLUENZA IMMUNITY BY STIMULATING TSLP RELEASE DURING INTRANASAL IMMUNIZATION**

L. Ye1,2, D. Schnept1, J. Becker1, K. Ebert2, Y. Tannir2, V. Berasconi3, H. H. Gad4, R. Hartmann4, N. Lycke3, P. Staeheli3

1Institute of Virology, Medical Center University of Freiburg, 2Institute of Medical Microbiology, Medical Center University of Freiburg, Freiburg, Germany, 3Department of Microbiology and Immunology, University of Gothenburg, Gothenburg, Sweden, 4Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark

**Introduction:** Interferon-λ (IFN-λ) is an important component of the innate immune system that primarily acts on epithelial cells and mediates innate antiviral protection of mucosal surfaces. In contrast to the well-documented role of IFN-λ during innate immune responses, less is known concerning the role of IFN-λ in adaptive immunity. In this study, we investigated the role of IFN-λ as a mucosal adjuvant following intranasal immunization.

**Methods:** Antigen-specific IgG subtypes and IgA levels were assessed from wild type and IFN-λ receptor deficient mice subjected to influenza virus infection and vaccination with subunit vaccines. T follicular helper cells and germinal center B cells in spleen and lymph nodes were assessed by FACS. Bone-marrow chimeric mice were generated to determine which cells require IFN-λ signals to support adequate antibody production. The regulation of the thymic stromal lymphopoietin (TSLP) gene after IFN-λ exposure and influenza virus infection of epithelial cells was assessed by FACS staining and RT-qPCR.

**Results:** Mice deficient in IFN-λ signaling showed an impaired antibody response after influenza virus infection. We further found that subunit vaccines enriched with IFN-λ induced strongly enhanced IgG1 and IgA antibody responses in wild-type mice compared with IFN-λ-free vaccines if administered by the intranasal route. No such adjuvant effect of IFN-λ was observed if the vaccines were administrated by the subcutaneous or intraperitoneal routes. IFN-λ triggered the synthesis of TSLP in epithelial cells of the upper airways which targeted migratory dendritic cells and boosted antigen-dependent germinal center reactions in draining lymph nodes and spleen. The IFN-λ/TSLP axis not only induced strongly increased responses to influenza subunit vaccines but also enhanced survival after lethal virus challenge.

**Conclusion:** Our study indicates that IFN-λ plays an important role in potentiating adaptive immune responses which initiate in the upper airways and it has great potential to increase the effectiveness of mucosal vaccines.

**Disclosure of Interest:** None Declared

**P279**

**LINGO3 IS A NOVEL RECEPTOR EXPRESSED ON EPITHELIAL CELLS THAT LIMITS COLITIC DISEASE SEVERITY**

K. Zullo1, S. Srivatsa4, Y. Ji1, K. Herbine1, C. Pastore1, Y. Wei2, M. Samsouk3, L. Y. Hung1, N. Cohen1, D. Herbert1

1University of Pennsylvania, Philadelphia, 2University of California, San Francisco, San Francisco, United States

**Introduction:** Molecules that control the balance between immunity and tissue repair are incompletely known. Through a biochemical screen for novel regulators of mucosal barrier function, we identified the orphan transmembrane receptor named leucine rich repeat and nogo-interacting protein 3 (LINGO3). This study demonstrates that LINGO3 is widely expressed on mucosal epithelial cells of mice and humans and serves a previously unappreciated role in regulating mucosal barrier integrity under steady-state and injurious conditions.

**Methods:** Lingo3−/− mice were generated using CRISPR-CAS9 gene editing and evaluated compared to WT C57BL/6 under both cohoused and non-cohoused conditions. Experiments were performed to evaluate intestinal and lung permeability, pro-inflammatory cytokine production, and activation state of myeloid and lymphoid subsets. Intestinal injury was induced following oral administration of dextran sodium sulfate (DSS) at 2.5% for 6 days prior to restoration with normal water. Colitic disease severity caused by DSS was evaluated by colon length, rectal bleeding, fecal consistency, lethargy, and weight loss.

**Results:** Immunofluorescence staining of mouse and human tissues revealed LINGO3 expression in mouse lung, small intestine, and colon, and human bronchus, nasal polyp, and rectal tissue. Studies of Lingo3−/− mice show that lung and intestinal organs had increased permeability to FITC-dextran as compared to WT mice under steady-state conditions. Consistent with a hypothesis that enhanced permeability drives microbial driven inflammation, flow cytometry experiments revealed...
Lingo3−/− mice had increased percentages and numbers of type 1 conventional dendritic cells cDC1, inflammatory macrophages, IFNγ+, TNFα+, and IFNγ+ TNFα+ CD4+ cells within the colon lamina propria under baseline conditions. Accordingly, DSS-induced intestinal injury resulted in more severe weight loss, shorter colon lengths, as well as enhanced IFNγ and TNFα production compared to WT mice.

Conclusion: Combined, these data imply that LINGO3 is a novel receptor which promotes barrier integrity and limits mucosal Tn1 inflammation. Further investigation of this dichotomy and identification of LINGO3 putative ligands may yield important insights to the regulation of inflammation at the mucosal barrier.

Disclosure of Interest: None Declared

P279.A
ROLE OF A NOVEL TRANSPORTER SLC46A3 IN MAINTENANCE OF INTESTINAL INTEGRITY AND MICROFLORA
A. Nandy1,*, K. OKUDA1, N. SILVERMAN1
1MEDICINE, UMASS MEDICAL SCHOOL, WORCESTER, United States

Introduction: The inflammatory response against infection and tissue damage is beneficial for the host as it limits the infection and contributes to tissue repair and maintenance. However, prolonged or uncontrolled inflammation may also be responsible for severe tissue injury, disease progression, and even early lethality. Numerous diseases are associated with chronic inflammation, including inflammatory bowel disease (IBD). IBD results from a sustained activation of mucosal inflammatory response that compromises the integrity of the gastrointestinal tract. Changes in commensal microflora are often linked with this disease progression. Individuals carrying mutations in the cytosolic innate immunity receptors NOD2 are genetically predisposed to IBD; however, it is still unclear how the NOD agonists such as muramyl dipeptide (MDP) can get access to the cytosol and trigger NOD receptors. We hypothesized that a novel family of a transporter is involved in that process.

Methods: We made SLC46A3 transporter knockout mice. The DSS model of colitis was used, where administration of DSS damages intestinal epithelial monolayer and induces inflammation. We compared colon integrity in both wild-type and knockout mice, treated with DSS

Results: We observed a significant loss of body weight in SLC46A3 knockout mice. Furthermore, the colony integrity was also compromised as evidenced by the significant reduction of colon length in mutant mice compared to the wild-type. Moreover, our experiments also suggested a change in the gut microbiota in the mutants compared to the wild-type.

Conclusion: These findings indicate that this particular transporter is involved in the maintenance of intestinal integrity.

Disclosure of Interest: None Declared

P279.B
ANTIGEN PRESENTING ILC3 REGULATE T CELL-DEPENDENT IGA RESPONSES TO COLONIC MUCOSAL-ASSOCIATED BACTERIA
1University Of Manchester, Manchester, United Kingdom, 2Karolinska Institute, Stockholm, Sweden, 3University of Birmingham, Birmingham, 4Roslin Institute, Edinburgh, United Kingdom, 5Childrens Hospital of Philadelphia (CHOP), Philadelphia, United States, 6University of British Columbia, Vancouver, Canada

Introduction: Intestinal immune homeostasis is dependent upon tightly regulated and dynamic host interactions with the commensal microbiota. Immunoglobulin A (IgA) produced by mucosal B cells acts to dictate the composition of commensal bacteria residing in the intestine and mediates responses against enteric pathogens. Emerging evidence suggests commensal and pathogenic species can elicit IgA via either T cell-independent or T cell-dependent mechanisms, orchestrated by T follicular helper cells. However, the mechanisms that modulate the magnitude and quality of T cell-dependent IgA responses in the intestinal tract and associated lymphoid tissues remain incompletely understood. Here we demonstrate that group 3 innate lymphoid cells (ILC3) regulate steady-state interactions between T follicular helper cells (TfH) and B cells to limit mucosal IgA responses.

Methods: Using immunofluorescence and flow cytometry approaches we dissected the interplay between ILC3, TfH and B cells in the intestinal draining lymph nodes. Moreover, using transgenic mice which lack ILC3-intrinsic antigen presentation via MHCII we characterised TfH, B cell and plasma cell responses in the intestine and associated lymphoid structures at steady state and during enteric pathogen infection.

Results: ILC3 were demonstrated to utilize conserved migratory cues to establish residence within the interfollicular regions of the intestinal draining mesenteric lymph nodes (mLN), where they co-localized with TfH at the border of the B cell zone. ILC3 in the mLN highly express MHCII and were found to limit TfH responses, and subsequently B cell class switching, through antigen presentation both in vitro and in vivo. Deletion of ILC3-intrinsic antigen presentation in mice resulted in increased class switching towards IgA and elevated numbers of IgA+ plasma cells in the colon, resulting in selective IgA coating of bacterial species residing within the colonic mucosa.

Conclusion: Taken together these findings implicate lymph node resident, antigen-presenting ILC3 as a critical regulatory checkpoint in the generation of T cell-dependent colonic IgA and suggest ILC3 act in this manner to maintain mutualism with the mucosal-dwelling commensal microbiota.

Disclosure of Interest: None Declared
P280
AN IN VITRO MODEL OF CD8+ MEMORY AND EFFECtor T CELL FATE BASED ON DIFFERENTIAL PRODUCTION OF INTERFERON-GAMMA.
M. Balood, J. Baillargeon, S. Talbot, M. Rangachari. 1Dept of Pharmacology and Physiology, Université de Montréal, Montréal, 2Department of Neurosciences, Centre de recherche de CHU de Québec, 3Dept of Pharmacology and Physiology, Université de Montréal, 4Laval University, 5Department of Neurosciences, CHU de Quebec Research Centre, Quebec, Quebec, Canada

Introduction: CD8+ T cells gain an exhausted phenotype during chronic infections and cancer. T cell exhaustion is defined as the hierarchical and progressive loss of T cell function characterized by loss of proliferation and the ability to produce cytokines such as IFN-γ, IL2 and TNF-α. Exhausted T cells may also be characterized by the expression of inhibitory immune checkpoint receptors (ICR) such as Tim3, PD-1 and Lag3. As loss of IFN-γ is an early event in T cell exhaustion, we hypothesized that by culturing and repeatedly stimulating IFN-γo CD8+ T cells, we might be able to generate an in vitro model T cell exhaustion, something that has eluded the field up until now.

Methods: We used GREAT (interferon-gamma reporter with endogenous polyA transcript) mice, which express yellow fluorescence protein (YFP) from a bicistronic reporter knocked in 3' to the Ilng STOP codon. This allows us to faithfully track IFN-γ+ cells by flow cytometry. We first stimulated naïve (CD62Lhi) mouse CD8+ T cells under inflammatory Tc1 conditions (plate bound agonistic anti-CD3+anti-CD28 plus rIL-12 and anti-IL4 blocking Ab). As it is believed that exhausted T cells arise from activated effector precursors that are high IFN-γ-producers, we purified IFN-γ+ (YFP+) Tc1 cells after 5 days of culture and restimulated them under conditions that varied the strength of signal 1 (CD3), signal 2 (CD28) or signal 3 (IL-12 and anti-IL4). After another 5 days (10 days total), we assessed expression of ICR (Tim-3, PD-1, Lag3) by flow cytometry. We then purified cells into IFNγ+ (YFP+) and IFNγo (YFP-) populations and restimulated them under the same conditions that were used at d5. ICR expression was re-assessed at d15 of the culture.

Results: Our data indicate that expression of ICR skews towards IFNγ+ cells at d5 and d10, likely reflecting a dual role of these receptors in both T cell activation and inhibition. By d15, ICR expression was detected at higher frequencies in YFP cells, suggesting that these cells might be exhausted. Cells were uniformly CD44+CD62L-effector memory cells by d15, regardless of the specific stimuli. We found that upon weaker signal 1+2 stimulation (soluble anti-CD3 and no anti-CD28), cells were unable to survive to d15, indicating that a baseline level of continual TcR/CD28 stimulation might be required to generate exhausted T cells.

Conclusion: The study of exhausted T cell biology currently requires the use of intricate and time-consuming cancer/viral inoculation paradigms. Our system could allow us to more easily define the signals and culture conditions that give rise to exhausted cells.

Disclosure of Interest: None Declared

P281
COMPARISON OF THE EFFECTS OF OBSTRUCTIVE SLEEP APNEA AND SLEEP DEPRIVATION ON THE IMMUNE SYSTEM
E. Said1, M. A. Al-Abrī2, I. Al-Said1, M. S. Al-Balushi1, J. Z. Al-Busaid1, I. Al-Reesi1, C. Y. Koh1, M. A. Idris1, A. A. Al-Jabri1, O. Habba3
1Microbiology and Immunology, 2Clinical Physiology, 3Clinical Anatomy, Sultan Qaboos University, Muscat, Oman

Introduction: The little information about the effect of sleep deprivation on the immune system is conflicting. Obstructive sleep apnea (OSA) is a sleep related breathing disorder that leads to an increased sleep fragmentation and the patient is therefore sleep deprived. OSA may affect the immune system for reasons that might not be related to sleep deprivation itself, e.g. hypoxia that is associated with inflammatory responses. Here we are comparing the effect of OSA on the immune system to that of sleep deprivation in healthy individuals (SDHI).

Methods: We investigated the association of OSA and sleep deprivation with levels of 14 Th1/Th2 and inflammatory cytokines and chemokines, CD4 T and NK cells, and the NADPH oxidase activation and phagocytic functions in neutrophils.

Results: Both OSA and SDHI were associated with a decreased capacity in neutrophils to phagocytose bacteria and activate NADPH oxidase (p<0.05). In contrast, CD4 T cell levels were increased in OSA but decreased in SDHI (p<0.05). This increase in OSA was associated with an increased expression of the nuclear protein Ki67 in CD4 T cells. Moreover, OSA was associated with an increase in the levels of IL-1beta and IL-6 (p<0.05), as well as a decrease in the levels of IFN-gamma and the ratio IFN-gamma/IL-4 in the blood (p<0.05). However, no changes in the levels of these cytokines was observed in SDHI, while an increase in the levels of CXCL9 and decrease in the CXCL10/CXCL9 and CCL5/CXCL9 ratios were detected in SDHI (p<0.05). Interestingly, the increase in CD4 T cell levels correlated with the levels of IL-1beta in OSA, while the changes in CD4 T cell in SDHI correlated with changes in the CXCL10/CXCL9 ratio (p<0.05). Additionally, an increase in the expression of Ki67 in CD8hi and CD8lo NK cells was observed in OSA only (p<0.05).

Conclusion: Our results indicate that there are differences in the changes that affect the immune response in OSA and SDHI. These differences indicate that sleep deprivation caused by OSA is responsible for only a part of these changes i.e. neutrophil functions, while changes affecting the cytokines, CD4 T lymphocytes and NK cells might be related to other factors associated with OSA.

Disclosure of Interest: None Declared
THE IMMUNOLOGICAL RESPONSE IN MEDIASTINAL LYMPH NODES IS EXACERBATED BY PPAR GAMMA DEFICIENCY IN A MURINE MODEL OF PULMONARY SARCOIDOSIS.

V. Sanderford, N. Letffer, D. Vargas, A. Malur, K. Kew, R. A. Barrington, B. P. Barna, A. Mohan, M. J. Thomassen

1Internal Medicine, 2Chemistry, East Carolina University, Greenville, 3Microbiology and Immunology, University of South Alabama, Mobile, United States

Introduction: Sarcoidosis is an inflammatory disease that is characterized by granuloma formation, primarily in the lungs, with unknown etiology. Exposure to environmental pollutants and mycobacterial antigens has been implicated as a risk factor for disease. We established a murine model for granuloma formation by oropharyngeal instillation of mice with multiwall carbon nanotubes (MWCNTs) with or without ESAT-6, a peptide derived from Mycobacterium tuberculosis. Peroxisome Proliferator Activated Receptor γ (PPARγ), a transcription factor associated with inhibition of pro-inflammatory molecules, was downregulated in MWCNT instilled mice. Additionally, PPARγ-KO mice presented with increased severity of granuloma formation and fibrosis compared to wild-type. Moreover, we noted a marked lymphadenopathy of the mediastinal lymph nodes (MLN) in mice instilled with MWCNT. Because lymphocyte reactivity to mycobacterial antigens may be associated with sarcoidosis, we hypothesized that MWCNT+ESAT-6 instillation of macrophage specific PPARγ-KO mice would lead to an adaptive immune response to ESAT-6 peptide in vitro, and this response would exceed the response of instilled wild-type C57BL/6 mice.

Methods: Macrophage specific PPARγ-KO and C57BL/6 mice were instilled with MWCNTs with or without ESAT-6, and sham mice were instilled with PBS/Surfactant or ESAT-6. After 60 days, the MLN were collected and measured for use in IHC staining, flow cytometric analysis, or culture for 5–72 hrs with 5–15 µg/mL ESAT-6 peptide. Gene and protein expression were measured with qPCR and ELISA analysis. Lungs were also collected after 60 days, and whole lung digest was analyzed by mass spectrometry. ANOVA or T tests were used for statistical analyses.

Results: Nanotubes were visible within the MLN of C57BL/6 and PPARγ-KO mice as soon as 3 days post-instillation, and frequency and aggregate size of nanotubes increased over time. This observation correlated with an increase in MLN volume and cell counts in MWCNT+ESAT-6 instilled mice compared to controls, which was exacerbated in PPARγ-KO mice compared to C57BL/6. Moreover, only mediastinal lymphocytes from MWCNT+ESAT-6 instilled PPARγ-KO mice, but no other treatment groups, upregulated IFNγ after challenge with ESAT-6 peptide in vitro for 5 hours. C57BL/6 lymphocytes failed to produce an IFNγ response within 5 hours; however, lymphocytes from both C57BL/6 and PPARγ-KO mice produced IFNγ in response to a polyclonal stimulus after 5 hours. Mass spectrometry of whole lung digest showed that ESAT-6 peptide was still present in the lungs of PPARγ-KO mice, but not C57BL/6, 60 days post-instillation.

Conclusion: Mediastinal lymphocytes from PPARγ-KO mice instilled with MWCNT+ESAT-6, but not other groups, produced a significant Th1 immune response in vitro in response to challenge with ESAT-6 peptide, suggesting an adaptive immune response which may parallel responses seen in human sarcoidosis.

Disclosure of Interest: None Declared

PD-1/PD-L1-MEDIATED FUNCTIONAL EXHAUSTION IS REQUIRED TO PROTECT IFNAR- AND IRF9-DEFICIENT MICE FROM LETHAL LCMV INFECTION

T. Suprunenko, T. Ashhurst, N. J. King, M. Hofer

1School of Life and Environmental Sciences, 2Sydney Cytometry Facility, 3Discipline of Pathology, The University of Sydney, Sydney, Australia

Introduction: Type I interferons (IFN-I) are critical in the host antiviral response against lymphocytic choriomeningitis virus (LCMV). The Armstrong strain of LCMV (LCMV-Arm) causes acute infection in wild-type mice. By contrast, mice lacking the IFN-I receptor (IFNAR) or the IFN-I signalling factor, interferon regulatory factor 9 (IRF9), develop a persistent infection. We have previously shown that this is accompanied by functional exhaustion of the CD8+ T cell response which is characterised by increased expression of co-inhibitory receptors, particularly programmed death (PD)-1, and a markedly reduced production of effector cytokines IFN-γ and TNF-α. Here, we aimed to clarify the contribution of PD-1/PD-L1-mediated exhaustion in the chronic LCMV-infection of IFNAR- and IRF9-deficient mice.

Methods: For this, we crossed IFNAR- and IRF9-deficient mice with mice lacking the primary ligand for PD-1, PD-L1, and examined their response to LCMV-Arm infection. Characterisation of T cells was determined by flow cytometry and the contribution of the CD8+ and CD4+ T cells in mediating infection was determined by neutralisation of either CD8 or CD4 by neutralising antibody injections.

Results: In contrast to IFNAR- and IRF9-deficient mice, which survived infection, IFNARxPD-L1 and IRF9xPD-L1 double deficient mice showed progressive weight loss and disease necessitating euthanasia. Accordingly, lack of PD-L1 in these mice was accompanied by a change in T cell function. Further, neutralisation of either CD8 or CD4 by neutralising antibody injections reversed the lethal phenotype of the double deficient mice following LCMV-Arm infection.

Conclusion: These results indicate that PD-1/PD-L1 dependent functional exhaustion is critical in moderating the antiviral immune response. Furthermore this data suggests a role for both CD8+ and CD4+ T cell responses in functional exhaustion during LCMV infection.

Disclosure of Interest: None Declared
Introduction: Novel cellular approaches to regulating transplantation immunity are being developed worldwide. IL-10-transfected dendritic cells (DCs) acquire the tolerogenic phenotype, induce Tregs, and suppress inflammatory response in vitro. Researchers have reported that IL-10-transfected DCs protect from autoimmune diseases. Here, we describe possibility of IL10-transfected DCs stimulate immune tolerance in mice with acute graft-versus-host-disease (GVHD).

Methods: In C57BL/6 mice, DCs were derived from bone marrow cells in the presence of rmGM-CSF and rmIL-4. Electroporation of DCs was performed on the 3rd day of cultivation according to the protocol with a plasmid encoding mouse IL10 (DCpIL10). Further, DCpIL10 was co-cultured with syngeneic splenocytes. The amount of Treg cells, IL10 expression, and inhibition of mixed lymphocytes reaction (MLR) was evaluated. CBF1 (C57BL/6 x CBA) mice were immunized with different type of cell culture (10^7 cells per 1 mouse) to induction of acute GVHD. IL-10-dependent tolerance was assessed by GVHD degree. Statistical data were processed using the GraphPad Prism 6.0 program.

Results: We demonstrated the advantage of using the IL-10 encoding construct in C57BL/6 mice for induction of a tolerogenic DC phenotype. IL-10-transfected DCs are characterized by increase CD11c+H2B+ cells in culture but decrease co-stimulation markers (such as CD86, CD40, CD83+CD40). Furthermore, we showed the effectiveness of DCpIL10 for induction of Treg differentiation and activation of regulatory property lymphocytes. DCpIL10 stimulated intracellular synthesis of IL-10 by CD4+ T lymphocytes and production of IL-10 in splenocyte culture. IL-10-transfected DCs and splenocytes mix cultures was reduces proliferations cells to alloantigens in MLR. As well, those mix cultures reduced acute GVHD degree indicated by normal mouse weight, lien and thymus size, WBS and RBS count.

Conclusion: The use of electroporation of a pDNA construct encoding the IL-10 is an effective approach for inducing tolerogenic DCs and regulatory T cells in splenocyte culture. DCs transfected with pDNA-IL-10 promote differentiation of naive CD4+ T cells into Treg cells, which is characterized by an increase in IL-10 production and a decrease in proliferation of splenocytes in response to addition of an alloantigen. This approach can be useful for modulation of various cells function in the cellular technologies of immunologic tolerance induction. This work was supported by RSF. Agreement №16-15-00086 (11.01.2016)
bound to the active promoter region of the Bhlhe40 locus, indicating Satb1 may control GM-CSF production in encephalitogenic tissue Th17 cells. Furthermore, we identified PD-1 as one of the up-regulated genes in Satb1-deficient Th17 cells. PD-1 blockade enhanced the production of IL-2 and IL-17 and restored the pathogenic function of Satb1-deficient Th17 cells. Notably, the differential regulation of Bhlhe40 and PD-1 in EAE Th17 cells was not seen in non-pathogenic Th17 cells in PPs, which barely secrete GM-CSF. Th17 cells from the draining LNs of control EAE mice had significantly higher levels of Satb1 than those from the PPs. RNA-seq analysis revealed that the majority of the differentially regulated genes in Satb1-deficient Th17 cells were specific to pathogenic Th17 cells.

**Conclusion:** Satb1 is dispensable for the non-pathogenic function of Th17 cells in the gut but plays a pivotal role in the effector functions of pathogenic Th17 cells, including GM-CSF production via regulation of Bhlhe40 and PD-1 expression in EAE mice.

**Disclosure of Interest:** None Declared

---

**P286**

**THE INSUFFICIENCY OF METABOLIC HORMONE LEPTIN IMPAIRS THE FUNCTION OF TFH CELLS AND CONFERS A RISK OF POOR VACCINE RESPONSES**

D. Yu1, J. Deng2, L. Lu3

1Australian National University, Canberra, Australia, 2Shanghai Renji Hospital, Shanghai, China, 3The University of Hong Kong, Hong Kong, Hong Kong

**Introduction:** Follicular helper T (TFH) cells are a specialized CD4+ T cell subset that critically controls antibody responses by supporting antibody affinity maturation and memory formation. Genetic mutations in ICOS, SH2D1A (encoding SLAM-associated protein, SAP), IL12RB1, IL21R and STAT3 impair the generation of TFH cells and result in immunodeficiency, whereas continuous stimulations from autoantigens and pro-inflammatory cytokines drive excessive TFH differentiation in autoimmune diseases. However, the mechanisms underlying the regulation of TFH cell function under non-pathogenic conditions are largely unknown. A deeper knowledge of these mechanisms will be critical to understanding why a significant proportion of the general population respond poorly to certain vaccines, since studies have identified TFH cells as the key limiting factor for effective vaccine responses.

**Methods:** In several human cohorts of diversified ages, the associations between low leptin levels and deficient antibody responses to influenza and hepatitis B virus (HBV) vaccines were examined. In parallel, vaccine-mediated protection was examined in mice in a state of physiological fasting (leading to leptin reduction) with or without leptin replacement. The mechanism of leptin in regulating TFH differentiation was investigated using leptin receptor (LepR)-deficient (db/db) mice and human T cell culture. The binding of Stat3, the key molecule downstream of leptin signal to the promoter of the II-21 gene was tested using chromatin immunoprecipitation (ChIP) and reporter assays.

**Results:** In different age groups, low leptin levels were consistently associated with deficient antibody responses to influenza and hepatitis B virus (HBV) vaccines. Similarly, mice subjected to fasting conditions failed to mount influenza vaccination-mediated protection, but this was largely rescued by leptin replacement. We revealed that leptin is required and sufficient to promote both mouse and human TFH differentiation, and potently induced IL-21 production in a STAT3-dependent manner.

**Conclusion:** We, for the first time, reveal a mechanism underlying the regulation of TFH cell function in general population. The metabolic hormone leptin plays an important role in determining TFH competency for effective vaccination, with leptin insufficiency being a profound risk factor for poor vaccine responses. The improved understanding of vaccine immunology and identification of a new biomarker for vaccine efficacy have far reaching implication for developing better strategies for personalized vaccination and vaccine development.

**Disclosure of Interest:** None Declared