ICIS International Cytokine & Interferon Society

Cytokines 2023
International Cytokine & Interferon Society ICIS

ABSTRACT BOOK

International Cytokine & Interferon Society • ICIS

Cytokines and interferons in the precision medicine era

Program Chair:
Evangelos Andreakos,
Biomedical Research Foundation,
Academy of Athens

Co-Chair:
George Pavlakis,
National Cancer Institute, (NIH)

Divani Caravel Athens / Greece

15 - 18 October 2023

athens.cytokinesociety.org

ORAL PRESENTATIONS

High throughput CRISPRi screen identifies the long noncoding RNA, LOUP, as a multi-functional loci important in immunity

Carpenter S[1], Halasz H, Malekos E, Covarrubias S, Liu S, Horlbeck M, Sudek L, Weissman J
[1]University Of California Santa Cruz

Plenary 1: Cytokines in precision medicine, targeted diagnosis and treatment

Advances in deep sequencing technologies have revealed that the majority of the human genome is actively transcribed into RNA. Our lab is focused on characterizing the largest group of RNA produced from the genome named long noncoding RNA (lncRNAs) and their associated protein binding partners. To date only 3% of lncRNAs have been functionally validated. Using both long and short read sequencing technologies we have generated an Isoform-level transcriptome atlas of macrophage activation characterizing all inflammatory inducible genes. Using CRISPR inhibition technology we have performed a systematic unbiased screen to identify functionally relevant lncRNAs involved in inflammatory functions within macrophages. We identified the lncRNA, LOUP, as a multifunctional gene involved in several aspects of innate immunity. We showed that LOUP can function as an enhancer to regulate its neighboring protein the pioneering factor SPI1 (PU.1). Interestingly SPI1 functions as a positive regulator of the transcription factor NF-kB yet we identified LOUP as a strong negative regulator of NF-kB. We found that LOUP localized to the cytosol and encodes a short open reading frame peptide. Ribo-seq data suggests that this region is actively translated. We inserted the peptide into a plasmid in frame with GFP and it was actively translated in HEK 293 cells. To determine if the peptide can function in innate immunity we utilized active CRISPR to specifically target the peptide and showed that indeed this region can function as a negative regulator of NF-kB. In conclusion we have identified LOUP as an important regulator of the immune response. It serves multiple functions, acting in cis to regulate SPI1 and encoding a small peptide that negatively regulates NF-kB signaling.

A novel E3 ligase of GILZ: a target to suppress multiple cytokine pathways and bypass the metabolic adverse effects of glucocorticoids in autoimmune disease treatment

Miceli I[1], Sherlock R[1], Cheang L[1], D'Cruz A[1], Bennett T[1], Pervin M[1], Cristofaro M[1], Lim Kan Sian T[1], Wilson T[2], Kandane-Rathnayake R[1], Morand E[1], Jones S[1]
[1]Monash University, [2]Hudson Institute of Medical Research

Plenary 1: Cytokines in precision medicine, targeted diagnosis and treatment

Glucocorticoids produce whole-scale changes in gene expression to suppress multiple cytokine pathways and induce immunological quiescence in inflammatory disease. However, glucocorticoids also come with an array of predictable and severe adverse effects, significantly limiting use. The glucocorticoid-induced leucine zipper (GILZ) mediates the anti-inflammatory effects of glucocorticoids but is independent of glucocorticoid-associated adverse effects, thus offering promise as a novel target for a glucocorticoid replacement therapy.

We identified “E3-X”, an E3 ubiquitin ligase which mediated GILZ proteasomal degradation. Here, we investigated whether E3-X deficiency would restore GILZ and amplify its anti-inflammatory effects.

We measured GILZ expression in E3-X knockout mice and human cell lines by flow cytometry. We performed inflammatory stimulations of plasmacytoid dendritic cells, bone marrow-derived dendritic cells and CD4+ T cells from wildtype and E3-X-deficient mice. To assess the effect of E3-X deficiency on TLR7/8-induced cytokine production in vivo, we performed intraperitoneal TLR challenges of wildtype and E3-X-deficient mice. Lastly, to determine the effect of E3-X deficiency on glucocorticoid-responsiveness, we conducted proteomic analysis of glucocorticoid-stimulated wildtype and E3-X knockout A549 cells.

E3-X deficiency increased the half-life of GILZ resulting in an accumulation of GILZ protein in the setting of inflammation. In plasmacytoid dendritic cells, absence of E3-X resulted in diminished IFN-I gene expression, reduced TNF and IL-6 production. E3-X-deficient dendritic cells demonstrated reduced secretion of Th17-inducing cytokines. Likewise, E3-X deficiency attenuated CD4+ T cell proliferation in response to Th17-inducing cytokines. In vivo, E3-X deficiency was protective against TLR7/8-induced inflammation, with marked suppression of IFN-I gene expression, IL-23, IL-6 and MCP-1 production. Absence of E3-X also resulted in a ten-fold glucocorticoid dose-sparing effect and enhancement of glucocorticoid-induced protein expression.

E3-X deficiency increased GILZ, reduced IFN-I and Th17-associated cytokine production and had a glucocorticoid-sparing effect. Therefore, E3-X is a promising therapeutic target to suppress inflammation while reducing reliance on glucocorticoids.

Ongoing Production of Tissue-Resident Macrophages from Hematopoietic Stem Cells in Healthy Adult Macaques

Brenchley J[1], Rahmberg A, Wu C, Dunbar C
[1]Niaid/nih

Symposium 1: Macrophages and dendritic cells at the single cell biology era

Macrophages orchestrate tissue immunity and repair of damaged tissue. Murine studies suggest tissue-resident macrophages are heterogenous, derived from yolk sac and bone-marrow. To understand the ontogeny and longevity of tissue-resident macrophages in nonhuman primates (NHPs), we use autologous hematopoietic stem progenitor cell (HSPC) transplantation with HSPCs modified to express individual bar-coded markers. We study the contribution of HSPC to tissue macrophages, their clonotypic profiles relative to leukocyte subsets in peripheral blood, and their transcriptomic and epigenetic landscapes. We also use in vivo bromodeoxyuridine infusions to monitor tissue macrophage turnover. We find that HSPC contribute to tissue-resident macrophage populations in all anatomic sites studied. Macrophage clonotypic profiles are dynamic and overlap with contemporaneous monocytes. Moreover, we find evidence of macrophage turnover at steady state. Epigenetic landscape and transcriptomics of HSPC-derived macrophages are similar to tissue macrophages isolated from non-transplanted NHPs. These data demonstrate the life span of tissue-resident macrophages can be limited and they can be replenished from HSPCs in NHPs.

The TCA cycle in macrophages is a central regulator of cytokine and interferon production

Peace C[1], Hooftman A[1], Ryan D[1,2,3], O'carroll S[1], Day E[1], Yang M[2,4], McGettrick A[1], Yin M[1], Montano E[5,6], Huo L[5,6], Toller-Kawahisa J[1,7], Zecchini V[2], Ryan T[1], Bolado-Carrancio A[8], Casey A[3], Prag H[3], Costa A[2,9], De Los Santos G[5], Ishimori M[5,10], Wallace D[5,10], Venuturupalli S[5], Nikitopoulou E[2], Frizzell N[11], Johansson C[12], Von Kriegsheim A[8], Murphy M[3], Jefferies C, Frezza C[2,4], O'Neill L[1]
[1]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, [2]MRC Cancer Unit, University of Cambridge, [3]MRC Mitochondrial Biology Unit, University of Cambridge , [4]CECAD Research Centre, Faculty of Medicine, University of Cologne, [5]Division of Rheumatology, Department of Medicine, Cedars-Sinai Medical Center, [6]Department of Biomedical Sciences, Cedars-Sinai Medical Center, [7]Department of Pharmacology, Riberao Preto Medical School, University of Sao Paulo, [8]Edinburgh Cancer Research UK Centre, University of Edinburgh, [9]Matterworks, [10]David Geffen School of Medicine, University of California Los Angelos, [11]School of Medicine, University of South Carolina, [12]National Heart and Lung Institute, Imperial College London

Metabolic rewiring facilitates macrophage effector function and cytokine production, however the mechanisms involved are currently being explored. Using unbiased metabolomics and stable isotope tracing, we show that an inflammatory aspartate-argininosuccinate shunt is induced following lipopolysaccharide stimulation. The shunt is supported via LPS-inducible expression of argininosuccinate synthase (ASS1) and leads to increased levels of cytosolic fumarate and protein succination. Pharmacological and genetic ablation of fumarate hydratase (FH) increases levels of fumarate, decreased respiration, and increased mitochondrial membrane potential. Transcriptomics and proteomics demonstrated strong immunoregulatory effects of impaired FH. Particularly, there was an observed increase in TNF production which was found to be through decreased IL-10 signalling. This effect was recapitulated with the addition of fumaric acid esters. Strikingly, in the context of FH inhibition, but not in the presence of fumarate esters, type I IFN production was found to be increased through the release of mitochondrial RNA activating the sensors TLR7, RIG-I, and MDA5. This pathway may also be conserved when the enzyme Succinate dehydrogenase (SDH) is impaired as the inhibitors Atpenin A5, TTFA, and dimethyl malonate all have been found to increase IFNβ production. Furthermore, whole blood from patients with the interferonopathy Systemic Lupus Erythematosus (SLE) also exhibit FH suppression, indicting a potential pathogenic role for this process in human disease resulting from a loss in the endosymbiotic tolerance of mitochondria in eukaryotic organisms. We therefore identify a protective role for FH in maintaining appropriate macrophage cytokine and interferon responses.

Macrophages control pathological type I interferon responses following viral respiratory infection

Hoagland D[1], Rodriguez-Morales P[1], Mann A[1], Lai A[1], Baez Vazquez A[1], Yu S[2], Medzhitov R[2], Franklin R[1,3]

[1]Harvard Medical School, [2]Yale University School of Medicine/HHMI, [3]Harvard University

Symposium 1: Macrophages and dendritic cells at the single cell biology era

Type I interferons (IFN-I) and their downstream effectors are critical mediators of antiviral immunity yet can become detrimental when dysregulated. Therefore, these host defenses must be tightly controlled to prevent immunopathology. To study mechanisms regulating inflammatory responses, we focused on macrophages due to their dual roles in activating inflammation and returning tissues to their homeostatic states. We found the cytokine, Oncostatin M (OSM), was upregulated in macrophages in response to cellular stress and pathogen recognition. OSM-deficient mice exhibited increased tissue damage and succumbed to challenge with influenza or a viral mimic due to heighted IFN-I responses. I will discuss our current work to uncover the mechanisms by which macrophages limit pathological IFN-I responses in the lung.

IL-12 drives the differentiation of human T follicular regulatory cells

Locci M[1], Castano D[1], Wang S[1], Sharpe H[1], Bustamante J[2], Romberg N[3], Utz P[4], Henrickson S[3], Casanova J[5]
[1]University Of Pennsylvania, [2]INSERM, [3]The Children's Hosp. of Philadelphia, [4]Stanford University, [5]The Rockefeller University

T follicular regulatory (Tfr) cells can counteract the B cell-helper activity of T follicular helper (Tfh) cells and hinder the production of antibodies against self-antigens or allergens. A mechanistic understanding of the cues initiating the differentiation of T regulatory (Treg) cells into Tfr cells, which is instrumental for the therapeutic manipulation of diseases associated with a Tfr cell imbalance, is still missing. Despite their opposed roles, Tfr and Tfh cell differentiation appears to be controlled by partially overlapping signals, including TCR stimulation and costimulatory molecules. However, it is still unknown if cytokines that have been shown to control the biology of human Tfh cells, including IL-12 and activin A, can influence the differentiation of Tfr cells. To address this question, we evaluated the impact of these two cytokines on the in vitro differentiation of Tfr cells. Herein, we report a role for IL-12 in driving, on activated Treg cells, the induction of molecules that belong to the Tfr cell program, including CXCR5, PD-1, BCL6 and ICOS meanwhile preserving Tfr regulatory function. Conversely, activin A presented a largely negligible effect on the in vitro differentiation of Tfr-like cells and only modestly strengthened the IL-12-driven differentiation of Tfr-like cells. Importantly, patients with inborn errors of immunity in IL12RB1 gene presented with a severe decrease in circulating Tfr cells and produced higher levels of anti-actin autoantibodies in vivo. Overall, this study unveils IL-12 as an inducer of Tfr cell differentiation in vivo and provides a novel approach for the in vitro generation of human Tfr-like cells.

Th1 cells instruct stromal responses to inflammation and tissue fibrosis by altering the transcriptional output of IL-6.

Millrine D[1], Cardus Figueras A[1], Andrews R[1], Szomolay B[1], Rice C[3], Fielding C[1], Jenkins B[2], Jones G[3], Williams N[1], Jones S[1]
[1]The School Of Medicine, Cardiff University, [2]Centre for Innate Immunity & Infectious Diseases, Hudson Institute of Medical Research, [3]School of Cellular & Molecular Medicine, University of Bristol

Symposium 2: Cytokines in T cell development and function

Fibrosis arising from recurrent or persistent inflammation is a pathological hallmark of many diseases. Using a model of acute peritoneal inflammation, we have examined how repeated inflammatory activation promotes fibrotic damage. In this context, fibrosis was dependent on IL-6, supporting an expansion of Th1 cells that disrupt the turnover of the extracellular matrix by metalloproteases. These data highlight a link between IL-6 and IFNγ in facilitating a shift from acute resolving inflammation to compromised tissue repair and fibrosis. IL-6 and IFNγ instruct decisions affecting tissue homeostasis, anti-microbial host defence, and inflammation-induced tissue injury. We propose that the transcriptional control of these processes involves a complex interplay between STAT1 and STAT3 transcription factors. To understand the coordination of these STAT activities, we applied RNA-seq, ChIP-seq, and ATAC-seq to identify the transcriptional output of STAT1 and STAT3 in peritoneal tissues from mice during acute resolving inflammation and inflammation primed to drive fibrosis. Bioinformatics focused on the transcriptional signature of IL-6 in both settings and tested how pro-fibrotic Th1 cells altered the interpretation of STAT1 and STAT3 cytokine cues. In resolving inflammation, STAT1 and STAT3 cooperated to drive stromal gene expression affecting anti-microbial immunity and tissue homeostasis. The introduction of IFNγ-secreting Th1 cells altered this transcriptional program and channelled STAT1 and STAT3 to a previously latent GAS motif in Alu-like elements. STAT1 and STAT3 binding to this conserved sequence revealed evidence of reciprocal cross-regulation and gene signatures relevant to pathophysiology. Thus, we propose that effector T-cells re-tune the transcriptional output of IL-6 by establishing a regulatory interplay between STAT1 and STAT3 that steers the course of inflammation.

Divergent molecular and cytokine networks program functionally distinct CD8+ skin-resident memory T cells

Park S[1,2], Christo S[2], Wells A[3], Gandolfo L[2,4,5], Zaid A[6], Alexandre Y[2], Burn T[2], Schroeder J[2], Collins N[3], Han S[3], Guillaume S[2], Evrard M[2], Castellucci C[2], Osman M[2], Obers A[2], Kannourakis G[7], Wang H[2], Berzins S[2,7], Mielke L[8], Carbone F[2], Kallies A[2], Speed T[4,5], Belkaid Y[3,9], Mackay L[2]
[1]Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, [2]Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, [3]Metaorganism Immunity Section, Laboratory of Host Immunity and Microbiome, National Institute of Allergy and Infectious Diseases, National Institute of Health, [4]School of Mathematics and Statistics, The University of Melbourne, [5]Walter and Eliza Hall Institute for Medical Research, [6]Institute for Glycomics, Griffith University, [7]Federation University Australia and Fiona Elsey Cancer Research Institute, [8]Olivia Newton-John Cancer Research Institute; La Trobe University School of Cancer Medicine, [9]NIAID Microbiome Program, National Institute of Allergy and Infectious Diseases, National Institute of Health

Skin-resident CD8+ T cells comprise functionally distinct IFN-γ- (TRM1) and IL-17- (TRM17) producing subsets that differentially contribute to immune protection against infection or cancer and autoimmune pathology. However, whether TRM1 and TRM17 cells employ common or divergent mechanisms to establish permanent tissue-residence is unknown. We find that while both subsets are non-migratory and maintained in the absence of local TCR signaling, CD8+ TRM1 and TRM17 cells navigate divergent developmental trajectories to establish tissue residency in the skin. Strikingly, while TRM1 cells uniquely depend on a T-bet-Hobit-IL-15 axis for their survival, TRM17 cells develop independently of these factors. Instead, we reveal that the transcription factor c-Maf commands a tissue residency program in TRM17 cells parallel to that induced by Hobit in TRM1 cells, and we identify a novel ICOS-c-Maf-IL-7 axis selectively required for TRM17 cell commitment and persistence. Accordingly, blockade of either ICOS or IL-7 signaling enables subset-selective ablation of skin TRM17 cells without compromising their TRM1 counterparts, with functional consequences for protective immune responses in the skin. Thus, we show that functionally diverse skin-resident T cells rely on distinct molecular circuitries including separate cytokine signalling pathways, which can be exploited to strategically modulate local immunity. Altogether, our results may inform the development of novel immunotherapies designed to selectively target and remove deleterious subsets of TRM cells that orchestrate autoimmune disease, whilst preserving protective TRM cells essential to uphold barrier immunity.

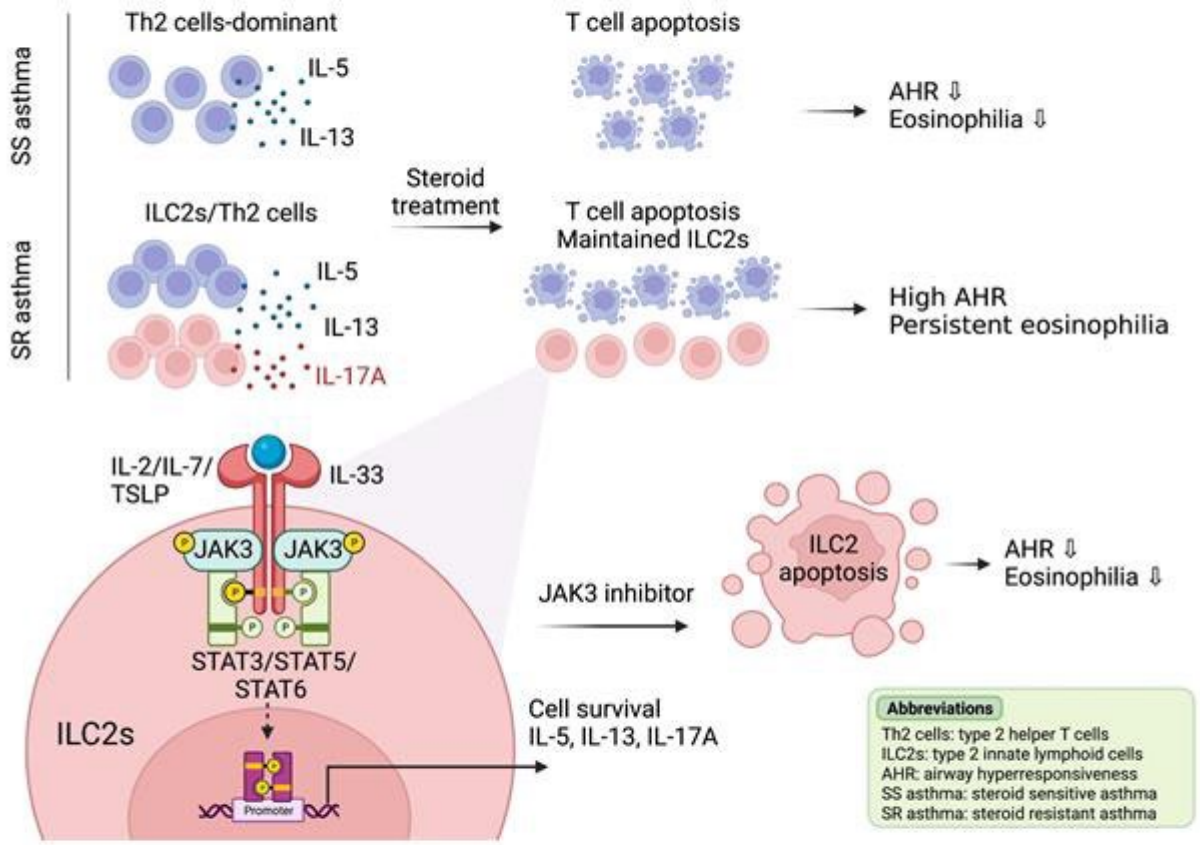
JAK3 inhibitor suppresses multipotent ILC2s and attenuates steroid-resistant asthma

Kim H[1]
[1]Seoul National University

Symposium 3: Innate lymphoid cells, heterogeneity and function

The standard treatment for the dominant asthma endotype, allergic-airway inflammation, is steroids. However, steroid-refractory asthma poses a significant problem. Innate lymphoid cells (ILCs) produce type-2 cytokines that are crucial in asthma pathogenesis similar to Th2 cells, and there is limited evidence from asthma-mouse models and human studies to suggest that ILC2s may play a role in steroid-resistant asthma. This study demonstrates that lung ILC2s, but not Th2 cells, can develop steroid resistance that allows them to survive, produce cytokines and maintain their pathogenic activity during steroid treatment. The presence of multiple ILC2-stimulating cytokines and the emergence of multipotent IL-5+IL-13+IL-17A+ ILC2s are associated with steroid-resistant ILC2s. The Janus-kinase (JAK) 3/signal-transducer-and-activator-of-transcription (STAT) 3, 5, and 6 pathways also contribute to the acquisition of steroid-resistant ILC2s. Treatment with a JAK3 inhibitor significantly reduces the survival, proliferation, and cytokine production of multipotent ILC2s in vitro and ameliorates ILC2-dependent Alternaria-induced asthma. Furthermore, combining a JAK3 inhibitor with steroids results in significant inhibition of steroid-resistant asthma. These findings suggest that chronic asthmatic conditions may induce multipotent ILC2s that promote steroid-resistant asthma and that combining a JAK3 inhibitor with steroids may be a viable therapeutic option for steroid-refractory asthma.

Graphic abstract



Dictionary of immune responses to 86 cytokines in vivo at single-cell resolution

Cui A[1,2], Huang T[3], Li S[2,3], Ma A[2], Perez J[2], Sander C[2,3], Keskin D[3], Wu C[2,3], Fraenkel E[2], Hacohen N[2]
[1]Harvard University, [2]Broad Institute of MIT and Harvard, [3]Dana-Farber Cancer Institute

Symposium 3: Innate lymphoid cells, heterogeneity and function

Background: Cytokines mediate cell-cell communication in the immune system and represent important therapeutic targets. Many studies have underscored their central role in immune function, yet we lack a global view of the cellular responses of each immune cell type to each cytokine.

Methods: We created the Immune Dictionary – a compendium of single-cell transcriptomic profiles of over 20 cell types in response to each of 86 cytokines in murine lymph nodes in vivo.

Results: The dictionary revealed that most cytokines induced highly cell type-specific responses. For example, the inflammatory cytokine IL-1β induced distinct gene programs in almost every cell type. A cell type-centric view of the dictionary identified over 65 cytokine-driven cell polarization states across immune cell types, including an IL-18-induced polyfunctional NK cell state. Based on the dictionary, we developed companion software, Immune Response Enrichment Analysis (IREA), for assessing cytokine activities and immune cell polarization from gene expression data, and applied it to reveal cytokine networks in tumors following immune checkpoint blockade therapy.

Conclusion: Our dictionary generates new hypotheses for cytokine functions, illuminates pleiotropic effects of cytokines, expands our knowledge of activation states of each immune cell type, and provides a framework to deduce the roles of specific cytokines and cell-cell communication networks in any immune response.

Targeted activation of IL-22-producing innate lymphoid cells enhances host resistance against Clostridioides difficile infection

Abt M[1], Denny J[1], Maslanka J[1], Mdluli N[1], Mears K[1]
[1]University of Pennsylvania

Symposium 3: Innate lymphoid cells, heterogeneity and function

Clostridioides difficile, the most common hospital acquired pathogen in the United States, infects the gastrointestinal tract following perturbation of the intestinal microbiota causing debilitating, potentially fatal colitis. Primary C. difficile infection has a high recurrence rate following antibiotic treatment, emphasizing the need to develop alternative treatment strategies to reduce the burden on the healthcare system. Innate lymphoid cells (ILCs) are necessary for acute host defense following C. difficile infection and represent a promising therapeutic target to harness the patient's innate immune defenses to limit disease. Here, using a murine model of C. difficile infection, we report that oral administration of Resiquimod (R848), a TLR-7 agonist previously reported to activate type-3 ILCs (ILC3s), protects mice from lethal C. difficile challenge. R848 treatment did not alter intestinal microbial communities or impact C. difficile burden. R848 treatment conveyed protection via activation of the host immune system in a TLR-7 dependent manner. R848-mediated protection was independent of T and B cells as well as type-I IFN signaling. Mice lacking ILCs, however, did not respond to R848 treatment and remained susceptible to infection, identifying these cells as the key targets of R848 activity. Last, R848 treatment robustly induced ILC3s in the large intestine to produce IL-22 and genetic ablation of IL-22 abrogated R848-mediated protection from C. difficile infection. Combined, these data identify an immunostimulatory molecule that activates the IL-22 producing ILC3s to support host defense against C. difficile infection without causing further dysbiosis of the intestinal microbiota.

Synovial CRTAM+ T-cells are a feature of lymphoid-driven synovitis.

Derrac Soria A[1,2], Monaco F[1], Lewis M[3], Choy E[1,2], Pitzalis C[3], Jones G[4], Jones S[1,2], Dimonte S[1]
[1]Division of Infection & Immunity, School of Medicine, Cardiff University, [2]Systems Immunity University Research Institute, Cardiff University, [3]Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, Queen Mary's School of Medicine & Dentistry, [4]School of Cellular & Molecular Medicine, University of Bristol

Symposium 4: Immune regulation in autoimmune and rheumatic diseases

Rheumatoid arthritis is a heterogeneous disease with synovial joint biopsies characterised by fibroblast-driven, myeloid-driven, and lymphoid-driven synovitis. The mechanisms controlling these pathologies are unknown but affect disease severity, progression, and response to biological medicines. To understand the inflammatory mechanisms contributing to these different forms, we hope to identify novel markers instructing the course of synovitis. Interrogating available RNA-seq data from human synovial biopsies, we have identified an immunoglobulin-like cell surface protein, Class I-restricted T-cell associated molecule; (CRTAM) highly expressed in lymphoid-rich synovitis. Analysis revealed positive correlates between synovial CRTAM and the expression of regulatory receptors (e.g., CD2, CTLA4, SLAMF7), chemokine receptors (e.g., CXCR3, CXCR4, CXCR6), granzymes (e.g., GZMA, GZMB, GZMK), effector cytokines (e.g., IFNG, IL21) and transcription factors (e.g., BATF, EOMES). Interrogating single-cell RNA-seq data from Accelerating Medicines Partnership Rheumatoid Arthritis Phase-1 Project, we identified CRTAM-positive lymphocytes characterised by granzyme and CCR7 or PD-1 expression. Thus, synovial CRTAM corresponds with cytokines involved in shaping antigen-specific responses and the effector properties of T-cells and B cells in lymphoid-rich synovitis. Extending these findings to the study of mice with antigen-induced arthritis (AIA), we observed that synovitis was associated with an accumulation of CD4+CRTAM+ T cells. These cells were abundant in Il27ra-/- mice, which display synovial ectopic lymphoid-like structures following AIA. We next considered the synovial expression of the CRTAM ligand CADM1. Unlike CRTAM, synovial CADM1 showed no correlation with clinical assessments of disease (e.g., DAS28 scores, ESR, tender joint scores and pain). Synovial cells expressing CADM1 included sub-lining fibroblasts and IL-1beta+ and NUPR1+ monocytes. CADM1 was unchanged by infiltrating leukocytes, and Il6ra-/- mice, which develop synovitis lacking a synovial infiltration, showed comparable Cadm1 expression to that of wt and Il27ra-/- mice. Thus, CRTAM and CADM1 may facilitate synovial interactions between stromal tissues and infiltrating immune cells that instruct lymphocyte responses during synovitis.

Inflammatory arthritis induced by immune checkpoint inhibitors: underlying mechanisms and therapeutic strategies

Nurieva R[1], Turner N[1], Rico R[1], Alekseev A[1], Kim S[1]
[1]The University of Texas MD Anderson Cancer Center

Symposium 4: Immune regulation in autoimmune and rheumatic diseases

Introduction: Although immune checkpoint inhibitors (ICIs) are revolutionizing cancer treatment, ICIs are associated with life- or organ-threatening complications, termed immune-related adverse events (irAEs), including arthritis-irAE. Arthritis-irAE is associated with either PD-1 inhibitor (PD-1i) monotherapy or combined ICI therapy (CTLA-4i and PD-1i). Arthritis-irAE can greatly impair quality of life, and lead to discontinuation of ICI(s) therapy. Steroids, the first line of treatment for arthritis-irAEs, significantly abrogate the anti-tumor efficacy of ICIs; however, our knowledge of the mechanisms and signs that underpin the onset and progression of arthritis-irAEs is very limited.

Methods: We have analyzed peripheral blood (PB) and/or synovial fluid (SF) samples from arthritis-irAE patients by single cell RNA sequencing, FACS analysis, and multiplex immunoassay. We have established a preclinical murine model of arthritis-irAE and assessed therapeutic strategies.

Results: We comprehensively analyzed PB and/or SF samples from arthritis-irAE patients and identified molecular and cellular hallmarks in ICI-arthritis, which are distinct from those in classical autoimmune arthritis or non-arthritic irAEs. Our analyses revealed that T helper (Th)1-CD8+ T cells play a pivotal role in arthritis-irAE disease pathogenesis, and Th17 cells and transient Th17 (Th1/Th17) cells, enriched in arthritis after combined ICI therapy, are involved in steroid resistance. Utilizing the novel pre-clinical model of arthritis-irAE, we found significant increase of arthritis severity in mice receiving combined ICIs compared to PD-1i alone. In addition, we detected enhanced number of antigen-specific Th17/T cytotoxic (Tc)17 and transient Th17/Tc17 cells within inflamed joints of mice post combined ICIs, suggesting that novel murine model recapitulates ICI-arthritis. Importantly, administration of Th17-cell targeted agents in arthritis-irAE mice led to improvement of arthritis severity, indicating the importance of preclinical model for testing of therapeutic strategies for ICI-arthritis without abrogating ICI-antitumor efficacy.

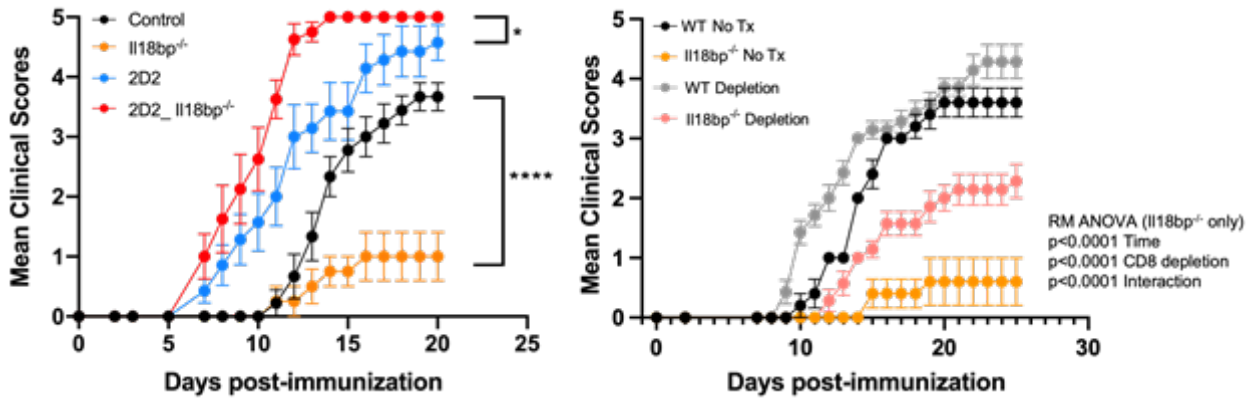
Conclusion: Our results thus for first time, provide insights into the mechanisms, predictive biomarkers, and therapeutic targets for arthritis-irAE.

Excess IL-18 augments suppressor/regulatory cell function to prevent Experimental Autoimmune Encephalomyelitis

Morrissette J[1,2], Dang V[1], Varghese J[1], Nguyen J[1], Lanzar Z[2], Landy E[3], Frank-kamenetskii A[1], Ring A[4], Canna S[1,2]
[1]Children's Hospital Of Philadelphia, [2]University of Pennsylvania, Perelman School of Medicine, [3]University of Pittsburgh School of Medicine, [4]Yale School of Medicine

Symposium 4: Immune regulation in autoimmune and rheumatic diseases

Interleukin 18 (IL-18) is an inflammasome-activated cytokine that canonically amplifies interferon-gamma (IFNg) production and cytotoxicity by CD8 T-cells (CD8Ts) and Th1 CD4T-cells (CD4Ts). It is inhibited by the high affinity antagonist, IL-18 binding protein (IL-18bp). In certain contexts, IL-18 amplifies non-Th1 responses including Th2, Th17, and Treg. Despite its likely pathogenic role in autoinflammation, clinical observations suggest a possible immunoregulatory role in CD4T-mediated autoimmunity. We sought to understand the role of IL-18 in the mixed Th1/17 EAE model of central nervous system autoimmunity. We immunized C57Bl/6 mice with CFA/MOG35-55 and pertussis toxin and assessed daily clinical scores and tissue cellular phenotypes. We hypothesized that systemic IL-18, either through genetic ablation of Il18bp (Il18bp-/-) or transgenic overproduction of mature IL-18 (Il18tg), would amplify the dominant inflammatory program and worsen EAE. However, Il18bp-/- and Il18tg mice, as well as mice treated with a recombinant decoy-resistant IL-18 (DR-18), were profoundly protected from EAE. Protection from EAE in Il18bp-/-mice was lost with neutralization of IFNg. In Il18bp-/-mice, draining lymph nodes demonstrated increased IFNg-producing, but equivalent IL-17-producing CD4Ts while spinal cords showed increased CD8Ts relative to CD4Ts. Mice expressing a transgenic T-cell receptor specific for I-Ab/MOG35-55 (2D2) have an abundance of myelin autoreactive CD4Ts and very few CD8Ts (due to allelic exclusion). Paradoxically, Il18bp-/-;2D2 mice develop more severe EAE than control 2D2 mice, suggesting that an increase in precursor autoreactive CD4Ts and/or loss of CD8Ts switches the effect of excess IL-18 from protective to pathogenic. Further, ex vivo IL-18 improved the ability of MOG-induced CD8Ts to delay EAE onset. Depletion of CD8Ts substantially, but incompletely, diminished protection in Il18bp-/- mice. Thus, unopposed IL-18 may preferentially augment CD8T suppressor function to mediate protection in EAE. This may inform novel strategies to amplify endogenous suppressive T cells or enhance cellular therapeutics in the treatment of autoimmune diseases.



Identification of a broadly fibrogenic macrophage subset induced by type 3 inflammation

Barron A[1], Fabre T[1], Christensen S[1], Asano S[1], Bound K[1], Lech M[1], Wadsworth M[1], Chen X[1], Wang C[1], Wang J[1], McMahon J[1], Schlerman F[1], White A[1], Kravarik K[1], Fisher A[2], Borthwick L[3], Hart K[1], Henderson N[4], Wynn T[1], Dower K[1]
[1]Pfizer, Inc., [2]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, [3]Newcastle Fibrosis Research Group, Biosciences Institute, Newcastle University, [4]MRC Human Genetics Unit, Institute of Genetics and Cancer, and Centre for Inflammation Research, the Queen's Medical Research Institute, University of Edinburgh

Macrophages are central orchestrators of the tissue response to injury, with distinct macrophage activation states playing key roles in fibrosis progression and resolution. Identifying key macrophage populations found in human fibrotic tissues could lead to new treatments for fibrosis. Here, we used human liver and lung single-cell RNA sequencing datasets to identify a subset of CD9+TREM2+ macrophages that express SPP1, GPNMB, FABP5, and CD63. In both human and murine hepatic and pulmonary fibrosis, these macrophages were enriched at the outside edges of scarring and adjacent to activated mesenchymal cells. Neutrophils expressing MMP9, which participates in the activation of TGF-β1, and the type 3 cytokines GM-CSF and IL-17A coclustered with these macrophages. In vitro, GM-CSF, IL-17A, and TGF-β1 drive the differentiation of human monocytes into macrophages expressing scar-associated markers. Such differentiated cells could degrade collagen IV but not collagen I and promote TGF-β1–induced collagen I deposition by activated mesenchymal cells. In murine models blocking GM-CSF, IL-17A or TGF-β1 reduced scar-associated macrophage expansion and hepatic or pulmonary fibrosis. Our work identifies a highly specific macrophage population to which we assign a profibrotic role across species and tissues. It further provides a strategy for unbiased discovery, triage, and preclinical validation of therapeutic targets based on this fibrogenic macrophage population.

A partial form of inherited human USP18 deficiency underlies infection and inflammation

Martin Fernandez M[1,2,3,4,5], Buta S[1,2,3,4,5], Le Voyer T[6,7], Li Z[8], Toftdal Dynesen L[8], Vuillier F[8], Franklin L[8], Ailal F[9,10], Muglia Amancio A[11,12], Malle L[1,2,3,4,5], Gruber C[1,2,3,4,5], Benhsaien I[9,10], Altman J[1,2,3,4,5], Taft J[1,2,3,4,5], Deswarte C[6,7], Roynard M[6,7], Nieto-Patlan A[6,7], Moriya K[6,7], Rosain J[6,7], Boddaert N[6,13], Bousfiha A[9,10], Crow Y[14,15], Jankovic D[11], Sher A[11], Casanova J[6,7,16,17,18], Pellegrini S[8], Bustamante J[6,7,16,18], Bogunovic D[1,2,3,4,5]

[1]Center for Inborn Errors of Immunity, Icahn School of Medicine at Mount Sinai, [2]Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, [3]The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, [4]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, [5]Microbiology Department, Icahn School of Medicine at Mount Sinai, [6]University of Paris, Imagine Institute, [7]Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut national de la santé et de la recherche médicale U1163, Necker Hospital for Sick Children, [8]Institut Pasteur, Cytokine Signaling Unit, Institut national de la santé et de la recherche médicale U1224, [9]Department of Pediatric Infectious Diseases, Clinical Immunology Unit, Children's Hospital, Centre Hospitalo-universitaire Averroes, [10]Laboratory of Clinical Immunology, Inflammation, and Allergy, Faculty of Medicine and Pharmacy of Casablanca, King Hassan II University,, [11]Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, [12]Hospital do Cancer de Muriae, Fundacao Cristiano Varella, [13]Department of Radiology, Assistance Publique - Hôpitaux de Paris, Necker Hospital for Sick Children, [14]Medical Research Council Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, [15]Laboratory of Neurogenetics and Neuroinflammation, Institut Imagine, Université de Paris, [16]St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, [17]Howard Hughes Medical Institute, [18]Center for the Study of Primary Immunodeficiencies, Assistance Publique - Hôpitaux de Paris, Necker Hospital for Sick Children

USP18 is an IFN-I-stimulated gene and a negative regulator of the IFN-I response. It is also an enzyme that removes covalently linked ISG15 from proteins it was conjugated to (deISGylation). Complete USP18 deficiency results in perinatal death with a diagnosis of pseudo-TORCH syndrome, due to uncontrolled IFN-I mediated inflammation. We report the occurrence of a homozygous hypomorphic USP18 mutation (p.I60N) in three siblings who presented with an adverse reaction to BCG vaccination, intracranial calcifications, and severe inflammation. We demonstrate that USP18 I60N had no impairment in stabilization by ISG15 and can deISGylate but is unable to effectively negatively regulate IFN-I signaling due to defective interaction with STAT2. When primed with type I IFN, patient fibroblasts are only partially refractory to a secondary IFN challenge unlike USP18 deficient cells, explaining the significantly milder disease. The patient has a steady-state type I IFN signature in whole blood, and IFN-I inflamed myeloid cells. We show that IFN-γ-dependent induction of IL-12 and IL-23 is reduced, due to IFN-I-mediated impairment of myeloid cells to produce both cytokines. Thus, insufficient negative regulation of IFN-I signaling by USP18-I60N underlies type I interferonopathy, which impairs IL-12 and IL-23 production by myeloid cells, explaining predisposition to mycobacterial disease.

IL-33-dependent upregulation of Serpine1 contributes to intestinal fibrosis in an animal model of Crohn's disease-like ileitis

De Santis S[1], Puca P[2], Wargo H[1], Williams J[1], Vidmar K[1], Chieppa M[3], Del Pinto R[4], Pietropaoli D[4], Cominelli F[5], De Salvo C[1], Pizarrro T[1]
[1]Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA, [2]IBD Unit - Digestive Disease Center - Surgical and Medical Sciences Department, Fondazione Policlinico Universitario "A. Gemelli" IRCCS - Università Cattolica del Sacro Cuore, Roma, Italy, [3]Department of Biological and Environmental Sciences and Technologies (DISTEBA), University of Salento, 73100 Lecce, Italy, [4]Department of Clinical Medicine, Public Health, Life and Environmental Sciences, University of L'Aquila, L'Aquila, Italy, [5]Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

IL-33 is a pleiotropic cytokine known to possess dichotomous roles during gut health and disease. We previously described that IL-33 promotes epithelial restitution/repair in otherwise healthy (C57BL/6) mice that have been challenged with dextran sodium sulphate (DSS) to induce acute colitis, with the end result of efficient resolution of inflammation. However, in SAMP1/YitFc (SAMP) mice that spontaneously develop Crohn's disease (CD)-like ileitis, elevated IL-33 levels persist as disease progresses, perpetuating chronic gut inflammation and severe fibrosis. While IL-33 has been implicated in the development of inflammation-associated fibrosis, the precise mechanism(s) by which this occurs remains unclear. The aim of this study was to determine IL-33-dependent events leading to intestinal fibrosis in ileitis-prone SAMP mice. Microarray data showed increased Il33 and its cognate receptor, Il1rl1 (ST2), as well as several profibrogenic genes, in strictured areas of full-thickness ilea from SAMP mice compared to parental (AKR) controls. IHC confirmed these findings and co-localized IL-33 to cells morphologically-consistent with both histiocytes and subepithelial myofibroblasts (SEMFs). Bulk RNA-Seq analysis of the human SEMF cell line, CCD-18Co, stimulated +/- IL-33 showed one of the highest-expressing transcripts as Serpine1, which codes for plasminogen activator inhibitor-1 (PAI-1), and has been previously reported to be highly enriched in IBD patients with active disease that do not respond to anti-TNF therapy. In fact, spatial transcriptomics revealed a progressive, concomitant increase in Il33 and Serpine1 in SAMP ilea, with a strong correlation of expression, particularly during later time points when fibrosis was evident. Currently, results are pending on SAMP mice treated with the PAI-1 inhibitor, MDI-2268, to determine its direct effect(s) on the development of inflammation-associated intestinal fibrosis. Taken together, these findings suggest IL-33-dependent regulation of Serpine1/PAI-1 that promotes intestinal fibrosis, commonly observed in CD patients, and may provide a novel target to treat IBD patients with fibrostenoic disease.

Cytokine-driven differentiation of the intestinal epithelium is countermanded by the helminth parasite H. polygyrus

Campillo-Poveda M[1], Cunningham K[1]
[1]University of Glasgow

Lightning Talks / Cytokines in infections

Many parasitic helminths establish long-term chronic infections, attributed to their ability to secrete products that alter their environmental niche to their advantage. Although immunomodulatory proteins have now been defined that target host immune cell populations, less is known of their impact on the epithelial layer, which serves as the first point of contact and a critical barrier to infection. In helminth infection, the epithelium also acts as a critical innate immune effector population through expansion of tuft cells and goblet cells, resulting from IL-4 and IL-13 stimulation of intestinal stem cells. In mice, the activation of this pathway results in expulsion of the rat parasite Nippostrongylus brasiliensis; however, in the natural mouse helminth, Heligmosomoides polygyrus, tuft cell expansion is more muted and chronic establishment results; furthermore, H. polygyrus suppresses tuft cell expansion either by N. brasiliensis or by administration of the metabolite succinate. We modelled these interactions in vitro using small intestinal organoid cultures and found that H. polygyrus excretory/secretory products (HES) reversed the effects of IL-4 and IL-13 on developmental pathways and suppressed the expression of tuft, Paneth, and goblet cell-associated gene sets. Moreover, organoid morphology was drastically altered, with HES causing a spheroid, proliferative phenotype devoid of crypts and differentiated cells. Among the components of HES are a group of TGFβ mimic proteins (TGMs) which bind receptors known to be expressed on intestinal stem cells, and fractionation of HES has identified a further subgroup of protein candidates that may interfere with differentiation of tuft and other specialised secretory cells. In this manner, H. polygyrus is able to prevent the development of a crucial epithelial cell for immune defence, allowing the parasite to survive and promoting a permissive environment for long-term chronic infection.

Interferon λ alters IL-17-mediated immunity during influenza, bacteria super-infection

Antos D[1], Rich H[1], Alcorn J[1]
[1]University Of Pittsburgh

Lightning Talks / Cytokines in infections

Each year, influenza infections result in a significant number of mortalities, which can be complicated by secondary bacterial super-infection. Primary influenza infection can increase susceptibility to secondary methicillin-resistant Staphylococcus aureus (MRSA) infection by altering the host immune response, leading to heightened mortality rates compared to infection with either pathogen alone. Macrophages are important in super-infection immunity as they engulf, degrade, and present bacterial antigens to adaptive immune cells, ultimately leading to activation of lymphocytes. Prior work by our group shows that interferon (IFN)α/β are involved in type 17 immune attenuation after primary influenza infection, which indicates that IFNλ may exhibit similar inhibitory functions due to overlapping signaling pathways. Though the effects of IFNλ on epithelial cells during super-infection have been previously outlined, the impact of IFNλ on immune cells is less defined. Using mouse models of IFNλR deletion globally and specifically in stromal and immune cells, we show a detrimental role for IFNλ in vivo during super-infection, both by altering macrophage function and by dampening type 17 immunity. Global IFNλ receptor (IFNλR) knockout mice had lower bacterial burden during super-infection compared to wild type mice with enhanced levels of IL-17 and IL-22 in the airways. Manipulation of type 17 cytokines, including IL-1β, resulted in modulation of bacterial burden in both wild type and IFNλR knockout mice. While macrophage-specific depletion of IFNλR also resulted in reduced bacterial burden, enhanced bacterial uptake by innate immune cells was only seen in global IFNλR knockout mice. Bone marrow chimera studies showed that IFNλR on stromal cells was required for the production of IL-1β and other cell-recruiting chemokines. In summation, we have shown that IFNλ signaling inhibited IL-17-dependent immunity during super-infection via regulating IL-17 production and immune cell recruitment to the lungs without impacting lung damage or efferocytosis by macrophages, which prolongs bacterial infection resolution.

Senescent cells in biopsies of GCA

VEROUTIS D[1], Argyropoulou O[2], Goules A[2], Kambas K[3], Palamidas D[2], Evangelou K[1], Chavaki S[1], Polyzou A[1], Karatza E[4], Boki K[5], Cavazza A[6], Kitas C[1,7], Thanos D[8], Ricordi C[9], Marvisi C[9], Muratore F[9], Croci S[10], Salvarani C[11], Gorgoulis V[1,12,13,14], Tzioufas A[2]
[1]Molecular Carcinogenesis Group, Department of Histology and Embryology, Medical School, National and Kapodistrian University of Athens, [2]Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, [3]Laboratory of Molecular Genetics, Department of Immunology, Hellenic Pasteur Institute, [4]Second Propaedeutic Department of Surgery, National and Kapodistrian University of Athens, Laikon General Hospital, [5]Rheumatology Unit, Sismanoglion Hospital, [6]Pathology Unit, Azienda Unità Sanitaria Locale-IRCCS Di Reggio Emilia, [7]Faculty of Health and Medical Sciences, University of Surrey, [8]Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, [9]Unit of Rheumatology, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, and University of Modena and Reggio Emilia, [10]Unit of Autoimmunità, Allergologia e Biotecnologie Innovative, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, [11]Unit of Rheumatology, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, and University of Modena and Reggio Emilia, Surgery, Medicine, Dentistry and Morphological Sciences with Interest in Transplant, Oncology and Regenerative Medicine, [12]Ninewells Hospital and Medical School, University of Dundee, [13]Biomedical Research Foundation, Academy of Athens, [14]Molecular and Clinical Cancer Sciences, Manchester Cancer Research Centre, Manchester Academic Health Sciences Centre, University of Manchester

Lighting Talks / Cytokines in chronic diseases

Background: Age is the strongest risk factor of giant cell arteritis (GCA), implying a possible pathogenetic role of cellular senescence. To address this question, we applied an established senescence specific multi-marker algorithm in tissue artery biopsies (TABs) of GCA patients.
Methods: Seventy five TABs from GCA patients and 22 from patients with polymyalgia rheumatica (PMR) were retrospectively retrieved and analyzed. Senescent cells and their histologic origin were identified with specific cellular markers; IL-6 and MMP-9 were investigated as components of the senescent associated secretory phenotype (SASP) by triple co-staining. GCA or PMR artery culture supernatants were applied to primary skin fibroblasts with or without IL-6 blocking agent to explore the induction of IL-6 associated cellular senescence.
Results: Senescent cells were mainly present in GCA arteries at higher proportion compared to PMR (9.50% vs 2.66% respectively, $p<0.0001$) and were mainly originated from fibroblasts, macrophages and endothelial cells. IL-6 was expressed by senescent fibroblasts and macrophages while MMP-9 by fibroblasts only. IL-6 positive senescent cells were associated with the extension of vascular inflammation (adventitial limited disease vs transmural inflammation: 10.02% vs 4.37% respectively, $p<0.0001$). GCA but not PMR artery culture supernatant could induce IL-6-associated senescence that was partially inhibited by IL-6 blockade.
Conclusions: Senescent cells are present in GCA arteries suggesting a potential implication in disease pathogenesis by perpetuating inflammation and affecting vascular remodeling via IL-6 dependent mechanisms.

Targeting metalloproteinases as a novel therapeutic strategy for pancreatitis

Saad M[1,2], Weng T[1,2], Kumar B[3], Rose-John S[4], Jenkins B[1,2]
[1]Centre for Innate Immunity and Infectious Diseases (CiiiD), Hudson Institute of Medical Research, [2]Department of Molecular and Translational Science, Monash University, [3]Monash Health, [4]Department of Biochemistry, Christian-Albrechts-Universität zu Kiel

Background: Pancreatitis is a multifactorial upper gastrointestinal inflammatory disorder that is associated with substantial morbidity, mortality, and economic burden worldwide. Currently, there are no effective therapeutic agents for pancreatitis. In this regard, the protease A Disintegrin And Metalloproteinase 17 (ADAM17) and the family of rhomboid pseudoproteases (iRhoms; which mainly controls ADAM17 activity) mediate inflammatory responses through shedding of bioactive inflammatory mediators, including tumour necrosis factor alpha (TNFα) and soluble IL-6 receptor (sIL-6R), the latter of which drives proinflammatory IL-6 trans-signaling. However, the role of ADAM17 and iRhoms in pancreatitis is ill-defined.
Methods: To address this, Adam17ex/ex mice (which are homozygous for the hypomorphic Adam17ex allele resulting in marked reduction in ADAM17 expression), Rhbdf2-/- mice (lacking iRhom2 expression), Adam17ex/ex:Rhbdf2-/- mice, and their wild-type (WT) littermates were exposed to the cerulein-induced acute pancreatitis model.
Results: Our data reveal that expression levels of ADAM17 and iRhoms was up-regulated in pancreatic tissues of animal models of pancreatitis. Moreover, the genetic targeting of ADAM17 and iRhom2 ameliorated experimental pancreatitis, which was associated with a reduction in the IL-6 trans-signaling/STAT3 axis. This led to reduced inflammatory cell infiltration and necrosis in the pancreas. Our data also revealed a hitherto unknown ADAM17-independent actions of iRhom2 in the context of pancreatitis.
Conclusion: Collectively, our findings indicate that the ADAM17 protease and the iRhom2 pseudoprotease play a pivotal role in the pathogenesis of pancreatitis, which could pave the way for devising novel therapeutic and biomarker options against this disease.

IL-32 is elevated in COVID-19 and synergizes with IFN-γ to drive macrophage inflammatory responses via an IRAK1-dependent pathway.

Ramon A[1], Skowyra A[1], Lee C[1], Crowley T[1], Lindsay A[2], Woznicki J[1], Stamou P[1], Velmurugan J[1], Lohan A[1], Albrich W[3], O'Mahony L[1], Melgar S[1], Macoritto M[4], Sadhukhan R[4], Levesque M[4], McRae B[4], Matzelle M[4], Nally K[1,2]
[1]APC Microbiome Ireland, University College Cork, [2]School of Biochemistry & Cell Biology, University College Cork, [3]Division of Infectious Diseases & Hospital Epidemiology, Cantonal Hospital St. Gallen, [4]Immunology Discovery, AbbVie Bioresearch Center

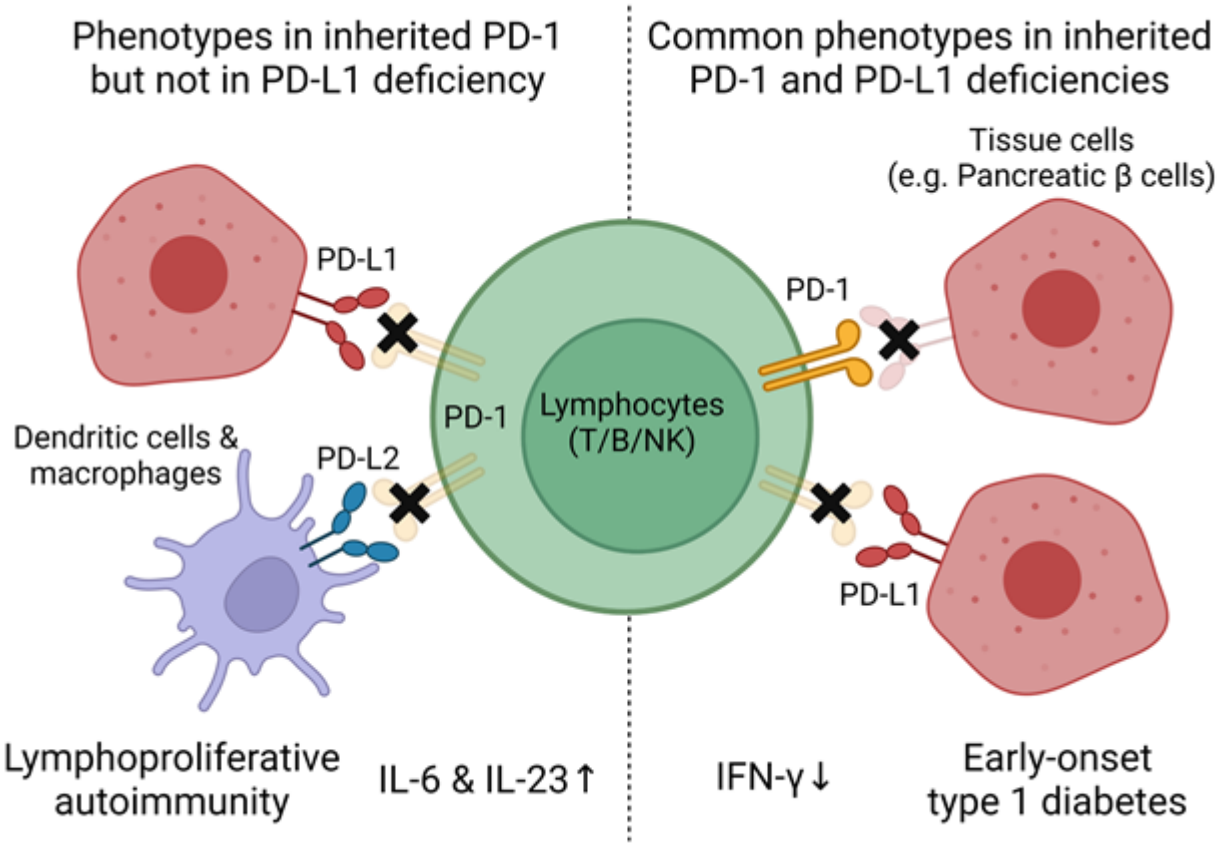
IFN-γ-driven M1-like inflammatory macrophage states are shared and abundant across several human immune-mediated inflammatory diseases (IMIDs) including, rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and COVID-19. To identify secreted proteins which drive or suppress M1-like macrophage states we screened >600 human proteins and peptides in cell-based assays using human monocyte-derived macrophages for four phenotypic states (M1-like, M1-like in IFN-γ-primed cells, IFN-γ-like and IL-10-like) based on production of inflammatory cytokines. We identified and validated several hits from our screens including the cytokines IL-32β and IL-32γ but not IL-32α which induced an M1-like inflammatory state in non-primed and IFN-γ-primed macrophages. IL-32β induced macrophage responses such as tolerance, cross-tolerance, activation of inflammatory signalling pathways and transcriptional responses which were overlapping yet distinct to those observed with LPS. Because IL-32 is an orphan cytokine with no rodent orthologue and without an identified receptor or downstream signalling pathway we used genome-wide RNAi screens, small molecule inhibitors and co-localization assays to identify the signalling pathway. We found that IL-32β uses components of the TLR4-MyD88-IRAK1 signalling pathway to mediate inflammatory signalling in human macrophages. Furthermore, by analysing the expression of IL-32β, IFN-γ, their pathway component genes and their specific transcriptional responses in macrophages across multiple human IMID single-cell datasets we identified a potential role for both IL-32β and IFN-γ signalling to macrophages in COVID-19. Analysis of serum from healthy controls and patients with mild and severe COVID-19 revealed elevated levels of IL-32 in severe COVID-19. Collectively, this work identifies IL-32 as a cytokine which synergises with IFN-γ, signals through a MyD88/IRAK-1 pathway, is elevated in severe COVID-19 and induces a transcriptional response which is observed in macrophages in mild and severe COVID-19.

Milder autoimmunity in humans with inherited PD-L1 than PD-1 deficiency

Johnson M[1], Ogishi M[2], Domingo C[3], Chen J[4], Boisson-Dupuis S[2], Anderson M[4], Casanova J[2], Tree T[3], Oram R[1]
[1]University of Exeter, [2]The Rockefeller University, [3]King's College London, [4]University of California San Francisco

Lighting Talks / Cytokines in chronic diseases

Background: Programmed death 1 (PD-1) expressed on lymphocytes restricts their activation when engaged by programmed death ligands 1 and 2 (PD-L1/2). We previously reported two siblings with inherited PD-1 deficiency, who manifested early-onset type I diabetes, thyroiditis, and lethal lymphoproliferative autoimmunity (deceased at ages 3 and 11 years). Although patients with malignancy treated with neutralizing antibodies against PD-1 and PD-L1 frequently manifest immune-related adverse events, the nonredundant roles of PD-1, PD-L1, and PD-L2 in preventing various forms of autoimmunity in humans remain poorly understood.
Methods: We studied two siblings (ages 11 and 10 years) with neonatal-onset type 1 diabetes (diagnosed at ages 1 day and 7 weeks, respectively) and a homozygous essential splicing variant in CD274 (encoding PD-L1).
Results: Exon trapping and bulk RNA sequencing analyses showed that the splicing variant resulted in an in-frame 51 amino acid deletion in the extracellular domain of PD-L1, leading to complete loss-of-function in an overexpression system. Surprisingly, cytometric immunophenotyping and single-cell RNA sequencing analysis at baseline showed largely normal development and transcriptional profiles across myeloid and lymphoid leukocyte subsets in the PD-L1-deficient siblings, whereas the previously reported PD-1-deficient child showed a reduction of Vδ2+ γδ T and MAIT cells, expansion of CD4-CD8- double-negative αβ T cells, and transcriptional dysregulation in Tregs and myeloid cells. Upon stimulation, PD-L1-deficient leukocytes did not show excessive production of IL-6 and IL-23, unlike PD-1-deficient leukocytes. Nevertheless, PD-L1-deficient leukocytes showed impaired IFN-γ production, like PD-1-deficient leukocytes, while showing excessive production of IL-4 and IL-10. Cultured PD-1- and PD-L1-deficient T lymphocytes also showed impaired IFN-γ production, suggesting cell-intrinsic imprinted alteration.
Conclusion: Our work suggests that both PD-1 and PD-L1 are essential for preventing early-onset type I diabetes, yet that PD-L1 is redundant for preventing unrestrained leukocyte dysregulation, possibly accounting for the more severe autoimmunity in patients with PD-1 deficiency.


Phenotypes in inherited PD-1 but not in PD-L1 deficiency
Common phenotypes in inherited PD-1 and PD-L1 deficiencies
Tissue cells (e.g. Pancreatic β cells)
PD-L1
PD-1
PD-1
Lymphocytes (T/B/NK)
Dendritic cells & macrophages
PD-L2
PD-L1
Lymphoproliferative autoimmunity
IL-6 & IL-23↑
IFN-γ↓
Early-onset type 1 diabetes

IL-1 alpha is required for T cell driven weight loss during respiratory viral infection.

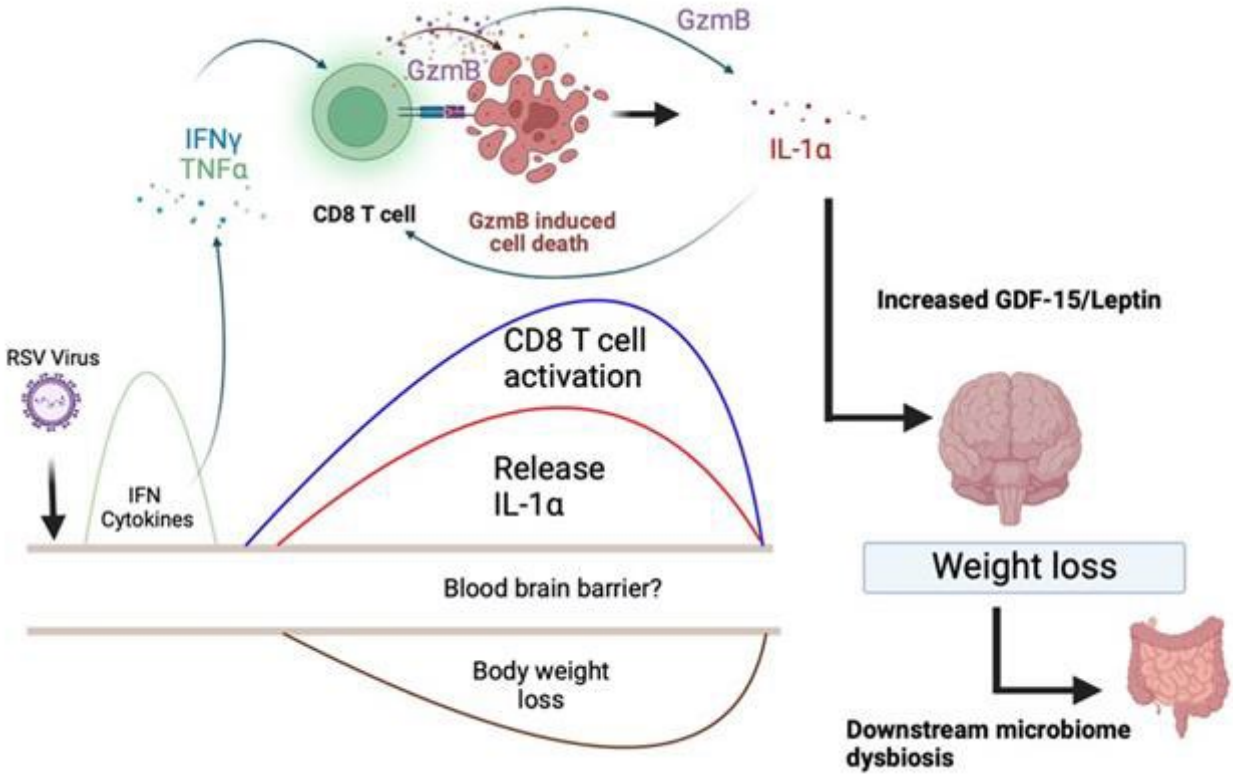
Wang Z[1], Tregoning J[1]
[1]Imperial College London

Lightning Talks / Cytokines in infections

Background
Respiratory viral infections remain a major cause of hospitalisation and death in infants, young children, and older adults worldwide. Patients with respiratory infections such as SARS-CoV-2, influenza, and respiratory syncytial virus (RSV) often lose weight. This weight loss is driven by the underlying host immune response to infection interfering with homeostatic regulation of appetite and metabolism. Whilst acute weight loss is speculated to be a tolerance mechanism to limit pathogen growth, severe weight loss following infection can lead to a deterioration in the quality of life and contributes to increased morbidity and mortality. Despite the clinical relevance of weight loss during infection, its causal mechanisms are not yet completely understood.

Methods and results
We utilised an in vivo model of CD8+ T cell-driven weight loss during RSV infection to dissect the immune regulation of weight loss. We observed significant enrichment of the IL-1 signalling pathway in the blood and airways after RSV. Infection associated weight loss was significantly reduced following IL-1α, but not IL-1β blockade, despite increased viral load. Direct nasal instillation of IL-1α led to weight loss and a downregulation of genes associated with lipid and glucose metabolism, of note we detected IL-1α in the brain after both infection and nasal delivery.

Conclusion
Together, these findings indicate a lung-brain-gut signalling axis for IL-1α in regulating weight loss after respiratory infection. Although the benefit of altering metabolism after infection is yet unknown, finding the balance between immunopathology and protection is critical against infectious diseases without fitness costs to the host. Dissecting the mechanism of IL-1α-driven infection-associated weight loss could contribute toward the development of novel therapeutic strategies.


GzmB
GzmB
IFNγ
TNFα
IL-1α
CD8 T cell
GzmB induced
cell death
Increased GDF-15/Leptin
RSV Virus
CD8 T cell
activation
Release
IL-1α
IFN
Cytokines
Blood brain barrier?
Weight loss
Body weight
loss
Downstream microbiome
dysbiosis

Prostaglandin E2-EP4 signaling in dendritic cells regulates intestinal homeostasis by controlling IL-6 production and Th17 differentiation

Kim J[1], Jung W[1], Song H[1], Park S[1], Kim S[1], Kim Y[1]
[1]Kaist

Lighting Talks / Cytokines in chronic diseases

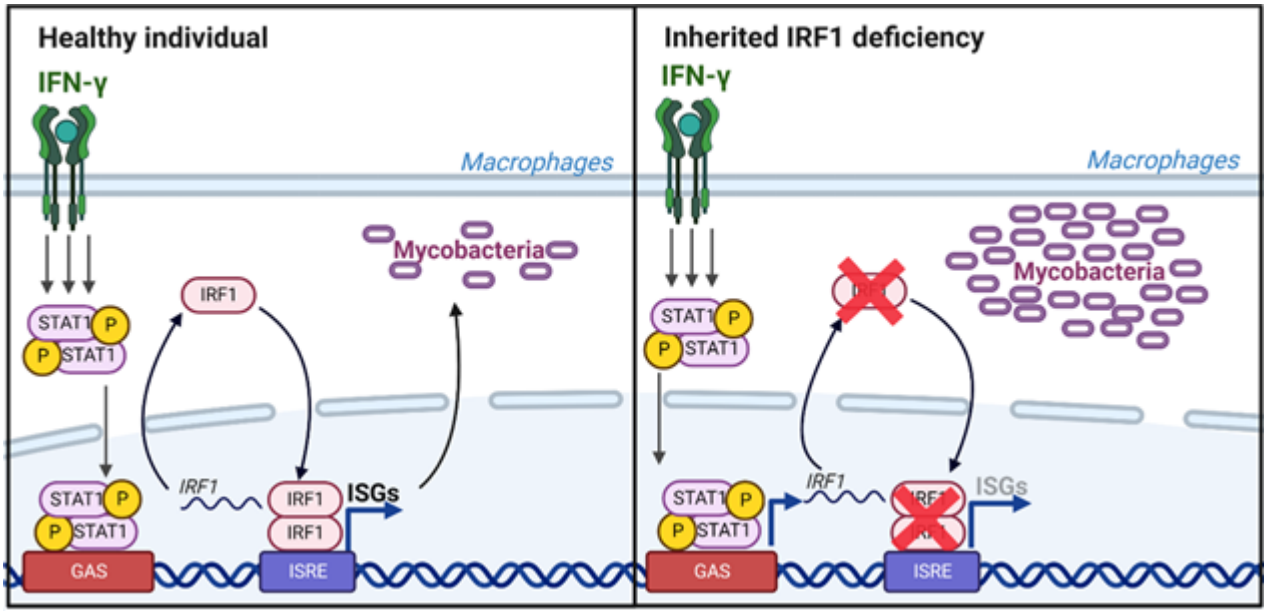
The intestine is constantly exposed to various environmental factors, including food, commensal microbiome, and harmful antigens that could trigger intestinal inflammation. Despite this, the intestine maintains immune tolerance by regulating immune cell activation, through dendritic cells (DCs) that play a crucial role in regulating the polarization and activation of regulatory T cells (Treg) and Th17 cells. The intestine has a higher proportion of Th17 cells compared to other organs, which is believed to be controlled by the unique characteristics of intestinal lamina propria dendritic cells (LP-DCs). However, the specific mechanisms underlying the distinct characteristics of LP-DCs remain unclear. It is well established that Prostaglandin E2 (PGE2), which is more abundant in the intestine than in other organs, regulates tolerogenic properties and cytokine production of immune cells. Based on this, we hypothesized that PGE2 plays a crucial role in regulating the function of LP-DCs and that its signaling in LP-DCs is critical for maintaining intestinal homeostasis. Our research has demonstrated that in vitro, PGE2 can enhance the Th17 differentiation ability and cytokine production, particularly IL-6, of DCs through the EP4 receptor, which is one of the PGE2 receptors (EP1-4). Additionally, DCs differentiated with PGE2 gained signature genes of LP-DCs and were associated with leading IL-6 production and Th17 differentiation. Moreover, DC-specific EP4 deficient mice (EP4ΔDC) had fewer intestinal Th17 cells compared to control mice and showed similar symptoms to those with a broken gut barrier, such as higher neutrophil infiltration into intestine and microbiota-specific serum IgA, and mesenteric lymphadenitis, in the steady state. In addition, EP4ΔDC mice had more susceptible to DSS-induced colitis than control mice. In conclusion, our results demonstrate that PGE2-EP4 signaling in DCs sets up the signature function of LP-DC for intestinal homeostasis, specifically by regulating intestinal Th17 differentiation.

Human IRF1 governs macrophagic IFN-γ immunity to mycobacteria

Rosain J[1], Neehus A[1], Manry J[1], Yang R[2], Le Pen J[8], Daher W[9], Liu Z[2], Chan Y[2], Tahuil N[3], Turel O[4], Bourgey M[5], Ogishi M[2], Doisne J[6], Izquierdo H[11], Shirasaki T[10], Le Voyer T[1], Guérin A[7], IRF1-Consortium[1], Tangye S[7], Di Santo J[6], Zhang Q[2], Zhang S[2], Rice C[8], Béziat V[1], Lachmann N[12], Langlais D[5], Casanova J[2], Gros P[5], Bustamante J[1]
[1]Laboratory of Human Genetics of Infectious Diseases, Imagine Institute, Paris Cité University Inserm U1163, [2]St. Giles Laboratory of Human Genetics of Infectious Diseases, The Rockefeller University, [3]Department of Immunology, Del Niño Jesus Hospital, [4]Department of Pediatric Infectious Disease, Bezmialem Vakif University Faculty of Medicine, [5]McGill University, Departement of Biochemistry, [6]Innate Immunity Unit, Institut Pasteur, [7]Garvan Institute of Medical Research, University of NSW, [8]Laboratory of Virology And Infectious Diseases, The Rockefeller University, [9]Infectious Disease Research Institute of Montpellier (IRIM), Montpellier University, [10]Department of Medicine, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, [11]Institut Curie, PSL Research University, Inserm U932, [12]Institute of Experimental Hematology, REBIRTH Center for Regenerative and Translational Medicine, Hannover Medical School

Lightning Talks / Cytokines in infections

Inborn errors of human IFN-γ-dependent macrophagic immunity underlie mycobacterial diseases, whereas inborn errors of IFN-α/β-dependent intrinsic immunity underlie viral diseases. Both types of IFNs induce the transcription factor IRF1. We describe unrelated children with inherited complete IRF1 deficiency and earlyonset, multiple, life-threatening diseases caused by weakly virulent mycobacteria and related intramacrophagic pathogens. These children have no history of severe viral disease, despite exposure to many viruses, including SARS-CoV-2, which is life-threatening in individuals with impaired IFN-α/β immunity. In leukocytes or fibroblasts stimulated in vitro, IRF1-dependent responses to IFN-γ are, both quantitatively and qualitatively, much stronger than those to IFN-α/β. Moreover, IRF1-deficient mononuclear phagocytes do not control mycobacteria and related pathogens normally when stimulated with IFN-γ. By contrast, IFN-α/β-dependent intrinsic immunity to nine viruses, including SARS-CoV-2, is almost normal in IRF1-deficient fibroblasts. Human IRF1 is essential for IFN-γ-dependent macrophagic immunity to mycobacteria, but largely redundant for IFN-α/β-dependent antiviral immunity.


Healthy individual
IFN-γ
Macrophages
Mycobacteria
IRF1
STAT1
P
STAT1
IRF1
IRF1
ISGs
GAS
ISRE
Inherited IRF1 deficiency
IFN-γ
Macrophages
Mycobacteria
STAT1
P
STAT1
IRF1
ISGs
GAS
ISRE

Aberrant levels of CXCL16 in severe COVID-19 patients: window into an aberrant immune response

Smieszek S[1]
[1]Vanda Pharmaceuticals Inc.

Lightning Talks / Cytokines in infections

Genome-wide association studies have recently identified 3p21.31, encompassing the CXCR6 gene, as the strongest thus far reported susceptibility locus for severe COVID-19 ((rs73064425, OR=2.14, discovery p=4.77 × 10-30 Pairo-Castineira et al., 2020). Nakanishi et al., reported that risk allele carriers experienced an increased risk of COVID-19 related mortality and complications: severe respiratory failure (odds ratio [OR] 2.0, 95%CI 1.6-2.6), venous thromboembolism (OR 1.7, 95%CI 1.2-2.4).
CXCL16 is synthesized as a transmembrane molecule that is expressed as a cell surface-bound molecule, and as a soluble chemokine. CXCL16 interacts with CXCR6 promoting chemotaxis or cell adhesion. The CXCR6/CXCL16 axis mediates homing of T cells to the lungs in disease and hyper-expression is associated with localised cellular injury.
To characterize the CXCR6/CXCL16 axis in the pathogenesis of severe COVID-19, plasma concentrations of CXCL16 from 47 hospitalized COVID-19 patients participating in ODYSSEY COVID-19 clinical trial (and 37 controls) were assessed. CXCL16 levels in plasma were determined with ELISA kit. We report a significant difference in plasma CXCL16 between COVID-19 hospitalized cases and controls (0.001 p-value). We furthermore report a larger variance within the cases, signifying further necessity of stratification based on clinical characteristics (ANCOVA p-value – 0.001, cov: age). We replicated the 3p21.31 association in our cohort of severe hospitalized patients) and stratified across carriers. The carriers had higher risk of mortality when compared to WT.

Previously it was demonstrated that circulating CD8+CXCR6+ T cells were significantly elevated with advanced age, yet virtually absent in patients with severe COVID-19. Pasma levels of CXCL16 were significantly upregulated in severe COVID-19. Differential expression of CXCR6 and CXCL16 mRNA was observed in severe COVID-19 compared to mild disease and significant functional polymorphisms in CXCR6 were linked to viral control. Our current study further supports a significant role of the CXCR6/CXCL16 axis in the immunopathogenesis of severe COVID-19.

Dysregulated Immune Responses via TLR7 Drive Maternal and Foetal Pathogenesis During Gestational Influenza Infection

Trollope G[1], Liong S[1], Coward Smith M[1], Miles M[1], Selemidis S[1]
[1]Centre for Respiratory Science and Health, School of Health and Biomedical Science, RMIT University

Background: Influenza A virus (IAV) is a significant cause of human disease, resulting in ~1 billion infections and>650,000 deaths annually. Pregnant individuals are particularly vulnerable to severe complications from IAV, leading to increased morbidity and mortality for both mother and foetus via a currently undefined mechanism. We have previously shown that gestational IAV infection triggers an inflammatory vascular storm in the maternal aorta. Here, we propose the pattern recognition receptor, Toll-like receptor 7 (TLR7) is the driver of a systemic hyper-immune phenotype, which disrupts the delicate immune balance required for healthy pregnancies ultimately driving cardiovascular complications in the maternal system and disrupting normal foetal development.
Results: Using an established mouse model of gestational IAV infection we have identified a new cardiovascular pathology of gestational IAV infection, characterised by bradycardia, systolic and diastolic hypotension. In this study, IAV-infected TLR7 knockout (-/-) mice displayed normal pulse rates compared to wild-type (WT) infected animals. Further, TLR7-/- prevented viral dissemination into the heart and aorta. This reduced inflammation in the maternal aorta and confined the infection to the respiratory tract, whilst simultaneously preserving the integrity of the lung tissue. Furthermore, TLR7-/- mice showed improved bodyweight growth in both the mother and the foetus, indicating that preservation of cardiovascular function protected foetal development. Finally, we show that TLR7-/- maintained a Th2 immune phenotype, which is crucial for maternal immune tolerance of the foetus, even in the presence of systemic infection.
Conclusion: In this study, we have shown TLR7 is central to gestational IAV infection pathogenesis via cardiovascular complications, which ultimately disrupts foetal development. The dysregulated immune response triggered by TLR7 promoted viral dissemination and systemic inflammation. These data offer insight into the pathogenic mechanisms of gestational IAV infections and indeed present TLR7 as a viable therapeutic target, which may reduce disease severity.

IL-1- and type III IFN-dependent regulation of GM-CSF promotes epithelial-immune cell crosstalk and orchestrates immunity to Aspergillus fumigatus in the lung

Mills K[2], Espinsoa V[3], Desai J[6], Aufiero M[1], Gjonbalaj M[1], Guo Y[1], Amariliz R[3], Lionakis M[4], Becher B[5], Hohl T[1]
[1]Memorial Sloan Kettering Cancer Center, [2]Weill Cornell Medicine, [3]Rutgers-New Jersey Medical School, [4]NIH, [5]University of Zurich, [6]Hackensack Meridian Center for Discovery and Innovation

Plenary 2: Lambda interferons at 20 years of age

Patients with defective granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling are susceptible to invasive aspergillosis, with Aspergillus fumigatus (Af) as the most common etiologic agent. The critical cellular source, regulation, and essential effector activities of GM-CSF (Csf2) have not been integrated into a comprehensive model of respiratory host defense against mold pathogens.

By analyzing mice with Csf2 deletion in distinct compartments as well as GM-CSF reporter mice, we show that lung epithelial cells (LECs) are the main GM-CSF producers after Af infection. Mice with conditional IL-1 receptor deletion in LECs have ~50% reduction in GM-CSF during infection. Using an Aspergillus bioreporter strain that quantifies fungal cell killing with single encounter resolution, we found that mice lacking Il1r1 on radioresistant cells exhibit defects in fungal killing by neutrophils, demonstrating that the IL-1-dependent anti-fungal program is mediated by structural cells of the lung. Stat1-/- mice also have reduced GM-CSF production, independent of IL-1. Interferons (which signal through Stat1) act on epithelial cells, and IFN-lambda receptor or IFN-lambda locus knockout mice have reduced GM-CSF production, consistent with parallel IL-1 and type III-dependent control of GM-CSF release during fungal pneumonia. Finally, uninfected Il1r1-/- and Stat1-/- mice have normal GM-CSF levels, indicating that these production pathways are induced by infection and, individually, are not essential for homeostatic GM-CSF functions, exemplified by alveolar macrophage maintenance. Finally, neutrophil GM-CSF responsiveness is essential for defense against Af.

We propose a model of cellular crosstalk in which IL-1 and type III interferon signaling through LECs licenses immune cells, particularly neutrophils, to kill Af via GM-CSF production. Building on work performed in other models of pathogenic infection, these findings deepen our understanding of organ-specific immunity and reveal remarkable functional complementarity between structural cells of the lung tissue and recruited innate immune cells in the anti-microbial response.

Type I and III interferons exert differential roles in immunity against the enteric bacteria Salmonella

Alphonse N[1,2], Wanford J[1], Alrehaili A[1], Dickenson R[1], Broggi A[3], Rosadini C[4], Wack A[2], Odendall C[1]
[1]King's College London, [2]Francis Crick Institute, [3]U1104 Centre d'immunologie de Marseille-Luminy (CIML) (INSERM), [4]Harvard Medical School and Division of Gastroenterology, Boston Children's Hospital

Plenary 2: Lambda interferons at 20 years of age

Type I and III interferons (IFNs) are immune cytokines secreted upon pathogen recognition by the host. Type I IFNs signal through the IFN-α-receptor (IFNAR) while type III IFNs signal through the IFN-λ-receptor (IFNLR). Unlike type I, type III IFNs are mostly restricted to epithelial cells (ECs), placing them as pivotal actors of mucosal immunity. Both IFNs induce interferon-stimulated-genes (ISGs) with well-known antiviral functions. However, their roles during bacterial infections remain largely understudied. In this study we analyse the roles of type I and III IFNs in colitis induced by the intestinal pathogen Salmonella.
We found that infected ceca lacking IFNAR exhibited similar pathology to WT ceca, suggesting that IFNAR may not modulate the histopathological changes associated with Salmonella infection. In contrast, mice lacking IFNLR exhibited different histopathological changes over time compared to WT mice. At 24-hour-post-infection (hpi), the absence of IFNLR resulted in reduced pathology but enhanced regeneration of ECs, suggesting that IFNLR may dampen the regenerative capacity of ECs during the early stages of infection. However, at 36hpi, both mice lacking IFNLR and WT exhibited similar pathology, and fewer crypts were observed in the ceca of IFNLR KO, suggesting that the initial protective effect of IFNLR deficiency was no longer present. By 48hpi, more pathology was observed in mice lacking IFNLR compared to WT. Crypt numbers were similar, but a decrease in crypt length was observed, suggesting that IFNLR may play a role in maintaining epithelium integrity during the later stages of infection. This dynamic process occurring during the course of infection was associated with a strong upregulation of ISGs at 36hpi, suggesting that ISGs may contribute to the observed changes. Overall, these findings demystify the role of type I and III IFNs at the intestinal mucosal barrier and emphasize their differential functions during bacterial colitis.

IFN ß-1a ring prophylaxis to reduce SARS-CoV-2 transmission

CONCORD-19 Consortium, Fish E[1]
[1]University of Toronto & University Health Network

Symposium 5: Cytokines and interferons in COVID-19 and beyond

While widespread vaccination has had success limiting the trajectory of the pandemic, emergence of Omicron variants demonstrates that despite mutations that appear to cause less severe disease, high transmission rates result in significant pressures on health services and society. There is no approved therapy to prevent SARS-CoV-2 transmission. A cluster randomized clinical trial was undertaken in Santiago, Chile, to determine whether IFNβ-1a administration limits SARS-CoV-2 household transmission from an infected individual (PCR +ve index case (IC)) to uninfected household contacts. Households were cluster randomized to receive 3 sc doses of 125µg pegylated IFNβ-1a or standard care. Analyses were undertaken to determine effects of treatment on viral shedding and viral transmission. 341 households (n=1172) were enrolled, 172 (n=607) assigned to receive IFNβ-1a and 169 (n=565) to standard care.
In households with ICs with viral load >10^6 treatment with IFNβ-1a significantly reduced transmission. Regardless of IC viral load, IFNβ-1a treatment significantly reduced median viral load at study Day 6 in household contacts and reduced incidence of hospitalization.
Bayesian analysis identified a 95% probability of reduction of infection within a household by IFNβ-1a treatment. During the active treatment period, there was a significant reduction in the odds of transmission with a probability that IFNβ-1a treatment is superior to standard care of 97.5%. The estimated Bayes factor indicated strong support that IFNβ-1a treatment reduces infection rate (~38 times more likely). The effect of IFNβ-1a on transmission was independent of household size. IFNβ-1a treatment was associated with a significant reduction (23%) in the odds of a SARS-CoV-2 positive saliva PCR for all household contacts (eligible contacts (18-80yrs) and ineligible contacts (<18yrs, >80 yrs, pregnant females, individuals with co-morbidities)) compared to standard care during the treatment period (study days 1-11). IFN regulation of IL-6, CRP, CD8+ T cells and platelets, associated with inhibition of SARs-CoV-2 infection.

Regulation of human IFN signaling by an exonized transposon in IFNAR2

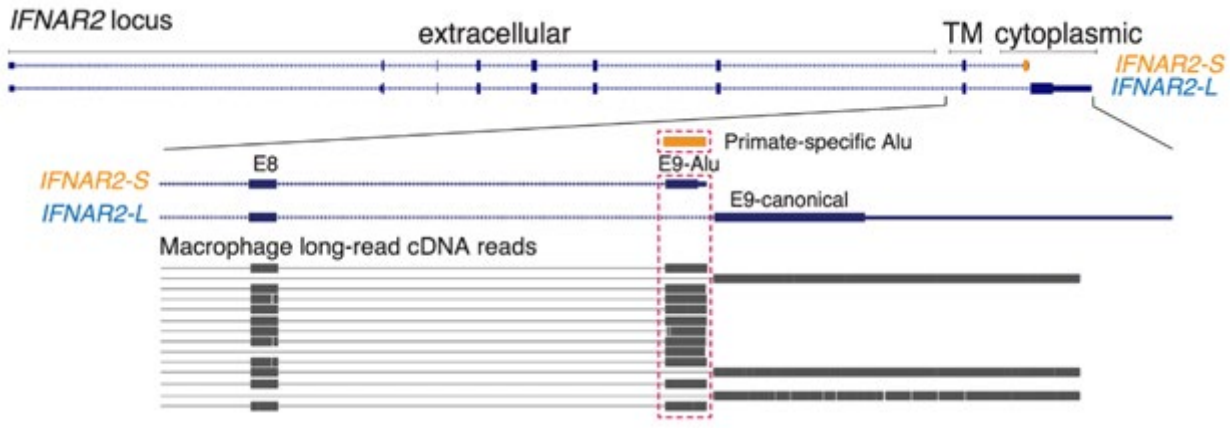
Chuong E[1], Pasquesi G[1]
[1]University Of Colorado Boulder

Symposium 5: Cytokines and interferons in COVID-19 and beyond

Type I interferon (IFN) signaling is crucial for eliminating pathogens and damaged cells. Although the core mechanisms of IFN signaling are evolutionarily conserved, the regulation and function of genes involved in IFN signaling have undergone rapid evolution, resulting in extensive species-specific differences in disease susceptibilities. However, the mechanisms underlying these changes remain poorly understood. Work by us and others has uncovered transposons as a powerful source of coding and non-coding elements that can be domesticated to drive immune evolution.

In this study, we analyzed long-read RNA-seq data from human cells to discover transcript isoforms of genes that are derived from the incorporation of transposons as alternative exons. This analysis revealed a poorly characterized isoform of IFN alpha/beta receptor 2 (IFNAR2), which uses a primate-specific transposon as an alternative terminal exon, generating a truncated isoform that lacks the intracellular signaling domain (IFNAR2-S). Surprisingly, our analysis revealed that this truncated isoform is expressed at 2-3 times higher levels than the canonical full-length IFNAR2 isoform in all human cell types. Therefore, we hypothesized that IFNAR2-S evolved to function as a decoy receptor that negatively regulates canonical IFNAR2 signaling. To test the potential physiological function of this isoform, we carried out isoform-specific functional studies using CRISPR genome editing and siRNA depletion in a panel of human cell lines. We found that deleting or depleting IFNAR2-S potently enhances cellular sensitivity to IFN, as evidenced by phosphorylation of STAT1 and activation of IFN-stimulated genes. Additionally, deletion of IFNAR2-S amplified both IFN-mediated cytotoxicity and antiviral effects to Dengue virus and SARS-CoV2.

These findings demonstrate that the IFNAR2-S isoform is a novel IFN decoy receptor with significant implications for understanding IFN regulation and dysregulation in human cells. More broadly, our work implicates transposon domestication as a prevalent mechanism driving isoform-level diversification of cytokine receptors throughout mammalian evolution.

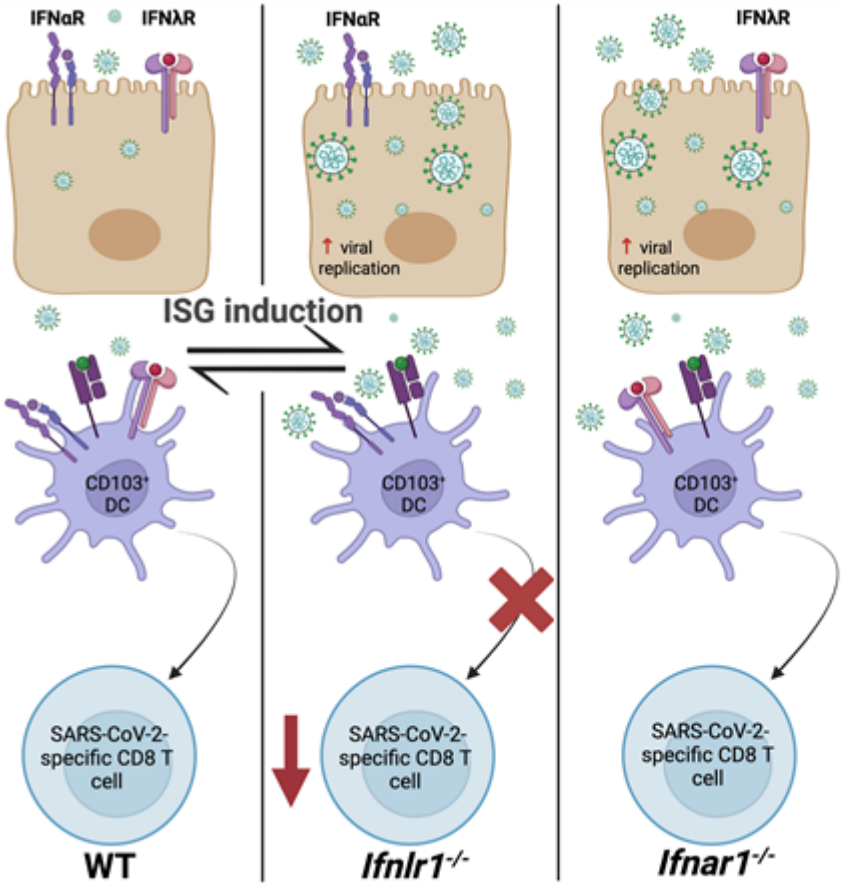


Interferon-lambda uniquely promotes CD8 T cell immunity against SARS-CoV-2 compared to type I interferon

Solstad A[1], Kenney A[1], Mahfooz N[1], Speaks S[1], Gong Q[1], Robinson R[1], Long M[1], Forero A[1], Yount J[1], Hemann E[1]
[1]Ohio State University

Symposium 5: Cytokines and interferons in COVID-19 and beyond, Olympia A

Optimization of protective immune responses against SARS-CoV-2 remains an urgent worldwide priority. In this regard, type III interferon (Interferon-lambda, IFNλ) restricts SARS-CoV-2 infection in vitro and treatment with IFNλ limits infection, inflammation, and pathogenesis in murine models. Further, IFNλ has been developed for clinical use to prevent illness during COVID-19. However, whether endogenous IFNλ signaling has an impact on SARS-CoV-2 antiviral immunity and long-term immune protection in vivo is unknown. In this study, we utilized a mouse adapted SARS-CoV-2 that allows for infection of WT C57BL/6 and mice lacking the IFNλ receptor (Ifnlr1-/-) to cause pulmonary disease without the need for overexpression of human ACE2. We identified a requirement for IFNλ signaling in promoting viral clearance and protective immune programming in SARS-CoV-2 infection. Interestingly, we found both IFN and IFN-stimulated gene (ISG) expression in the lungs following infection was independent of IFNλ signaling. Instead, IFNλ promoted generation of protective CD8 T cell responses against SARS-CoV-2 by facilitating accumulation of CD103+ DC in lung-draining lymph nodes. Conversely, CD8 T cell immunity to SARS-CoV-2 is independent of type I IFN signaling, revealing a unique dependence on IFNλ. Overall, these studies demonstrate that IFNλ is critical for adaptive immune responses upon infection with SARS-CoV-2, and suggest that IFNλ serves as an immune adjuvant to support CD8 T cell immunity.


IFNαR
IFNλR
IFNαR
IFNλR
↑ viral replication
↑ viral replication
ISG induction
CD103⁺ DC
CD103⁺ DC
CD103⁺ DC
SARS-CoV-2-specific CD8 T cell
SARS-CoV-2-specific CD8 T cell
SARS-CoV-2-specific CD8 T cell
WT
Ifnlr1-/-
Ifnar1-/-

Tumor-activated PD1-targeted IL-2 increased antigen specific T cells in tumor models and demonstrated anti-tumor activity in mice

Eryilmaz E[1], Guzman W[1], Johnson P[1], Hsiao S[1], Quinn L[1], Lakhani J[1], Whalen B[1], Quiroz R[1], Jenkins K[1], Yerov O[1], Scott W[1], Greene J[1], Liu Z[1], McLaughlin M[1], Vu S[1], Turcotte C[1], Rao H[1], Taylor J[1], O'Toole C[1], Pederzoli-Ribeil M[1], Duprey H[1], Malkova N[1], Fantini D[1], Crowe D[1], Smith K[1], Lee J[1], Smith K[1], Card J[1], Coleman S[1], Nicholson b[1], O'Neil J[1], Bialucha C[1]
[1]Xilio Therapeutics

Utilizing our proprietary Xilio Advanced Cellular Targeting (X-ACT) platform, we have developed an innovative approach to enhance PD-1/PD-L1 therapies. By combining a PD-1 blocker with a tumor-activated, engineered IL-2 agonist, we have developed PD1/IL2, a multifunctional molecule designed to improve upon existing PD-(L)1 therapies. PD1/IL2 is designed to block the activity of IL-2 by a protein domain that prevents IL-2Rβγ binding until it is activated within the tumor microenvironment (TME) by matrix-metalloproteinases. In preclinical studies, the selective activation of IL-2 enabled inhibition of immunosuppressive PD1/PD-L1 signaling while minimizing systemic IL-2 toxicity. PD1/IL2 is specifically designed to engage IL-2 receptors on antigen-experienced PD-1+ CD8+ T cells within the TME in cis. PD1/IL2 induced effector T cell functions and limited stimulation of immune suppressive Tregs relative to wild-type IL-2. In both anti-PD-1 sensitive and insensitive preclinical models, PD1/IL2 demonstrated significantly greater tumor growth inhibition compared to anti-PD1 therapy alone. Furthermore, PD1/IL2 exhibited better tolerability compared to a PD-1-directed non-masked IL-2 in the same preclinical models. Pharmacodynamic studies revealed that PD1/IL2 had minimal impact on immune cell profiles in the peripheral blood but significantly increased antigen specific CD8+ T cells within the TME. These data align with the preferential engagement of PD1+ effector T cells at the tumor site. Ex vivo protease cleavage assays performed in human tumor samples demonstrated activation of PD1/IL2 with minimal activation in plasma. In a pharmacokinetic study in nonhuman primates, PD1/IL2 achieved antibody-like half-life, suggesting PD1/IL2 has the potential to achieve exposure levels comparable to existing PD-1 blocking immunotherapies. In this study, PD1/IL2 was well tolerated at doses up to 30mg/kg. Collectively, these data support our hypothesis that PD1/IL2 has the potential to broaden the activity of existing immunotherapies by simultaneously inhibiting PD1/PDL1 and delivering an immunostimulatory cytokine preferentially to antigen-experienced T cells in the TME.

T cell factor 1 (TCF-1) is a critical regulator of intraepithelial lymphocytes in colorectal carcinoma

Raghu D[1], Yakou M[1], Tran K[1], Liao Y[1], Ghilas S[1], Afshar-Sterle S[1], Kumari A[1], Schmid K[1], Dijkstra C[1], Inguanti C[1], Ostrouska S[1], Wilcox J[1], Smith M[2], Parathan P[1], Allam A[1], Xue H[3], Belz G[4], Mariadason J[1], Behren A[1], Drummond G[5], Ruscher R[2], Pal B[1], Williams D[6], Ernst M[1], Shi W[1], Mielke L[1]
[1]Olivia Newton-John Cancer Research Institute and La Trobe University School of Cancer Medicine, [2]Centre for Molecular Therapeutics, Australian Institute of Tropical Health and Medicine, James Cook University, [3]Center for Discovery and Innovation, Hackensack University Medical Center, [4]University of Queensland Frazer Institute, Faculty of Medicine, The University of Queensland, [5]Centre for Cardiovascular Biology and Disease Research, Department of Microbiology, Anatomy, Physiology & Pharmacology, School of Agriculture, Biomedicine and Environment, La Trobe University, [6]Department of Anatomical Pathology, Austin Health

Intraepithelial lymphocytes (IELs), including γδ and αβ T cells (T-IELs) constantly survey and play a critical role in maintaining the gastrointestinal (GI) epithelium. We show that cytotoxic molecules important for defense against cancer, were highly expressed by T-IELs in the small intestine. In contrast, colon T-IELs were promoted by the microbiome, had higher expression of TCF-1/TCF7 and a reduced effector and cytotoxic profile, with low expression of granzymes. Deletion of TCF-1 led to the activation of a unique T-IEL effector profile and resulted in decreased tumor growth. In human colorectal cancers (CRCs), we observed decreased TCF-1 expression in γδ T-IELs compared to normal healthy colon, which strongly correlated with improved patient survival and increased γδ T-IEL effector phenotype, including production of Xcl1 and granzymes. These findings identify TCF-1 as a transcriptional regulator specific to colon T-IELs and provide valuable insights for the development of novel immunotherapeutic approaches for treating CRC.

Mechanism of cooperation of mutant IL-7Ra and mutant NRAS in T-cell acute lymphoblastic leukemia (T-ALL): Role of MYC stabilization

Durum S[1], Winer H[1], li w[1], Rodrigues G[1], gower t[1], Meyer T[1], hixon j[1]
[1]NCI NIH USA

Symposium 6: Targeted Therapies in Cancer & Beyond

Abstract
T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive leukemia derived from T-cell precursors. With current treatments, the survival rate is high, but the treatments are highly toxic with severe side effects. Individual mutations in IL7Rα and RAS pathways have been previously shown to be prevalent in T-ALL and especially in relapsed patients. The relationship of IL-7Rα and RAS was investigated by transducing immature mouse thymocytes with the combination of these mutants. The resultant T-ALL cells were analyzed to identify the regulators and the oncoproteins that are upregulated or downregulated by the combination of IL7Rα with NRAS. Leukemia cells showed a significant increase in IL7Rα-mediated BCL2 expression, and an increase in MYC protein levels, induced by the combination of NRAS and IL7Rα signaling. MYC was both necessary and sufficient to replace mutant NRAS and drugs targeting the MYC pathway showed a therapeutic benefit in IL-7Rα/NRAS T-ALL. We suggest that MYC protein stability is regulated by PLK-1 kinase, which was increased mainly by the NRAS signal and can lead to inhibition in MYC proteasomal degradation. These studies identify novel pathways of oncogenesis and new targets for intervention that could lead to better therapeutic development. One such therapeutic to be discussed is a novel anti-IL-7Rα that has shown efficacy against patient-derived T-ALL xenografts and will enter clinical trials this year.

Dysregulated STAT3 signaling leads to amplified expression of CD39 and CD8+ T cell dysfunction in STAT3 gain-of-function (STAT3 GOF)

Campos Duran J[1,2], Sayed S[2], Hay C[1,2], Conrey P[2], Knight M[2], Schmitt E[3], Thelin M[4], Mauracher A[2], Dalalo M[1,2], Romberg N[1,2], Holland S[5], Freeman A[5], Bergerson J[5], Su H[5], Leiding J[6,7], Forbes L[8,9], Anderson M[4], Cooper M[3], Vogel T[8,9], Henrickson S[1,2]
[1]University of Pennsylvania, [2]Children's Hospital of Philadelphia, [3]Washington University in St. Louis, [4]University of California, San Francisco, [5]NIAID, NIH, [6]Johns Hopkins University, [7]Arnold Palmer Hospital for Children, [8]Baylor College of Medicine, [9]Texas Children's Hospital

Background: Monogenic primary immune regulatory disorders alter the balance of adaptive and innate immune responses, including CD8+ T cell activation, by mistuning T cell receptor and/or cytokine signaling pathways. Here, we investigate the impact of STAT3 gain-of-function (STAT3 GOF) variants on CD8+ T cells.

Methods: We performed high dimensional immune profiling (via CyTOF and spectral flow cytometry), quantification of effector function, and single-cell transcriptional analysis of CD8+ T cells from untreated STAT3 GOF patients and age-matched healthy controls.

Results: STAT3 GOF patients have altered CD8+ T cell differentiation (e.g., decreased naïve and increased effector memory and TEMRA cells), increased expression of activation markers and effector molecules (e.g., PD-1, CD39, T-bet, Granzyme B), and decreased memory and stemness markers (e.g., CD127 and TCF-1). However, STAT3 GOF CD8+ T cells demonstrate decreased IL-2 production and proliferation upon ex vivo stimulation. CD39, which is involved in purinergic signaling and highly expressed on exhausted CD8+ T cells, was globally upregulated on patient CD8+ T cells and on CD8+CD44+ T cells in two STAT3 GOF mouse models (STAT3+/G421R and NOD-STAT3+/K392R). In vitro, STAT3-activating cytokines (e.g., IL-6, IL-21, IL-27, IL-10) augment CD39 expression in a TCR-dependent manner in healthy donor CD8+ T cells; inhibiting STAT3 signaling reduces CD39 levels. Finally, acutely activated and sorted CD39+CD8+ T cells from healthy donors hydrolyze ATP and suppress responder CD8+ T cell proliferation and cytokine production.

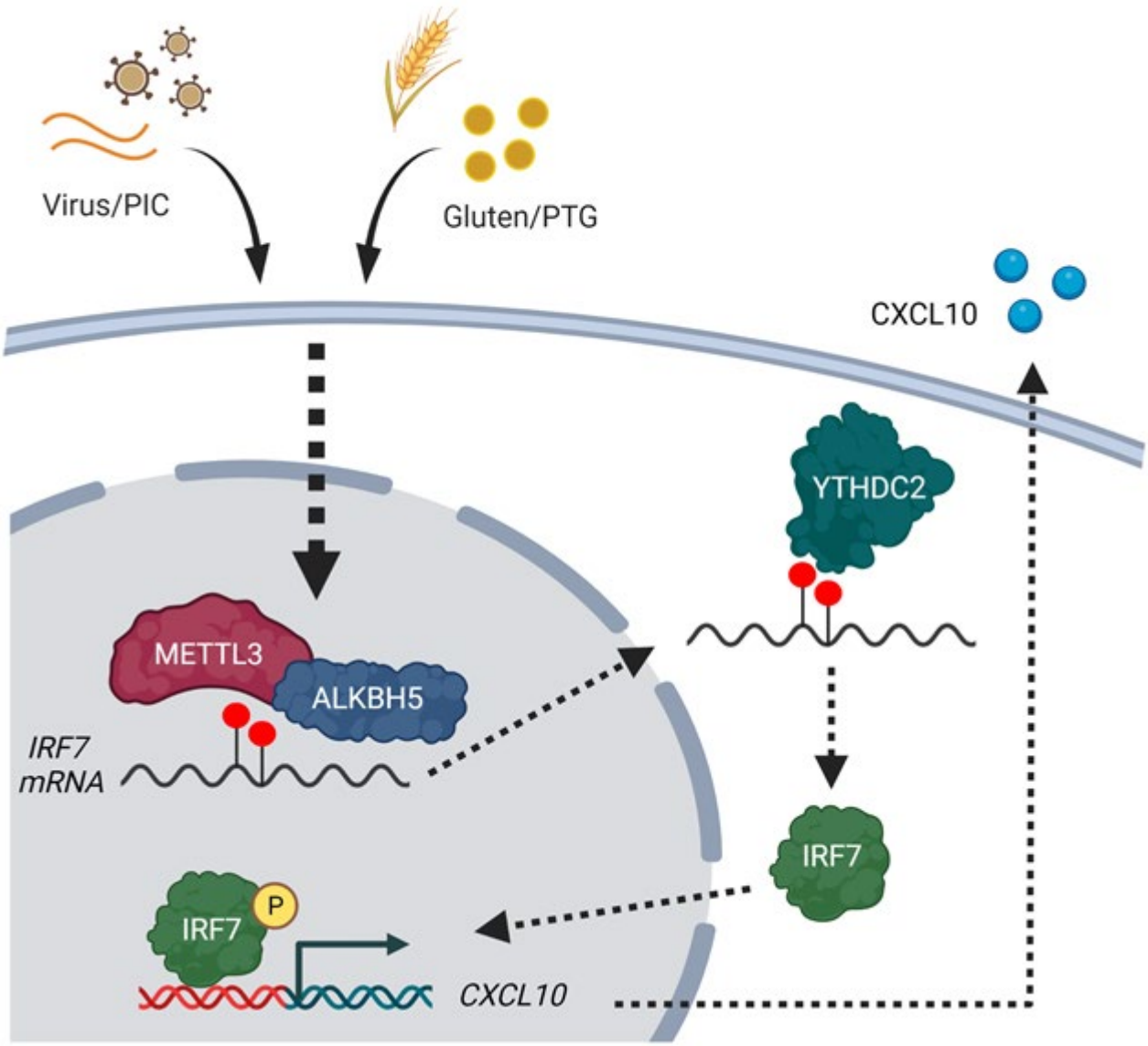
Conclusions: In these studies, we demonstrate that amplified STAT3 signaling, in STAT3 GOF primary CD8+ T cells, increases CD39 expression. In addition, we provide evidence that STAT3 may directly regulate CD39. We hypothesize that CD39 may represent a compensatory regulatory mechanism in these patients. We will next assess the suppressor capacity of STAT3 GOF patient CD39+CD8+ T cells, as well as directly test the consequences of inhibiting CD39 activity in STAT3 GOF in vivo.

IRF7 epitranscriptomics: linking viral infections and autoimmunity

Sebastian-Delacruz M[1], Olazagoitia Garmendia A[1,2], Rojas Márquez H[1,2], Mendoza L[2], Legarda M[3], Tutau C[3], Ghosh S[4], Postler T[4], Bilbao J[1,2,5], Castellanos Rubio A[1,2,5,6]
[1]University Of The Basque Country, [2]BioCruces Health Research Institute, [3]Pediatric Gastroenterology Section, Cruces Hospital, [4]Columbia University, College of Physicians & Surgeons, [5]CIBERDEM, [6]IKERBASQUE, Basque Foundation for Science

Symposium 7: Genetics of cytokines

IRF7 is a master regulator of the IFN-I pathway that plays an important role in the maintenance of the antiviral response and in the development of autoimmunity. Epitranscriptomics is the field studying post-transcriptional RNA modifications. N6-methyladenosine (m6A) is the most common on mRNA molecules and it has been implicated in autoimmune processes and innate immune response to infections.
Given the connection between viral infections and autoimmunity and the presence of m6A motifs in IRF7 RNA sequence, we hypothesized that IRF7 could connect these two immune processes by an m6A-dependent mechanism. We studied celiac disease (CeD) as an autoimmune disease model since viral infections have been implicated in its pathogenesis and its main triggering agent, gluten, is known. Moreover, IRF7 is upregulated in CeD and alterations in m6A regulation have been observed in the intestine of patients. Using intestinal cell cultures, we first confirmed the methylation within IRF7 mRNA. Moreover, we observed altered expression of m6A machinery genes and increased total m6A levels in response to a viral mimic. Interestingly, when the viral mimic was combined with gluten, IRF7 induction was stronger. Additionally, m6A methylation increase by METTL3 writer overexpression or ALKBH5 eraser silencing, resulted in IRF7 upregulation at RNA and protein level. Mutation of IRF7 m6A motifs confirmed its m6A-mediated regulation, as m6A mutant overexpression vectors presented reduced capacity of IRF7 protein synthesis. Furthermore, we found that YTHDC2 reader, which is involved in mRNA translation, recognizes IRF7 m6A motifs increasing protein levels. In conclusion, our results show that in the context of viral infection, gluten consumption may lead to autoimmunity by altering m6A machinery and inducing the innate immune response via the epitranscriptomic regulation of IRF7 mRNA. These results enlighten the link between autoimmunity and viral infections and open the door to novel therapeutic approaches focused on RNA modifications.


Virus/PIC
Gluten/PTG
CXCL10
YTHDC2
METTL3
ALKBH5
IRF7
mRNA
IRF7
P
IRF7
CXCL10

NLRP3 autoactivation in cryopyrin-associated periodic syndromes (CAPS) is HSP90β-selective

Spel L[1], Hou C[1], Theodoropoulou K[1,2], Zaffalon L[1], Bertoni A[3], Volpi S[3], Gattorno M[3], Martinon F[1]
[1]Dept. of Immunobiology, University of Lausanne, [2]CHUV, [3]Gaslini

Background
Gain-of-function mutations in inflammasome sensors, including NLRP3, are responsible for a spectrum of monogenic autoinflammatory diseases, such as cryopyrin-associated periodic syndromes (CAPS).
NLRP3 auto-activation is associated with inflammatory cytokine release, but the molecular mechanisms of this auto-activation remain unclear.

Methods
We set out to identify the regulators of NLRP3 inflammasome complex formation using a functional genetic screening approach. We generated a doxycycline-inducible expression system for GOF NLRP3, reflecting CAPS disease.

Results
In the CAPS cell model, NLRP3 was activated as evidenced by the detection of inflammasome 'specks', the release of cleaved IL-1β, cleaved GSDMD, and induction of cell death. We identified the HSP90β-SGT1 chaperone complex to be essential for NLRP3 inflammasome formation and activation in CAPS. HSP90β deficient cells, but not HSP90α-deficient cells, were unable to form inflammasome specks.
HSP90β and SGT1 depletions decrease but not abolished NLRP3 activity upon exposure to alum and nigericin, highlighting a disease-specific regulation. In patient-derived PBMCs, IL-1β secretion was totally repressed by an HSP90β pharmacologic inhibitor.

Conclusion
Together, our data indicate that HSP90β may represent a novel therapeutic approach for CAPS without altering infection-driven NLRP3 activity.

IL-2 synchronizes with T cell derived IL-10 to initiate lung-specific Th2 responses to inhaled allergen

He K[1], MacDonald W[1], Meta I[1], Xu Z[1], Ray A[1], Chen W[1], Das J[1], Lambrecht B[2], Poholek A[1]
[1]University of Pittsburgh, [2]Ghent University

Symposium 8: Cytokines in mucosal immunity and allergic diseases (SMI Session)

Allergic asthma is driven by Th2 cytokines and TFH-mediated IgE triggered by inhalation of environmental antigens. Although many drivers of Th2 cells have been described, how Th2 and TFH cells form to inhaled allergens remains unclear. Using temporal, spatial and single cell transcriptomic tracking of house dust mite (HDM) specific T cells in the lung draining lymph node, we demonstrate IL-2 signaling together with IL-10 is required for upregulation of Blimp-1 to repress Bcl6 and support GATA3 expression. Several feedback loops were identified leading to commitment to TFH or Th2 respectively. First, IL-2 signaling via STAT5 repressed Bcl6, leading to upregulation of IL-2Ra, Blimp-1, and Gata3, as loss of STAT5 led to upregulation of Bcl6 and a decrease in IL2Ra+ Gata3+ Blimp-1+ population. Next, IL-10 via STAT3 from antigen specific T cells enforces a second feedback loop to further upregulate Blimp-1, enforcing Bcl6 repression and Th2 commitment. Both putative Th2 and TFH cells are found at the T-B border within 3 days of activation, suggesting local microniches support both cytokine feedback loops that enforce T cell commitment. Our findings illuminate the early molecular details of how inhaled allergens promote Th2 and TFH differentiation and identify an unexpected early requirement for autocrine/paracrine IL-10 from responding T cells to support Th2 differentiation via Blimp-1 and subsequently drive allergic asthma.

GPR43 signaling in eosinophils restrains neutrophilic airway inflammation by preventing the emergence of pathogenic Siglec-Fhi neutrophils

Yu J[1], Kim S[1], Kim Y[1]
[1]KAIST

Symposium 8: Cytokines in mucosal immunity and allergic diseases (SMI Session)

In asthma, two distinctive endotypes - eosinophilic and neutrophilic - have been characterized based on their different etiology and pathophysiology. In eosinophilic asthma, eosinophils activated by type 2 cytokines are considered as the major cell type that mediates inflammation in the lung. However, recent studies suggest that eosinophils can also play immune-regulatory roles in tissues. We found that eosinophils in the asthmatic lung upregulate the expression of GPR43, a short-chain fatty acid receptor with anti-inflammatory functions. To examine the role of GPR43 signaling in eosinophils, we compared wild-type (WT) mice and eosinophil-specific GPR43 knock-out (GPR43△EOS) mice in the house dust mite extract-induced asthma model. The lung inflammation and airway hyperresponsiveness were severer in GPR43△EOS mice. Moreover, induction of asthma in GPR43△EOS mice led to an increase in neutrophil and Th17 cell counts in the lung, in addition to the increase in eosinophils and Th2 cells normally observed in WT mice. By performing scRNA-seq analysis of asthmatic lungs, we identified a distinct Siglec-Fhi neutrophil subset in GPR43△EOS mice, which has recently been described and found to be more pathogenic than the regular Siglec-Flow neutrophil subset. Based on in vitro co-culture experiments using eosinophils purified from asthmatic lungs and neutrophils from bone marrow, respectively, we found that GPR43-deficient eosinophils directly induce Siglec-Fhi neutrophils in an IL-4/GM-CSF- and contact-dependent manner. Indeed, GPR43-deficient eosinophils produced a higher level of neutrophil chemo-attractants and made more contact with neutrophils compared to WT eosinophils. We also revealed that Siglec-Fhi neutrophils have a greater ability to promote Th17 differentiation compared to Siglec-Flow neutrophils. Meanwhile, WT and GPR43-deficient eosinophils comparably induced Th17 differentiation. Together, these results demonstrate that GPR43 signaling in eosinophils restrains the emergence of Th17-promoting Siglec-Fhi neutrophils and prevents eosinophilic asthma from progressing to neutrophilic asthma, which is more difficult to manage due to steroid-resistance.

Circadian Control of Enteric Viral Infection Through Rhythmic IRF1-Mediated Innate Immune Responses

Maples R[1], Brooks II J[2], Acevedo Woods M[4], McCune B[1], Dende C[3], Pendse M[3], Lowe A[1], Behrendt C[3], Green C[5], Takahashi J[5], Hooper L[3], Pfeiffer J[1]
[1]Microbiology Department, UT Southwestern Medical Center, [2]Molecular Biology Department, Princeton University, [3]Immunology Department, UT Southwestern Medical Center, [4]Pediatrics Department, University of Pittsburgh School of Medicine, [5]Neuroscience Department, UT Southwestern Medical Center

Symposium 8: Cytokines in mucosal immunity and allergic diseases (SMI Session)

Circadian rhythms have been intertwined with the evolution of life and control immunological function. However, how circadian rhythms influence enteric infection remain unclear. To interrogate how circadian rhythms impact intestinal host response to a pathogen, we orally infected mice at different times of day with coxsackievirus B3 (CVB3), an enteric virus in the Picornaviridae family. We found that CVB3 had reduced viral titers in multiple tissues when mice were orally infected in the morning compared to the evening. To understand what factor(s) are rhythmic and may contribute to circadian effects on infection, we performed a transcriptomic analysis on intestinal tissues across time in uninfected mice. We focused our efforts on one rhythmic factor, IRF1, a transcription factor that drives antiviral gene expression. We found that protein levels of IRF1 and its downstream antiviral effector targets, IFIT1 and OAS2, were rhythmic, with peak effector protein levels in the morning. However, expression of IRF1, IFIT1, and OAS2 was lost in uninfected IRF1 knockout mice as well as mice lacking the circadian transcription factor BMAL1. Importantly, rhythmic CVB3 infection efficiency was lost in mice lacking IRF1, in mice expressing a dominant negative version of the CLOCK transcription factor, and in mice lacking expression of the circadian transcription factor BMAL1ΔLysM in myeloid cells. Our data suggest a model where circadian transcription factors drive rhythmic expression of IRF1 and its downstream antiviral effectors, to limit enteric virus infection in the morning in a myeloid-dependent manner. Overall, our work may uncover how circadian rhythms influence immunological control of enteric viral infections.

Repression of Id3 aggravates lupus phenotypes in murine models via aberrant B cell differentiation

Park Y[1], Jeon H[2], Kim D[3,4], Cho M[3], Kwok S[1,3]
[1]Division of Rheumatology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, [2]Division of Rheumatology, Department of Internal Medicine, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, [3]The Rheumatism Research Center, Catholic Research Institute of Medical Science, The Catholic University of Korea, [4]Department of Biomedicine & Health Sciences, College of Medicine, The Catholic University of Korea

Background: Id3 is a member of the inhibitor of DNA binding (Id) family which is a helix-loop-helix protein acting as a transcriptional regulator. Previous studies have reported that Id3 plays an important role in development and function of regulatory T cells in lupus. However, its role in B cells remains unclarified.

Methods: To investigate a role of Id3 in B cells, we generated C57BL/6 mice with a CD19Cre-mediated B cell-specific depletion of Id3 as well as mice (Id3-/-) with conventional knockout of Id3. In these mice, we evaluated presentation of lupus-mimicking phenotypes and changes of immune cell populations. Additionally, we assessed influences of B cell-specific Id3 depletion in lupus-induced mice by R848.

Results: In Id3-/- mice, proportions of effector T cells such as Th1, Th2, and Th17 cells were elevated whereas those of regulatory T cells were lower than in control mice. Furthermore, proportions of plasma cells were significantly elevated in Id3-/- mice. These mice presented with increased inflammation in kidney tissues, resembling lupus nephritis. Elevated proportion of plasma cells was replicated in mice with B cell-specific Id3 depletion. Induction using R848 resulted in exacerbated lupus-like manifestations including increased proteinuria and higher serum immunoglobulin levels in B cell-specific Id3-depleted mice than in control mice. In an in vitro study, CD19+ B cells from Id3-/- mice were more differentiated into plasma cells than those from control mice whereas there were no significant differences in other B cell subsets.

Conclusion: Genetic suppression of Id3 in murine models exacerbated lupus-like phenotypes with aberrant B cell differentiation. These findings may imply a potential role of Id3 in the pathogenesis of lupus.

PRC2 complex regulates immunogenicity by shaping cell-intrinsic type-II interferon (IFN) responses in treatment naïve Small Cell Lung Cancer (SCLC).

Bartley O[1], Hood S[1], Simpson K[1], Blackhall F[2], Kilgour E[1], Millrine D[1], Dive C[1]
[1]CRUK Manchester Institute Cancer Biomarker Centre, [2]Christie Foundation Trust

Lightning Talks / Novel therapeutics

Small cell lung cancer (SCLC) represents an aggressive neuroendocrine (NE) tumour characterised by reduced antigen presentation and immune infiltration. Patients typically present with extensive stage SCLC and are rarely eligible for surgical resection. Furthermore, limited stage biopsies are scarce and necrotic in nature making SCLC difficult to investigate. In order to circumvent these issues, our lab pioneered the development of circulating tumour cell-derived explant (CDX) models. Whilst SCLC is classified as a NE tumour, endogenous activation of the Notch signalling pathway promotes transition towards a non-NE (NNE) phenotype, fuelling disease heterogeneity. CDX models recapitulate this phenotypic switch, which coincides with epigenomic remodelling, enhanced STAT signalling and restored MHC-I expression. IFN-γ enhances antigen presentation by virtue of upregulating MHC-I expression through stimulation of the JAK-STAT pathway. We screened pharmacological inhibitors of chromatin remodelling enzymes in NE cultures and found PRC2 regulates immunogenicity by shaping cell-intrinsic IFN-γ responses. Allosteric inhibition of PRC2 induced global chromatin remodelling in both baseline and progression models. In the treatment naïve setting, catalytic inhibition of EZH2 followed by IFN-γ stimulation demonstrated up to 11-fold increases in MHC-I expression (P=0.0098). However, upon disease progression, this response is significantly impaired (P=0.0379). Our findings identify a context-dependant cell-intrinsic regulator of type-II IFN signalling and a potential therapeutic strategy to enhance tumour immunogenicity in the context of immune checkpoint blockade.

Loss of the ARID1A Tumor Suppressor Activates an R-loop Driven STING-Type I Interferon Signaling Axis that Promotes Anti-Tumor Immunity

Maxwell M[1,2], Hargreaves D[2]
[1]The University Of California, San Diego, [2]The Salk Institute for Biological Studies

Lightning Talks / Novel therapeutics

ARID1A is a core protein subunit of the mammalian Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex and among the most frequently mutated tumor suppressors human cancers. Recent checkpoint blockade immunotherapy clinical trials have identified ARID1A mutations as enriched among patients who respond favorably to immunotherapy in a diverse array of solid tumor types in a manner that is independent of microsatellite instability and confers an added benefit to high tumor mutational burden. Thus, the molecular mechanisms that dictate improved immunotherapy responsiveness in ARID1A mutant tumors remain incompletely understood. To investigate the mechanisms underlying improved immunotherapy response in ARID1A mutant tumors, we developed ARID1A deficient melanoma and colon cancer murine tumor models that recapitulate anti-tumor immunity phenotypes such as improved immunotherapy response and increased CD8+ T and NK cell infiltration observed in ARID1A mutant human cancers. Leveraging these tumor models and human tumor RNA-seq data, we discovered that both mouse and human ARID1A deficient cancers show prominent upregulation of an immunogenic Type I interferon (IFN) gene expression signature that includes interferon stimulated genes known to mediate cytotoxic immune cell recruitment and activation. Mechanistically, we have demonstrated that the ARID1A deficient Type I IFN gene expression signature is driven by STING dependent cytosolic DNA sensing and that both Type I IFN and STING are required for ARID1A-deficient anti-tumor immunity. Moreover, we have demonstrated that the source of cytosolic DNA in ARID1A-deficient cancer cells is derived from transcription-replication conflicts called R-loops which generate increased levels of cytosolic single stranded DNA (ssDNA) and RNA:DNA hybrids that trigger cytosolic DNA sensing and type I IFN induction. These findings represent a novel molecular mechanism underlying increased tumor immunogenicity in ARID1A-mutant cancers and provide a possibility for combining ARID1A-mutation status with R-loop-Type I IFN gene expression signature status to determine which patients may benefit most from immunotherapy.

Combinations of heterodimeric IL-15 with a fatty acid metabolism mobulator or a FLT3 inhibitor enhance anti-tumor effects in mouse breast cancer

Karaliota S[1,2], Stellas D[1], Stravokefalou V[1,3], Myers B[1], Felber B[1], Pavlakis G[1]
[1]National Cancer Institute at Frederick , [2]Basic Science Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, [3]University of Thessaly

Lightning Talks / Novel therapeutics

Introduction: hetIL-15, a cytokine directly affecting lymphocytes and inducing cytotoxic cells, also has a significant effect on the recruitment of myeloid cells, initiating a cascade for tumor elimination through innate and adoptive immune mechanisms. Due to its anti-cancer activities, the native heterodimeric form of IL-15 (hetIL-15), has advanced in clinical trials. We have identified novel hetIL-15 based drug combinations that enhance its anti-tumor effects in triple negative breast cancer (TNBC) models.
Study design and methods: We have evaluated the therapeutic efficacy of hetIL-15 immunotherapy in combination with a fatty acid (FA) metabolism modulator (FAMM) or Quizartinib (AC220), a Fms-like tyrosine kinase-3 (FLT3) inhibitor in the murine EO771 orthotopic TNBC model. We have monitored the effect of the treatment on tumor growth and survival and we have evaluated the immune cell profile in the tumors using flow cytometry and transcriptomics. We also assessed the metabolic profile of tumor-infiltrating CD8+T cells.
Results: Combination therapy of hetIL-15 and FAMM resulted in increased Oxygen Consumption Rate (OCR), mitochondrial function and FA uptake revealing an increased metabolic fitness compared to the tumor-infiltrating CD8+T cells of the hetIL-15 monotherapy. Combined treatment of hetIL-15 immunotherapy and FAMM resulted in significant EO771 tumor growth delay and eradication in 85% of mice. Combined administration of hetIL-15 and AC220 also resulted in significant EO771 tumor growth delay, compared to hetIL15; AC220 monotherapy did not show any anti-tumor activity. Combination treatments reshaped the cytokine/chemokine milieu and promoted myeloid recruitment into the tumor.
Conclusions: Our results show that hetIL-15 synergizes with metabolic reprogramming of T cells or FLT3 inhibition to achieve superior antitumor efficacy and complete cures. Therefore, novel combinations with hetIL-15 enhance its anti-tumor effects and may enhance its clinical applications in immunotherapy.

Single cell transciptional profiling of innate immune receptor agonstists reveal distinct cell type specific cytokine responses

Ireton R[1,2], Davis M[1], Knoll M[1], Smith E[1], Cornelius J[1], Whitmore L[1], Gale, Jr. M[1,2,3]
[1]Department of Immunology, University Of Washington, [2]Center for Innate Immunity and Immune Disease, University of Washington, [3]Department of Microbiology & Global Health, University of Washington

Introduction: RIG-I-like receptor (RLR) and STING pathways mediate response to pathogen associated molecular pattern (PAMP) nucleic acid including RNA and DNA respectively. RIG-I senses RNA PAMPs and undergoes signaling activation and MAVS interaction while cGAS senses cytosolic DNA and mediates STING interaction and signaling. These pathways signal downstream transcription factor activation, innate immune gene expression, and production of types I and III interferons and interferon stimulated gene (ISG) expression to mediate the innate immune response. We have developed small molecule agonists of RIG-I for antiviral therapeutic applications. Here we evaluated the response to RIG-I agonist compounds with STING agonist in treated human peripheral blood mononuclear cells (PBMCs).

Methods: We used the 10X Genomics Chromium fixed single cell RNA profiling platform to evaluate the transcriptional response generated in human PBMCs after ex vivo treatment with RIG-I agonists or STING (DiABZI). We used the Seurat pipeline along with clustermole and sc-type to determine cell identity in order to evaluate differential gene expression. We then used Ingenuity Pathway Analysis (IPA) of differential gene expression patterns to determine the functional impact of RIG-I or STING activation on differential gene expression. Data from the single cell profiling were computationally compared with existing published gene expression datasets.

Results: Our studies reveal that innate immune receptor agonists of RIG-I and STING generate unique gene expression profiles in human PBMCs. RIG-I agonist induced a unique IRF3 target gene set signature featured prominently in myeloid cells while DiABZI induces focused gene expression responses in T cells compared to monocytes, NK cells and B cells. Comparison analyses of transcriptomics datasets show an array of distinctive gene expression patterns that could inform therapeutic host-targeted antiviral therapies and vaccines through each pathway.

Conclusion: Our findings show that host innate immune agonists differentially activate distinct immune populations in the peripheral blood.

The BiST of Burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis

Ariel A[1], Schif-Zuck S[1], Saqib U[1], Ben Jashar N[1]
[1]The Department of Human Biology, University of Haifa

Lightning Talks / Novel therapeutics

Stimulator of IFN Genes (STING) is a cytosolic DNA sensor that plays a central role in host protection against pathogens upon binding of DNA-derived ligands. STING primarily acts by controlling the transcription of type I interferons (IFNs) and pro-inflammatory cytokines. Notably, STING can be inhibited or activated pharmacologically to control STING-associated pathologies. 5, 6-Dimethylxanthenone-4-acetic Acid (DMXAA) is a pharmacological activator of murine STING that induces IFN-β and its affected genes. Here, we report that macrophages from DMXAA-treated mice engulfed significantly higher numbers of apoptotic cells ex vivo, and exhibited enhanced reprogramming reflected by an increased IL-10 and reduced inflammatory cytokine secretion upon LPS exposure. Macrophage reprogramming was significantly hampered in STING and IFN-β-deficient macrophages. Furthermore, we found used virtual docking and batch screening to identify biased STING agonists (BiSTs) that enhanced IL-10 and IFN-β production by splenocytes while inhibiting TNF-α. One of these compounds, termed BiST 2.1, also induced the murine STING pathway in vivo and in human macrophages. Finally, we found BiST 2.1 to enhance the resolution of liver fibrosis induced by CCl4.
Conclusions: Thus, our findings indicate that STING can be harnessed to drive IFN-β-mediated IL-10 secretion by resolution phase macrophages and consequently shape macrophage function to enhance the resolution of inflammation and treat fibrotic disorders.

Type III interferons control tissue restitution in the intestine by inducing pyroptosis in gut epithelial cells.

Sposito B[4,2], Mambu J[1], Jena K[2], Millet V[1], Boehmer D[2], Spinelli L[1], Galland F[1], Naquet P[1], Mitsialis V[3], Bugda Gwilt K[3], Thiagarajah J[3], Snapper S[3], Zanoni I[2,3], Broggi A[1]
[1]Centre d'Immunologie Marseille Luminy (Aix Marseille Université-CNRS-INSERM), [2]Harvard Medical School, and Boston Children's Hospital, Division of Immunology, [3]Harvard Medical School, Boston Children's Hospital, Division of Gastroenterology, [4] Dep. of Biotechnology and Biosciences and Ph.D. program in Molecular and Translational Medicine (DIMET), University of Milano - Bicocca,

While there is an abundance of knowledge on the mechanisms that cause tissue damage during inflammatory diseases, the mechanisms by which inflammatory cues influence tissue repair are less understood. In our study, we investigated how interferons influence tissue restitution after damage to the intestinal mucosa.

Here we demonstrate that type III interferons can delay tissue repair by activating a novel signaling pathway. This pathway induces the upregulation of ZBP1, which leads to Caspase-8 activation and cleavage of Gasdermin C, ultimately resulting in epithelial cell death by pyroptosis. Importantly, we found that this pathway is activated in patients with inflammatory bowel disease.

These findings highlight the crucial role of interferons in regulating tissue repair and the outcome of the immune response. Moreover, they provide insight into a novel molecular mechanism that has important implications for human
inflammatory disorders.

Stat5 antagonizes Tox and CD8 T-cell exhaustion to foster a durable effector-like state during chronic antigen exposure

Beltra J[1,2,3], Abdel-Hakeem M[4], Manne S[1,2], Zhang Z[5], Huang H[5], Kurachi M[6], Su L[7], Picton L[7], Foong Ngiow S[1,3], Muroyama Y[1,3], Casella V[8], Huang Y[1,3], Giles J[1,2,3], Mathew D[1,2,3], Belman J[1,3], Klapholz M[1,3], Decaluwe H[9,10,11], Huang A[2,3,12,13], Berger S[5], Garcia K[2,7,14,15], Wherry J[1,2,3]
[1]University Of Pennsylvania - Department of Systems pharmacology and translational therapeutics, [2]Parker Institute for Cancer Immunotherapy, [3]Institute for Immunology and Immune Health, [4]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, [5]Department of Cell and Developmental Biology, Penn Institute of Epigenetics, Perelman School of Medicine, [6]Department of Molecular Genetics, Graduate School of Medical Sciences, Kanazawa University, [7]Departments of Molecular and Cellular Physiology and Structural Biology, Stanford University School of Medicine, [8]Infection Biology Laboratory, Department of Medicine and Life Sciences, Universitat Pompeu Fabra, [9]Cytokines and Adaptive Immunity Laboratory, Sainte-Justine University Hospital Research Center, [10]Department of Microbiology and Immunology, Faculty of Medicine, University of Montreal, [11]Immunology and Rheumatology Division, Department of Pediatrics, Faculty of Medicine, University of Montreal, [12]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, [13]Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, [14]Stanford Cancer Institute, Stanford University School of Medicine, [15]Howard Hughes Medical Institute, Stanford University School of Medicine

Lightning Talks / Novel therapeutics

CD8 T-cell exhaustion is a leading cause of cancer immunotherapy failure. Overcoming this process therapeutically remains an unmet challenge due to the epigenetic stability of the exhaustion lineage. However, flexibility in the exhaustion program was recently highlighted with evidence of an “effector-like” TEX subset (TEX intermediate; TEXint) suggesting opportunities for interventions. Examining TEXint biology revealed a reciprocally antagonistic circuit between the transcription factors Stat5a and Tox, the master regulator of exhaustion. Stat5-signals directed TEXint development and the effector-biology in this subset. Constitutive Stat5a activity rewired CD8 T-cells away from exhaustion to acquire a durable effector/NK-like state with superior anti-tumor potential. Temporal induction of Stat5-activity in TEX using an orthogonal IL-2/IL2Rβ-pair fostered TEXint accumulation, particularly upon PD-L1 blockade and manipulating the IL-2-Stat5 axis partially reprogrammed the epigenetic landscape of exhaustion and restored polyfunctionality. Together, these data highlight therapeutic opportunities of manipulating the IL-2-Stat5 axis to rewire TEX towards more durably protective states.

This work is supported by the National Institute of Health (NIH) and the Parker Institute for Cancer Immunotherapy. JC-Beltra is a Parker Institute for Cancer Immunotherapy Bridge fellow.

A therapeutic strategy to eliminate all sources of IgE and abolish allergy

Limnander A[1], Kaur N[1], Asrat S[1], Tasker C[1], Ben L[1], Godin S[1], Daniel B[1], Chin H[1], DeVeaux M[1], Stahl N[1], Sleeman M[1], Murphy A[1], Yancopoulos G[1], Orengo J[1]
[1]Regeneron Pharmaceuticals

Background: Immunoglobulin E (IgE) is a key driver of type 1 hypersensitivity reactions and allergic disorders; however, no therapeutic strategy reported to date can fully ablate IgE production. While IL4Rα signaling is required for class switching from IgM or IgG to IgE, IL4Rα blockade gradually reduces, but does not eliminate, IgE. Multiple observations suggest that IgE+ long-lived plasma cells (LLPCs), which secrete IgE independently of antigen encounter, play a role in maintaining serological IgE levels to allergens.

Methods: We evaluated the combination of transient depletion of plasma cells (PCs) using a PC-depleting bispecific antibody (BCMAxCD3) that specifically ablates PCs with concomitant, persistent IL-4Rα blockade to prevent IgE class switching in mouse models of chronic allergen exposure previously shown to generate IgE+ LLPCs, as well as in cynomolgus monkeys. We also perform in vitro cytotoxicity assays to evaluate BCMAxCD3-mediated killing of human IgE+ bone marrow PCs.

Results: Transient plasma cell ablation with a BCMAxCD3 bispecific antibody eliminates LLPCs that support serological memory to allergen, while continuous, concomitant IL-4Rα blockade prevents re-emergence of IgE by blocking IgE class switching of memory B cells. This combination treatment prevents anaphylaxis in mice and results in profound and durable depletion of circulating IgE even during allergen re-exposure. These findings were confirmed in cynomolgus monkeys. We further demonstrate that the BCMAxCD3 bispecific antibody induces in vitro T cell-mediated killing of human primary PCs, including IgE+ BMPCs from allergic donors.

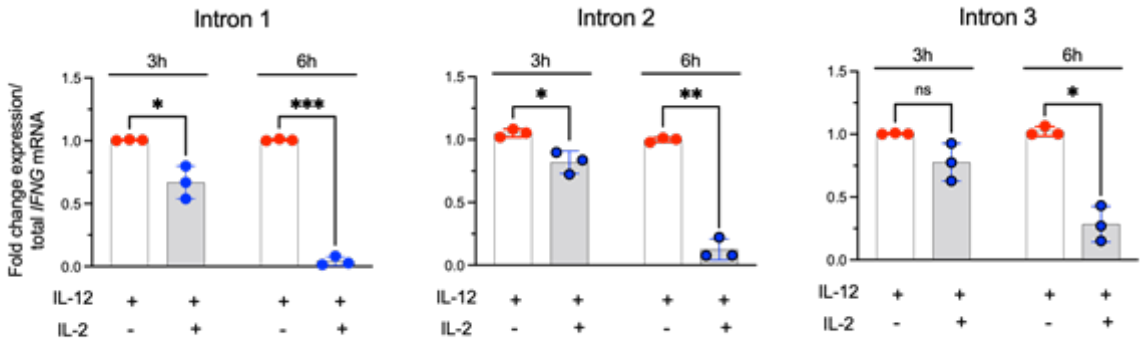
Conclusion: Allergic memory is largely maintained by IgG+ memory B cells and IgE+ LLPCs, and both sources of IgE can be targeted with the combination approach of transient BCMAxCD3 bispecific antibody with concomitant, persistent IL-4Rα blockade. We demonstrate the cross-species translatability of this approach and validate a therapeutic strategy for complete elimination of pathogenic IgE.

Intron retention allows for rapid NF-kB-mediated IFNγ expression in stimulated natural killer cells

Van Gelder R[1], Gokhale N[1], Omelia D[1], YOUNG H[2], Savan R[1]
[1]University Of Washington, [2]National Cancer Institute

Lightning Talks / Cytokines in immunobiology

Interferon-gamma (IFNγ) is a potent inflammatory cytokine produced by natural killer (NK) cells during the early response to infection. It is crucial that IFNγ expression is carefully modulated to ensure rapid sterilizing immunity while preventing excess inflammation and resulting pathology. Post-transcriptional regulation (PTR) of mRNA resolves expression of inflammatory cytokines, and several PTR mechanisms dampen IFNγ expression via IFNG mRNA degradation. However, whether PTR is involved in the rapid induction of IFNγ protein remains unknown. The combination of IL-12, produced by dendritic cells upon sensing pathogens, and IL-2, expressed by activated CD4+ T cells, causes rapid synergistic upregulation of IFNγ protein. Interestingly, we observe that NK cells treated with IL-12 alone transcribe IFNG mRNA with its introns intact. However, when NK cells are treated with both IL-2 and IL-12, we find that IFNG transcript is spliced to form mature mRNA (Fig 1), correlated with the observed synergistic increase in IFNγ protein expression. We hypothesize that IFNG transcript is post transcriptionally regulated by delayed intron splicing, allowing immature mRNA to persist until IL-2 signaling induces splicing and translation. We have observed that IL-2-dependent intron splicing occurs independently of nascent transcription and translation, as cells treated with Actinomycin D or cyclohexamide after IL-12 stimulus readily splice IFNG upon IL-2 treatment. Additionally, we have found that IL-2 mediated splicing is NF-kB dependent; treatment with the IkB phosphorylation inhibitor BAY-11 blocks IL-2-mediated IFNG splicing, transcriptional upregulation, and protein expression. Interestingly, IL-2 treatment does not significantly affect the nuclear export or translation of IFNG mRNA, but rather increases IFNG mRNA stability. We propose that while IL-12 transcriptionally induces IFNG mRNA expression, IL-2 signaling stabilizes IFNG mRNA in an NF-kB dependent manner by swift splicing of detained introns, ensuring that NK cells are poised for rapid and robust IFNγ protein expression.



N=3 multiple pairwise T tests *p<.05 **p<0.01 ***p<0.001.

TGFβ differentially specifies Tfh versus Th17 cell fates of murine CD4+ T cells

Chang Y[1,2], Bach L[1], Hasiuk M[3], Tsui C[4], Wen L[4], Gutiérrez-Melo N[1], Utzschneider D[4], Raj T[2], Heink S[5], Elmzzahi T[4,6], Cheng J[7], Zeiträg J[2], Jost P[8], Alterauge D[2], Dahlström F[2], Becker J[1], van Uelft M[9], Becker M[6], Reschke S[10], Krebs S[10], Blum H[10], Ohnmacht C[11], Neumann C[12], Hasenauer J[8], Meissner F[7,8], Beyer M[6], Korn T[5,14], Heissmeyer V[2,13], Kallies A[4], Jeker L[3], Baumjohann D[1,2]
[1]University Hospital Bonn, [2]LMU Munich, [3]Basel University Hospital, [4]The Peter Doherty Institute for Infection and Immunity, [5]Technical University of Munich, [6]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), [7]Max Planck Institute of Biochemistry, [8]University of Bonn, [9]Life & Medical Sciences (LIMES) Institute, [10]Laboratory for Functional Genome Analysis (LAFUGA), [11]Center of Allergy and Environment (ZAUM), [12]Charité – Universitätsmedizin Berlin, [13]Helmholtz Zentrum München, [14]Munich Cluster for Systems Neurology (SyNergy)

Lightning Talks / Cytokines in immunobiology

Background
T follicular helper (Tfh) cells are essential for effective antibody responses. However, deciphering the cell-intrinsic wiring of Tfh cells has long been hampered by the lack of reliable in vitro protocols for murine CD4+ T cells.

Methods
We used in vitro culture of naive CD4+ T cells from various conditional gene knock-out mouse strains and combined it with bulk and single-cell RNA-seq, CRISPR/Cas9-mediated gene ablation in naive CD4+ T cells, and in vivo experiments.

Results
We found that TGFβ was able to induce robust protein expression of the Tfh hallmark molecules CXCR5 and Bcl6 in vitro. TGFβ-induced CXCR5+ Tfh cells were functional and provided critical help to B cells in a contact-dependent manner. Dissection of the TGFβ-induced molecular pathways revealed that CXCR5 expression was independent of Bcl6. Notably, classical TGFβ-induced Th17 cultures also yielded separate CXCR5+ and IL-17A-producing cells, thus highlighting shared and distinct cell fate trajectories of Tfh and Th17 cells, respectively.

Conclusion
Our data establish the long-awaited requirements and molecular pathways for the generation of murine CXCR5+Bcl6+ Tfh cells in vitro, underscoring the pleiotropic functions of TGFβ and opening up new avenues for future research.

Whole-body analysis of tissue-resident immune cells

Clatch A[1], Lee H[1], Gordon C[1,2], Christo S[1], Haque A[1], Mackay L[1]
[1]University Of Melbourne - Peter Doherty Institute, [2]Austin Hospital

Lightning Talks / Cytokines in immunobiology

Tissue-resident memory T (TRM) cells are a non-circulating lymphocyte population that are principally located in peripheral tissues. TRM cells provide rapid protection against a wide range of infections and cancer; hence, enhancing TRM cell formation and persistence is an attractive means for establishing durable immunity. While many studies have dissected the properties of TRM cells within peripheral tissues in mice, our knowledge of human T cells has been largely derived from blood sampling. In collaboration with Austin Health, we have established the first Australian Donation and Transplantation Biobank that provides access to a wide range of healthy human organs. Using this resource, we performed a whole-body analysis of TRM cells across barrier and non-barrier tissue sites. We employed multiparameter flow cytometry and scRNAseq to resolve distinct TRM cell populations across the gut, skin, liver and spleen. We observed intra- and inter-organ TRM cell heterogeneity based on the expression of tissue residency markers CD69 and CD103, and inhibitory molecules such as PD-1 and CD244. Furthermore, we have demonstrated how the tissue microenvironment influences various TRM cell functional capabilities. Together, this holistic characterisation of TRM cells across solid organs underscores the importance of investigating local tissue immunity which cannot be discovered by conventional blood sampling. The results of this study will direct novel tissue-specific immunotherapies aimed to promote and establish durable tissue immunity.

Differential role of CD4+ T cells in programming tissue-resident versus circulating memory CD8+ T cells

Osman M[1], Park S[2,3], Park S[3], Mackay L[1]
[1]The Peter Doherty Institute, The University of Melbourne, [2]Institute for Immunology, Perelman School of Medicine, University of Pennsylvania , [3]Department of Pharmacology, Perelman School of Medicine, University of Pennsylvania

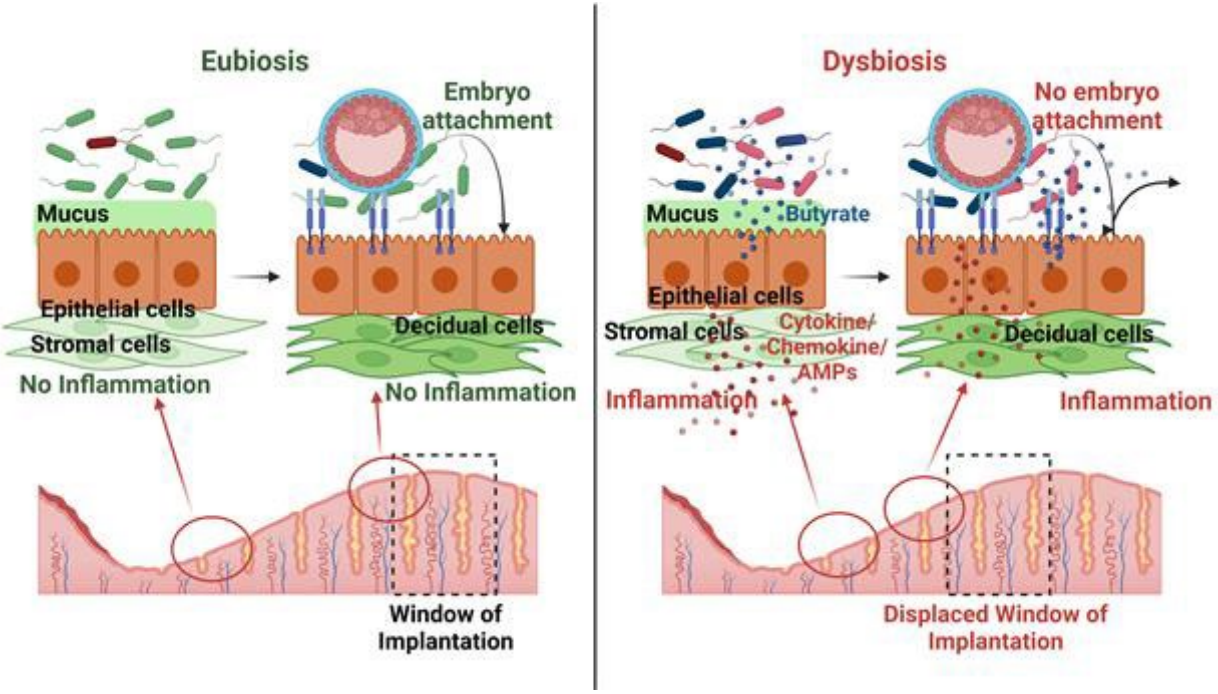
Tissue-resident memory T cells (TRM) are a non-recirculating population of cells lodged within barrier tissues such as the skin. Due to their location at barrier sites and key role in frontline immunity against invading pathogens and tumours, CD8+ TRM provide superior immune protection compared to their circulating memory T cell (TCIRC) counterparts, making their generation an attractive goal of vaccination and cell therapies. However, our understanding of the environmental factors and cellular interactions that drive CD8+ TRM development and functionality remain incomplete, limiting efforts to harness their therapeutic potential. CD4+ T cells play an important role in coordinating CD8+ T cell responses by promoting CD8+ T cell expansion and the generation of functional TCIRC populations. While the role of CD4+ T cell help in CD8+ TCIRC formation is well documented, their contribution to TRM formation across tissues is less clear. Using CD4+ T cell help-dependent models of viral skin infection, we found a diametric contribution of CD4+ T cell help to TRM versus TCIRC responses, with skin CD8+ TRM formation greatly enhanced in the absence of CD4+ T cells. Critically, unlike TCIRC, un-helped skin TRM were able to generate robust secondary responses, displaying proficient cytokine production and local proliferation following rechallenge. Moreover, un-helped CD8+ TRM exhibited a phenotype distinct to that of helped CD8+ TRM, showing differential expression of phenotypic markers that may influence their protective potential. Our findings imply CD4+ T cells restrain the formation of skin CD8+ TRM by conditioning the local microenvironment and altering the availability of TRM-inducing factors. Together, these results highlight a fundamental discordance between TRM and TCIRC responses and highlight a novel role for CD4+ T cells in controlling local immunity, with implications for rational design of therapeutics aimed at boosting local T cell memory.

The uterine microbiome and the microbial metabolite butyrate stimulate pro-inflammatory responses in endometrial epithelial cells, suggesting a possible impact on female fertility

Giangrazi F[1], Sugrue J[1], Crosby D[2], Horan M[2], Wingfield M[2], Glover L[2], O'Farrelly C[1]
[1]Trinity College Dublin, [2]Merrion Fertility Clinic

Symposium 9: Metabolic control of cytokine-mediated inflammation

Background: The endometrial microbiome has not been fully characterised, however its communication with the specialised epithelium may shape female fertility outcomes.
Methods: We assessed the endometrial microbiome in biopsies collected from a cohort of women with unexplained infertility undergoing assisted reproductive technologies (ART) with either successful or unsuccessful outcomes. Using in vitro models of endometrial epithelium, we analysed whether microbial-derived metabolites can promote innate immune responses in the endometrial epithelial cells and how these effects can impact female fertility.
Results: We identified a dysbiotic microbiome in endometrial biopsies from women with unexplained infertility and unsuccessful outcome following ART. 16S analysis showed higher diversity of short chain fatty acid (SCFA)-producing bacteria in this group of women. Butyrate, a well-known SCFA, was shown to induce pro-inflammatory activation in both tumoral endometrial epithelial cells and primary human endometrial epithelial cell models. The effects induced by butyrate included increased expression of cytokines, chemokines and antimicrobial peptides. Butyrate was also shown to impair barrier integrity and induce the expression of leaky-epithelial barrier Claudin 2. We also assessed the effect of butyrate on endometrial receptivity and stromal decidualisation by treating cells with progesterone with or without butyrate. Butyrate was shown to drastically enhance stromal cells decidualisation and endometrial epithelial receptivity markers, leading to possible changes in the window of implantation in the presence of this SCFA.
Conclusion: We identified a different endometrial microbiome in the biopsies collected from women undergoing ART with unsuccessful outcomes. This showed enriched composition of butyrate-producing bacteria and we also showed that butyrate induces inflammation as well as changing stromal cell decidualisation and endometrial receptivity markers. Further investigation of the microbiome in healthy endometrium and on physiological levels of butyrate would clarify the role of this metabolite, since we have shown that excessive endometrial levels of butyrate can impact female fertility.


Eubiosis
Embryo attachment
Mucus
Epithelial cells
Stromal cells
Decidual cells
No Inflammation
No Inflammation
Window of Implantation
Dysbiosis
No embryo attachment
Mucus
Butyrate
Epithelial cells
Stromal cells
Cytokine/ Chemokine/ AMPs
Decidual cells
Inflammation
Inflammation
Displaced Window of Implantation

An innate immune signalling axis that licenses bacterial killing and suppresses inflammatory cytokine production via regulated immunometabolism

Sweet M[1], Das Gupta K[1], Ramnath D[1], Wang Y[1], Abrol R[1], Gunther K[1], von Pein J[1], Curson J[1], Raven K[1]
[1]IMB, The University Of Queensland

Symposium 9: Metabolic control of cytokine-mediated inflammation

The innate immune system senses danger signals in different contexts, thus enabling tailoring of immune responses matched to the threat encountered. He we show that histone deacetylase 7 (HDAC7) is a cytoplasmic lysine deacetylase in macrophages that triages danger signals depending on the level of threat. HDAC7 deacetylates the glycolytic enzyme PKM2 and drives glycolysis, HIF-1alpha activation, and proinflammatory IL-1beta production in macrophages that sense soluble danger signals that are indicative of far-away or distal danger. In contrast, when macrophages sense proximal or nearby danger signals, such as Gram-negative bacteria, HDAC7 triggers the pentose phosphate pathway (PPP), NADPH production, and phagocyte oxidase-mediated reactive oxygen species generation for bacterial killing. HDAC7-mediated activation of the PPP also suppresses proinflammatory IL-1beta production, thus enabling cells to focus on bacterial killing. Consequently, genetic or pharmacological targeting of HDAC7 severely compromises antibacterial host defense and exacerbates inflammatory responses in vivo. The mechanism for PPP engagement involves HDAC7-mediated activation of the PPP enzyme 6-phosphogluconate dehydrogenase (6PGD) via a mechanism that is distinct to that of PKM2 activation. Remarkably, ribulose-5-phosphate (RL5P), an enzymatic product of 6PGD, synergises with reactive oxygen species for bacterial killing and suppresses selective inflammatory responses in both human and mouse macrophages, implicating this metabolite as a key mediator of HDAC7-mediated antibacterial defense and inflammation suppression. The HDAC7-PPP-6PGD-RL5P axis thus selects for direct antibacterial defense over proinflammatory cytokine production in macrophages and may be amenable to targeting for host-directed therapies against antibiotic-resistant bacterial infections.

A metabolic switch that boosts the fitness of anti-tumor immunity

Ergin I[1,3], Maher T[1,3], Bunk P[1,3], Chung C[1], Shah V, Moresco P[1], Shao S[1], Habel J[1], Rubino R[1], Wilkinson E[1], Weinberg S[2], Beyaz S[1]
[1]Cold Spring Harbor Laboratory, [2]Northwestern University, [3]These authors have contributed equally

Symposium 9: Metabolic control of cytokine-mediated inflammation

Tumor-infiltrating immune cells and cancer cells rely on similar metabolic pathways, leading to a "metabolic tug-of-war" in the tumor microenvironment (TME) that dampens immune cell function. We previously showed that high-fat diet (HFD) promotes tumorigenicity through lipid-sensing transcription factor PPARδ activation in cancer cells. However, little is known how PPARδ activation influences anti-tumor immunity. Here, we found that selective PPARδ activation in immune cells using genetic or pharmacological approaches boosts the fitness of anti-tumor immunity in mice irrespective of tumor antigenicity, immune infiltration status, tumor stage or tumor type. We corroborated these findings in humans using autologous colorectal cancer patient-derived organoids – immune cell coculture models. Notably, PPARδ activation in immune cells reprograms TME to boost anti-tumor immunity without any adverse effects to the host. PPARδ activity correlates with the fitness of tumor infiltrating immune cells in mice and humans. CD8 T cells are necessary and sufficient in mediating PPARδ-mediated boost on anti-tumor immunity. Mechanistically, PPARδ activation reprograms mitochondrial metabolism through Cpt1a-mediated fatty acid oxidation in CD8 T cells. Ablation of Cpt1a in immune cells promotes tumor growth and impairs the fitness of anti-tumor immunity. Finally, human CAR-T cells engineered to overexpress active PPARδ are superior in controlling tumor growth. Thus, PPARδ – Cpt1a axis constitutes a metabolic switch that boosts the fitness of anti-tumor immunity.

CXCL4 synergizes with TLR8 for TBK1-IRF5 activation, epigenomic remodeling and inflammatory response in human monocytes

Yang C[1], Bachu M[1], Du Y[1], Brauner C[1], Yuan R[1], Ah Kioon M[1], Chesi G[1], Barrat F[1], Ivashkiv L[1]
[1]Hospital for Special Surgery

Symposium 10: Latest developments and novel therapeutics

Regulation of endosomal Toll-like receptor (TLR) responses by the chemokine CXCL4 is implicated in inflammatory and fibrotic diseases, with CXCL4 proposed to potentiate TLR responses by binding to nucleic acid TLR ligands and facilitating their endosomal delivery. Here we report that in human monocytes/macrophages, CXCL4 initiates signaling cascades and downstream epigenomic reprogramming that change the profile of the TLR8 response by selectively amplifying inflammatory gene transcription and interleukin (IL)−1β production, while partially attenuating the interferon response. Mechanistically, costimulation by CXCL4 and TLR8 synergistically activates TBK1 and IKKε, repurposes these kinases towards an inflammatory response via coupling with IRF5, and activates the NLRP3 inflammasome. CXCL4 signaling, in a cooperative and synergistic manner with TLR8, induces chromatin remodeling and activates de novo enhancers associated with inflammatory genes. Our findings thus identify new regulatory mechanisms of TLR responses relevant for cytokine storm, and suggest targeting the TBK1-IKKε-IRF5 axis may be beneficial in inflammatory diseases.

Dietary tryptophan metabolite released by intratumoral Lactobacillus reuteri facilitates immune checkpoint inhibitor treatment

Meisel M[1], Bender M[1], McPherson A[1], Phelps C[1], Pandey S[1], Shapira J[1]
[1]University of Pittsburgh

Symposium 10: Latest developments and novel therapeutics

The use of probiotics by cancer patients is increasing, including among those undergoing immune checkpoint inhibitor treatment (ICI). Here, we elucidate a critical microbial-host crosstalk between probiotic-released aryl hydrocarbon receptor (AhR) agonist, indole-3-aldehyde (I3A), and CD8 T cells within the tumor microenvironment that potently enhances antitumor immunity and facilitates ICI in preclinical melanoma. Our study reveals that probiotic Lactobacillus reuteri (Lr) translocates to, colonizes, and persists within melanoma, where via its released dietary tryptophan catabolite I3A, it locally promotes interferon-□-producing CD8 T cells, thereby bolstering ICI. Moreover, Lr-secreted I3A was both necessary and sufficient to drive antitumor immunity, and loss of AhR signaling within CD8 T cells abrogated Lr's antitumor effects. Further, a tryptophan-enriched diet potentiated both Lr- and ICI-induced antitumor immunity, dependent on CD8 T cell AhR signaling. Finally, we provide evidence for a potential role of I3A in promoting ICI efficacy and survival in advanced melanoma patients.

Interferon lambda confers protection against cardiometabolic diseases

Salagianni M[1], El Hamidie A[1], Thanopoulou K[1], Siouti E[1], Koronaiou E[1], Manioudaki M[1], GALANI I[1], Kalafati N[2], Dedoussis G[2], Kaltsas G[3], Chavakis T[4], Tzoulaki I[5], Andreakos E[1]
[1]Biomedical Research Foundation Academy of Athens, [2]Department of Nutrition and Dietetics, School of Health Sciences and Education, Harokopio University of Athens, [3]1st Propaedeutic Department of Internal Medicine, National and Kapodistrian University of Athens, [4]Institute for Clinical Chemistry and Laboratory Medicine, Faculty of Medicine, Technische Universität Dresden, [5]Center for Systems Biology, Biomedical Research Foundation of the Academy of Athens

Symposium 10: Latest developments and novel therapeutics

The interferon-λ(IFNλ) family of cytokines consisting of interleukin-28A (IFNλ2), IL-28B (IFNλ3), and IL-29 (IFNλ1)share low homology with the interferon (IFN) and IL-10 cytokine families, yet they exhibit common and uniqueactivities. IFNλs have been extensively studied for their antiviral activities, however, it is now appreciated that they also mediate many other diverse immunomodulatory effects with their full spectrum of activities still remaining poorly understood. In the present study, we sought to investigate the role of IFNλs in low-grade chronic inflammation which underlies cardiometabolic diseases such as obesity, type 2 diabetes mellitus, and cardiovascular disease. For the purposes of this study, wegenerated an IFN receptor 1-deficient (IFNLR1-/-) mouseand areporter mouse expressing dt Tomato under the promoter of IFNλR1. We found that impairment of IFNλ function led to the development of metabolic disease in wild type mice fed a normal chow diet (NCD) which was markedly aggravated when these mice were fed a high fat diet (HFD). Impairment of IFNλ function also exacerbated atherosclerosis in APOE-/-mice. Conversely, IFN administration abrogated diet-induced metabolic disease in HFD-fed wild type mice and atherosclerosis in APOE-/-mice. Moreover, in humans SNP polymorphisms in IFNLR1 and IFNL genes were associated with cardiometabolic disease in humans. Our data thus reveal a previously unidentified function of IFNλs in regulatingmetabolic homeostasis and related diseases.

Engineered tumor-matrix targeted IL-12 to achieve safe and efficacious therapies

Ishihara J[1]
[1]Imperial College London

Plenary 3: Cytokines In cancer immunity and immunotherapy

Background
Although checkpoint-inhibitor (CPI) immunotherapy has achieved remarkable clinical success, its efficacy in 'immunologically cold' tumours has been modest. New therapies are needed to treat >80% of CPI-unresponsive cancer patients. Interleukin-12 (IL-12) is a powerful cytokine that activates the innate and adaptive arms of the immune system; however, the administration of IL-12 has been associated with immune-related adverse events.
Method We have recombinantly fused collagen-binding domain (CBD) derived from con Willebrand factor A3 domain, which we previously found that CBD protein assembles in the tumour after intravenous injection.
Result
Here we show that, after intravenous administration of a collagen-binding domain fused to IL-12 (CBD–IL-12) in mice bearing aggressive mouse tumours, CBD–IL-12 accumulates in the tumour stroma due to exposed collagen in the disordered tumour vasculature. In comparison with the administration of unmodified IL-12, CBD–IL-12 induced sustained intratumoural levels of interferon-γ. CBD-fusion to IL-12 substantially reduced its systemic levels as well as organ damage. CBD-IL-12 provided superior anticancer efficacy compared to unmodified IL-12, eliciting complete regression of CPI-unresponsive breast tumours and melanoma after single injection. CBD-IL-12 shows remarkable efficacy in several difficult-to-treat tumours, such as prostate and ovarian tumours. Furthermore, CBD–IL-12 recruited CD8+ T cells, M1 macrophages, DCs into immunologically cold tumours. CBD-IL-12 potently synergised with CPI to eradicate large established melanomas, induced antigen-specific immunological memory and controlled tumour growth in a genetically engineered mouse model of melanoma. We have recently found that CBD-IL-12 synergises with CART cell therapies. Also, human version of CBD-IL-12 was found safe in healthy beagles. Human version of CBD-IL-12 is extremely efficacious for several orthotopic patient-derived human cancers in mice with human immune system.
Conclusion Tumour-matrix binding localised IL-12 is beneficial in both safety and efficacy, with high translational promise.

Combining an IL-12-based Immunocytokine (PDS0301) with Docetaxel in Metastatic Prostate Cancer: Preliminary Safety and Immune Data

Donahue R[2], Tsai Y[2], Atiq M[1], Chandran E[1], Meininger L[3], Karzai F[1], Bilusic M[1], Marte J[2], Arlen P[1], Cordes L[1], Hausler M[1], Hankin A[1], Williams N[1], Figg W[1], Schlom J[2], Gulley J[2], Madan R[1]
[1]Genitourinary Malignancies Branch, National Cancer Institute, [2]Center for Immuno-oncology, National Cancer Institute, [3]Walter Reed National Military Medical Center

Plenary 3: Cytokines In cancer immunity and immunotherapy

Background: PDS0301 (formerly M9241) is an IL-12 immunocytokine that targets necrotic tumor tissue with preclinical data suggesting synergy with docetaxel. This is the first study in prostate cancer to evaluate docetaxel and an immunocytokine.

Methods: Patients (pts) enrolled at 3 dose levels of PDS0301 (8mcg/kg, 12mcg/kg, and 16.8mcg/kg every 3 weeks) combined with docetaxel (75mg/m2) every 3 weeks. 3-6 pts enrolled with metastatic castration-sensitive prostate cancer (mCSPC) or metastatic castration-resistant prostate cancer (mCRPC). PDS0301 was started with the second cycle of treatment. The dose-limiting toxicity (DLT) window spanned the 6 weeks after initiating docetaxel.

Results: 18 pts are evaluable for toxicity. Median age is 69 years (range 39-82). Eleven pts have mCSPC and 7 have mCRPC. All mCRPC pts had stable disease (SD) for >6 months. Grade 3 toxicity related to docetaxel and/or PDS0301 included anemia, elevated AST, dehydration, diarrhea, fatigue, febrile neutropenia, hypertension, hypotension, neutropenia, sepsis, and leukopenia. The most frequent of these were anemia (19%) and neutropenia (13%). Grade 2 fatigue was the most common toxicity seen in 5/6 pts at 16 mcg/kg, 2/6 at 12 mcg/kg and 1/6 at 8 mcg/kg. The addition of PDS0301 was associated with increases in peripheral activated NK cells, central memory CD8, proliferating CD4 and CD8 cells and cytokines INFg and IL-10. Unlike single agent PDS0301, the changes in immune responses were primarily dose independent.

Conclusions: As demonstrated in this first-in-human trial to combine docetaxel and an immunocytokine in prostate cancer, PDS0301 can safely be administered with docetaxel every 3 weeks. Since immune responses were dose independent, and likely because PDS0301 was given in combination with a necrosis inducing agent, the phase 2 cohorts of this study (NCT04633252) will proceed at 12mcg/kg in both mCSPC and mCRPC.

IL-10 Surrogate Cytokine Agonists (SCAs) Tune Receptor Signaling and Engineer Cell Type Specificity

Ramadass M, Vivona S, Rokkam D, Gangopadhyay A, Totagrande M, Buffone C, Balasubrahmanyam P, Greb H, Riener R, De Waal Malefyt R, McCauley S, Kastelein R, Oft M, Lupardus P
[1]Synthekine Inc.

Symposium 11: Novel approaches for the development of cytokine/anti-cytokine therapies

IL-10 is a pleiotropic homodimeric cytokine that suppresses inflammatory cytokine production by monocytes, while increasing granzyme and IFNγ secretion by activated CD8+ T cells. Each IL-10 subunit signals through a high affinity IL-10Rα and low-affinity IL-10Rβ heterodimer. Here, we establish a new means of bridging IL-10 receptors in a cytokine-independent manner using hIL-10Rα- and hIL-10Rβ-specific camelid heavy chain single domain antibodies (VHHs), resulting in functional dimerization of the receptors and downstream signaling.

Seven IL-10Rα VHHs and seven IL-10Rβ VHHs were identified, linked together in two confirmations (IL-10Rα amino/ IL-10Rβ carboxy and IL-10Rα-carboxy/IL-10Rβ-amino) to create 98 IL-10Rα/IL-10Rβ surrogate cytokine agonists (SCAs) and tested for signaling in phospho-(p)STAT3 reporter cells. Twenty-three IL-10Rα/IL-10Rβ SCAs were able to heterodimerize IL-10Rα and IL-10Rβ subunits and generate pSTAT3 signaling.

IL-10Rα/IL-10Rβ SCAs that were biologically active on primary human cells had relatively higher pSTAT3 signaling in human monocytes compared to lymphocyte populations such as B cells, NK cells, CD4 and CD8 T cells with varying Emax values. These IL-10Rα/IL-10Rβ SCAs inhibited monocyte LPS-induced secretion of IL-1β, TNFα and IL-6. Furthermore, nanostring analyses showed a similar transcriptional profile between an IL-10Rα/IL-10Rβ SCA and WT IL-10 in monocytes. However, unlike WT IL-10, IL-10Rα/IL-10Rβ SCAs showed minimal induction of IFN-γ and Granzyme production in CD3/CD28 activated CD8 T cells, decoupling the immunosuppressive and immunostimulatory activities of IL-10. In cynomolgus monkeys, an IL-10Rα/IL-10Rβ SCA demonstrated selective myeloid cell but not T cell activation and showed antibody-like PK properties.

Designing synthetic ligands with varying signaling strengths allows the possibility of generating molecules that can decouple the immunosuppressive and immunostimulatory pleiotropy of IL-10. Here, we have generated IL-10Rα/IL-10Rβ surrogate cytokine agonists that are biologically active and signal with varying strengths, potentially providing an improved therapeutic window to optimize the anti-inflammatory properties of IL-10 for treating inflammatory diseases.

Structural insights into the mechanism of leptin receptor activation

Saxton R[1], Caveney N[2], Garcia K[2]
[1]UC Berkeley, [2]Stanford University School of Medicine

Symposium 11: Novel approaches for the development of cytokine/anti-cytokine therapies

Leptin is an adipocyte-derived cytokine that regulates satiety and energy homeostasis by activating the leptin receptor (LepR)–JAK2–STAT3 signaling axis in a subset of hypothalamic neurons. Leptin signaling is dysregulated in obesity, however, leading to a state of leptin resistance in which appetite remains elevated despite high levels of circulating leptin. Thus, the use of leptin as an anti-obesity medication has been limited to rare cases of leptin deficiency. To better understand the mechanisms of leptin signaling and resistance, we determined the structure of a stabilized leptin-bound LepR signaling complex using single particle cryo-EM. Our findings reveal an asymmetric complex architecture in which a single leptin induces LepR dimerization via two distinct receptor-binding sites. Analysis of the leptin–LepR binding interfaces uncovered both shared and unique features of receptor activation relative to related cytokines such as IL-6 and IL-27. Structure-based design of leptin mutants that destabilize the LepR dimer yielded both partial and biased agonists that decouple stimulation of STAT3 from activation of LepR negative regulators, even in the presence of wild-type leptin. Collectively, our results shed light on the structural basis for LepR activation and offer insights into the differential plasticity of leptin signaling pathways.

An optimally engineered, cis-signaling, anti-PD1/IL-2 bispecific expands pre-exhausted CD8+ T (Tpex) cells in the tumor microenvironment and markedly inhibits growth of poorly immunogenic tumors

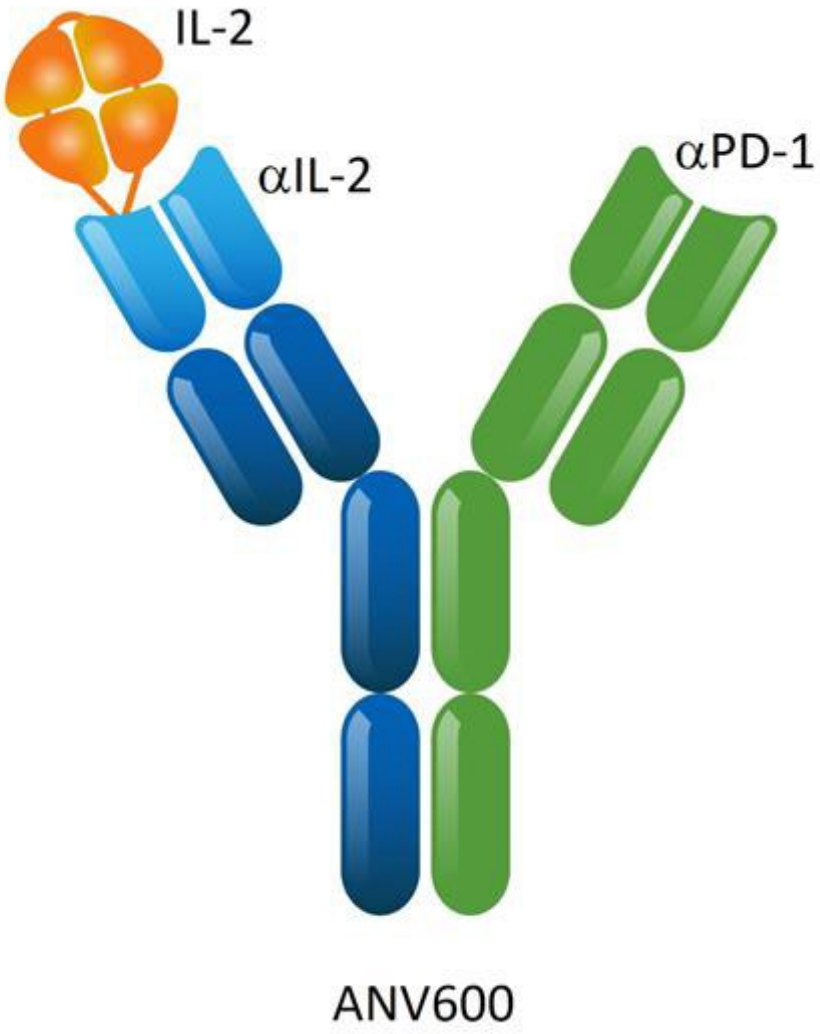
Murer P[1], Salazar U[1], Egli N[1], Petersen L[1], Neubert P[1], Stocker C[1], Rau A[1], Richter K[1], Katopodis A[1], Huber C[1]
[1]Anaveon AG

Symposium 11: Novel approaches for the development of cytokine/anti-cytokine therapies

Background:
The presence of tumor infiltrating lymphocytes (TILs) is closely associated with disease prognosis in cancer. However, multiple pathways lead TILs to various exhausted phenotypes making them unable to respond to tumor antigens. Targeting activating cytokines to TILs is an attractive strategy to re-invigorate the immune response against cancer.

Methods and Results:
We present a novel bispecific molecule, named ANV600, which delivers IL-2Rβγ directed IL-2 to Tpex. The structure of ANV600 comprises two arms, each serving a distinct purpose. One arm features an αIL-2/IL-2 fusion protein, in which the circularly permuted cytokine is embedded within the CDR1 of the antibody light chain. This strategy effectively prevents IL-2Rα from binding to the cytokine, ensuring a selective activation of IL-2Rβ/γ expressing effector cells. The second arm consists of a high affinity PD-1 antibody which binds to a unique epitope on PD1. ANV600 has been optimized using a minimal engineering strategy which leads to a remarkably stable bispecific molecule with drug-like qualities and low immunogenicity potential.
Extensive in vitro and in vivo data demonstrate that ANV600 exhibits the desired cis-signaling mode of action with increased activation of PD-1 expressing effector cells and reduced signaling on Tregs. In transgenic human PD-1 mice, ANV600 treatment resulted in a marked tumor growth retardation in the B16F10 and MC38 subcutaneous tumor models compared to untargeted compound or vehicle. TILs immunophenotyping demonstrated a dose-dependent increase of Tpex, as well as cytotoxic GrzB+PD-1+T cells in mice treated with ANV600.

Conclusions:
ANV600 may be a promising anti-tumor therapeutic against poorly immunogenic tumors and a prototype for delivering stimulatory cytokines to TILs. Further pharmaceutical development and clinical investigations are currently undertaken to explore the full therapeutic potential of ANV600 as a targeted immunotherapy against cancer.


IL-2
αIL-2
αPD-1
ANV600

Influence of the Chemokines CCL17 and CCL22 on IL-33-induced Adipose Tissue Fibrosis

Thiem M[1,2], Majlesain Y[1], Kallies A[2], Weighardt H[1], Villadangos J[2,3], Strugnell R[2], Förster I[1]
[1]Immunology and Environment, Life & Medical Sciences (LIMES) Institut, University of Bonn, [2]Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, [3]Department of Biochemistry and Molecular Biology, Bio21 Institute, The University of Melbourne

Symposium 12: Cytokines in neuro-immune interactions, inflammation and repair

The chemokines CCL17 and CCL22 are primarily expressed by dendritic cells (DC) and macrophages. As ligands of CCR4, both chemokines recruit T cells, facilitate the T cell-DC interaction, and sensitise DC for migration. Furthermore, CCL17 promotes the pathogenesis of allergic and inflammatory diseases. In comparison, the functions of CCL22 are mostly unknown although it appears to play a more immunosuppressive role. While several studies have analysed the CCL17/CCL22/CCR4-axis in vitro, we aim to elucidate the differential function of both chemokines in vivo in the context of bacterial infections and IL-33-mediated immune responses.
WT, CCL17-, CCL22- and CCL17/CCL22-deficient mice were intraperitoneally injected with 0.5 µg of recombinant murine IL-33 every second day (day 0, 2, 4 and 6) and analyzed on day 8. Using this IL-33-injection model, we observed that both chemokines are involved in adipose tissue homeostasis of the inguinal white adipose tissue and brown adipose tissue with a dominant role for CCL22. In the absence of CCL22 morphology of the adipose tissue was altered in naïve state with the inguinal white adipose tissue appearing more fibrotic and the brown adipose tissue showing reduced lipid contents. Furthermore, CCL22 appears to play a detrimental role in IL-33-induced adipose tissue fibrosis as adipose tissue morphology exhibited less signs of inflammation and fibrosis in CCL22-deficient mice. Absence of CCL17 or CCL22 also impaired eosinophil recruitment from the bloodstream, potentially contributing to the observed tissue phenotypes.
Uncovering both protective and detrimental functions of CCL17 and CCL22 in disease pathogenesis opens new aspects to consider in future applications, including therapeutic approaches.

Beyond the Barriers: Delving into Interferon Lambda's Role in the Brain

Koronaiou E[1], GALANI I[1], Salagianni M[1], Polissidis A[2], Andreakos E[1]
[1]Laboratory of Immunobiology, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, [2]Laboratory of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Symposium 12: Cytokines in neuro-immune interactions, inflammation and repair

The interferon lambda (IFN-λ) system has been traditionally studied in the respiratory and gastrointestinal tracts, due to its pivotal role in regulating innate immune response at mucosal barriers. However, recent data have also implicated it in the regulation of the blood-brain barrier integrity in viral infection models. Moreover, in clinical trials with a pegylated form of IFN-λ, adverse neuropsychiatric effects have been reported, while other members of the interferon family exert various effects on brain function, including microglia-dependent synapse maintenance and cognitive behaviors, such as learning and memory, and sociability. Therefore, we sought to investigate the role of the IFN-λ system in the brain.
Herein, we used newly-generated knock-in reporter mice expressing Td Tomato and cre recombinase following IFNλR1 gene, and under the control of the IFNLR1 promoter, in order to map the receptor's expression in the brain. We identified brain regions expressing the IFNλR1 receptor, namely the hippocampus, hypothalamus and amygdala, and subsequently, specific cell types including neurons, ependymal cells, and tanycytes. Furthermore, in order to gain insight into IFNλR1's role in the brain, we performed a full behavioral evaluation of IFNλR1 knock-out mice, assessing locomotor activity, anxiety levels, sensorimotor gating and various other aspects, including spatial, contextual and emotional learning and memory. Abrogation of IFNλR1's expression resulted in prominent behavioral deficits, including impaired spatial and discrimination recognition, and deficient prepulse inhibition (endophenotype of schizophrenia). Finally, using HPLC with electrochemical detection, we quantified neurotransmitter levels in the relevant brain regions and observed dysregulated serotonin and glutamine neurotransmission.
Our data provide the first evidence of IFNλR1 expression in the mammalian brain. We conclude that deletion of IFNλR1 results in significant behavioral and neurochemical abnormalities, and investigation of the underlying mechanisms is currently under way.

MAVS signaling in microglia is critically needed to define the transcriptional fate of CNS infiltrating CD8+ T cells in viral encephalitis

Pavlou A[1,2], Ghita L[1], Mulenge F[1], Waltl I[1], Gern O[1,4], Larsen P[1], Costa B[1], Duran V[1], Robertson S[5], Lueder Y[6], Halle S[6], Förster R[6,7], Best S[5], Stangel M[2,3,7], Kalinke U[1,7]
[1]Institute for Experimental Infection Research, TWINCORE, Centre for Experimental and Clinical Infection Research, a joint venture between the Helmholtz Centre for Infection Research and the Hannover Medical School, [2]Clinical Neuroimmunology and Neurochemistry, Department of Neurology, Hannover Medical School, [3]Center for Systems Neuroscience, University of Veterinary Medicine Hannover, [4]Department of Pathology, University of Veterinary Medicine Hannover, [5]Innate Immunity & Pathogenesis Section, Laboratory of Virology, NIAID/NIH, [6]Institute of Immunology, Hannover Medical School, [7]Cluster of Excellence - Resolving Infection Susceptibility (RESIST, EXC 2155), Hannover Medical School, Carl-Neuberg-Straße 1

Viral infections of the central nervous system (CNS) emerge as a global research priority. A plethora of viruses show neurotropic potential and may cause viral encephalitis. Currently, the therapeutic arsenal to treat neurotropic virus infection of the CNS is limited and many affected individuals develop severe post-encephalitis neurological sequelae or succumb to the infection.
The nasal epithelium that contains olfactory sensory neurons is constantly exposed to a broad range of viruses that may gain access to the CNS under specific conditions. Upon virus propagation to the olfactory bulb (OB), microglia undergo a morphological shift, proliferate and eventually get recruited to the site of infection in order to restrict virus dissemination within the CNS. Recent data point towards microglia cross-presenting antigens to CNS infiltrating virus-specific CD8+ T cells. However, it is not yet clear which pathways coordinate cross-presentation in response to infection.
Our experiments revealed the importance of the adaptor molecule of RIG-I like helicases, which is termed MAVS, selectively in microglia, in promoting survival against lethal VSV infection. Microglia specific MAVS deficiency was associated with impaired gene expression of cross-presentation related genes, suggesting a loss of microglia cross-presentation capacity to T cells. To address this option, we bulk-sequenced CNS-infiltrating CD8+ T cells from mice with microglia that are either MAVS competent or deficient from CNS and draining lymph nodes. The data revealed normal CD8+ T cell priming in the lymph nodes in all mice while CNS-infiltrating CD8+ T cells sorted from mice with a microglia-selective MAVS deficiency showed less activated gene expression profiles from CD8+ T cells of control mice. Mechanistically, MAVS signaling enhanced T cell proliferation in the OVA-OT1 system upon infection with a replication deficient OVA-expressing virus. In conclusion, MAVS signaling in microglia is essential for cross-presentation within the infected CNS, relicensing the CNS infiltrating CD8+ T cells.

TISSUE RESIDENT B1 CELLS IN THE LUNGS PERFORM LOCAL IMMUNOREGULATORY FUNCTIONS AND CONTRIBUTE TO HOMEOSTASIS

Carroll K[1], Sulka K[1], Sawden M[1], Hopkins J[1], Sharma S[1,2]
[1]Graduate School of Biomedical Sciences, Tufts University, [2]Tufts University School of Medicine, Immunology Department

Symposium 13: Cytokines in T and B cell responses to vaccination and beyond

Tissue-resident innate immune cells contribute to lung homeostasis by orchestrating local immunity and regulating inflammation in a tissue-specific manner. Thus far, characterization of homeostatic mechanisms has been largely focused on myeloid populations, but the contribution of other innate immune cells to homeostasis is still in its infancy and requires further inquiry. A population of innate-like B-cells, B1-cells, has been critically implicated in the innate immune response to inflammatory lung diseases and infections. Notably, B1 cells are a resident immune cell described only in the peritoneal cavity and are thought to be recruited to the lungs during inflammation. In the peritoneal cavity, B1 cells perform immunoregulatory functions essential for maintaining homeostasis including spontaneous secretion of large amounts of IL-10 and polyreactive, self-antigen directed 'Natural' antibodies. Recently, B1 cells have been identified as an immunoregulatory resident immune population in the small intestinal lamina propria, suggesting a homeostatic role of B1 cells in mucosal tissues. Yet, their pre-existence in resting lungs and putative contribution to lung homeostasis remain unknown. To address this gap in knowledge, we leveraged spectral flow cytometry, confocal-imaging and single-cell RNA- sequencing (scRNA-seq) of lungs from mice at various developmental timepoints. Our preliminary studies identify a transcriptionally distinctive B1-cell subset in scRNA-seq data from embryonic lungs that persists throughout aging. High-dimensional spectral flow-cytometry and in situ confocal imaging confirm the presence of a phenotypically distinct B1-cell subset, expressing the characteristic markers CD19, IgM, and CD43, in developing and adult lungs. Finally, deeper scRNA-seq analysis revealed key homeostatic immune signatures in these resident B1- cells, supporting putative immunoregulatory functions. Herein, we provide the first evidence for a tissue-resident population of B1-cells at single cell resolution in the lungs of healthy mice. These highly novel observations suggest that resident B1-cells are a significant contributor to the resting lungs' immune-composition throughout lifespan.

Early cytokine interactions and association with antibody levels upon nucleic acid vaccination in macaques and humans

Rosati m[1], Khandhar A[2], Reed S[2], Dimopoulos M[3], Terpos E[3], Pavlakis G[1], Felber B[1]
[1]National Cancer Institute At Frederick, [2]HDT Bio, [3]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens

Symposium 13: Cytokines in T and B cell responses to vaccination and beyond

Early responses to vaccination are important for shaping protective immunity. Dissecting innate vaccine signatures may provide biomarkers predicting immunogenicity and assist optimization of vaccine strategies. We have previously reported a proteomic signature after BNT162b2 mRNA vaccination (NCT04743388; PMID: 34352226). These results were compared with the proteomic analysis of different RNA or DNA vaccines in macaques (RM), which allowed frequent blood and lymph node (LN) measurements and in-depth flow analysis. Specifically, we compared different vaccine formulations: mRNA/liposome nanoparticles, replicating RNA/LION™, DNA/LION™ and DNA/electroporation (EP). Our RM studies showed a rapid innate response within 4-24 hrs, and a delayed in peak response (day 6) after DNA/EP. We identified a systemic transient signature upon BNT162b2 mRNA vaccination including IL-15, IFN-gamma, IP-10/CXCL10, enriched by TNF-a and IL-6 (2nd vaccination), and similar induction after the 3rd vaccination. Importantly, we found correlations of IL-15 and IFN-gamma responses to binding and neutralizing Spike antibody (0.5-3 months after booster). Using different platforms in RM, a unifying finding has been the transient increase of IL-15, IFN-gamma, CXCL10. The DNA/LION vaccine induced a similar co-ordinated transient increase in IL-15 signature and a myeloid-cell associated signature (MIP-1b, MDC-1, IL-1Ra). Both platforms also induced CXCL13, a biomarker for Germinal Center (GC) activation. Phenotypic analysis of LN showed strong activation of distinct CD4 subsets (CD4 TfH; CD4 TfH-GC) and a CXCR5+CD279+PD-1+ CD8 subset cells. We also found a robust increase of dividing Bcl-6+ B cells. The functional status of these subsets appeared to be activated by networks of chemokines including CXCL11 and CXCL13, and cytokines like IL-7 and IL-15. Our data provide evidence of strong activation of LN GC by these vaccines. The data reveal coordinated cascades of cytokine responses to the mRNA/DNA vaccines and highlight pathways of innate responses to vaccination modulating adaptive immunity.

Hyperinflammation due to chronic exposure to excess IL-18 arises from both CD8 T-cell hyper-responsiveness and virus-specific immunodeficiency rather than NK dysfunction.

Varghese J[1], Canna S[1,3], Landy E[2], Eisenlohr L[3,1], Peauroi E[3], Dang V[1], Frank-kamenetskii A[1], Morrissette J[1,3], Nguyen J[1]
[1]Children's Hospital of Philadelphia, [2]University of Pittsburgh School of Medicine , [3]University of Pennsylvania Perelman School of Medicine

Symposium 14: Cytokines in HIV and novel targeted therapies

Systemic Juvenile Idiopathic Arthritis (SJIA) and Macrophage Activation Syndrome (MAS) are associated with highly elevated peripheral blood levels of the inflammasome-activated cytokine IL-18 and chronic exposure to free IL-18. IL-18 canonically acts on NK and activated T-cells to amplify the effects of other cytokines (like IL-12) on Interferon gamma (IFNg) production and cytotoxicity. We assessed lymphocyte phenotypes, distribution, and function via flow cytometry and RNAseq in mice with transgenic expression of mature, excretable IL-18 (Il18tg) and relevant controls. Like SJIA/MAS patients, Il18tg mice demonstrated decreased numbers of NK cells and a concomitant increase in activated CD8 T-cells in peripheral blood, spleen, and liver. Splenic and hepatic NK cells from Il18tg mice were enriched for cell cycle and general gene transcription programs, suggesting rapid cellular turnover. Il18tg mice cleared CD8 T-cell dependent LCMV (Armstrong) infection similarly to WT, but thereafter developed CD8 T-cell and IFNg-mediated immunopathology. To more stringently assess the relevance of these changes in NK populations, we infected mice with the DNA poxvirus ectromelia, which causes an abortive infection in WT mice but severe viral immunopathology in perforin-deficient or NK-depleted mice. NK cells from infected Il18tg mice failed to expand and upregulate activation markers, but Il18tg mice cleared virus similarly to WT mice at early timepoints suggesting that NK cells role is not necessary in Il18tg mice because they are “pre-primed” for a robust CD8 T-cell expansion. As with LCMV, Il18tg mice developed hyperinflammation at the peak of the response, but surprisingly showed negligible clearance of viral DNA between days 5 and 10. These data suggest excess IL-18 induces both CD8 T-cell hyper-responsiveness and a virus-specific immunodeficiency. Thus, although “pre-primed” T-cell responses may help compensate for NK dysfunction at early timepoints, both hyperinflammation and an unexpected immunodeficiency may collude to drive immunopathology at the peak of CD8 T-cell expansion.

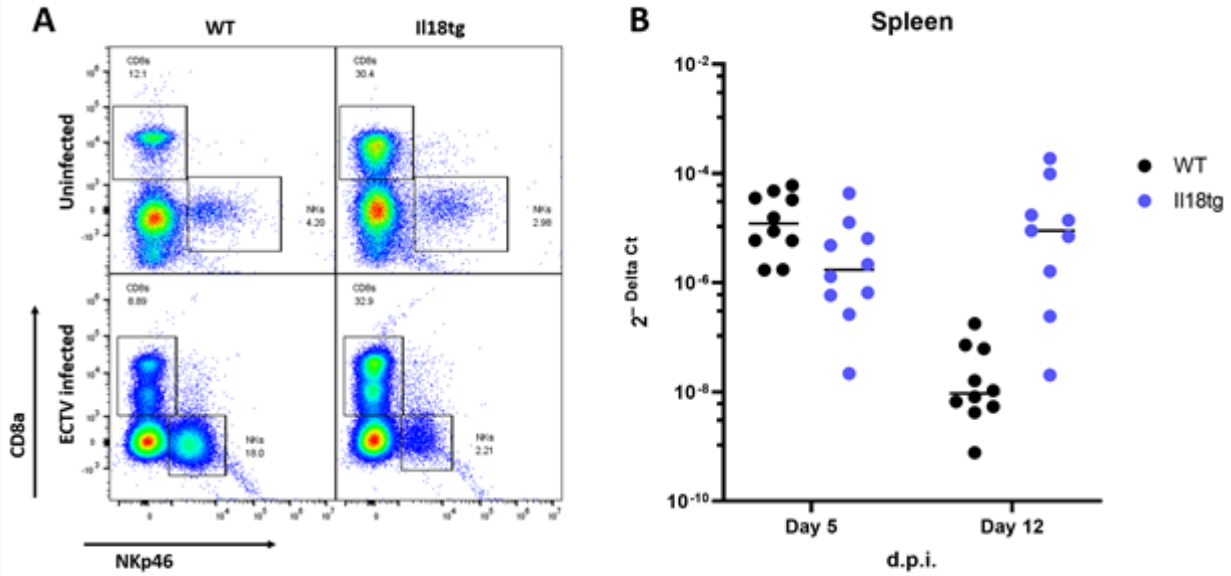


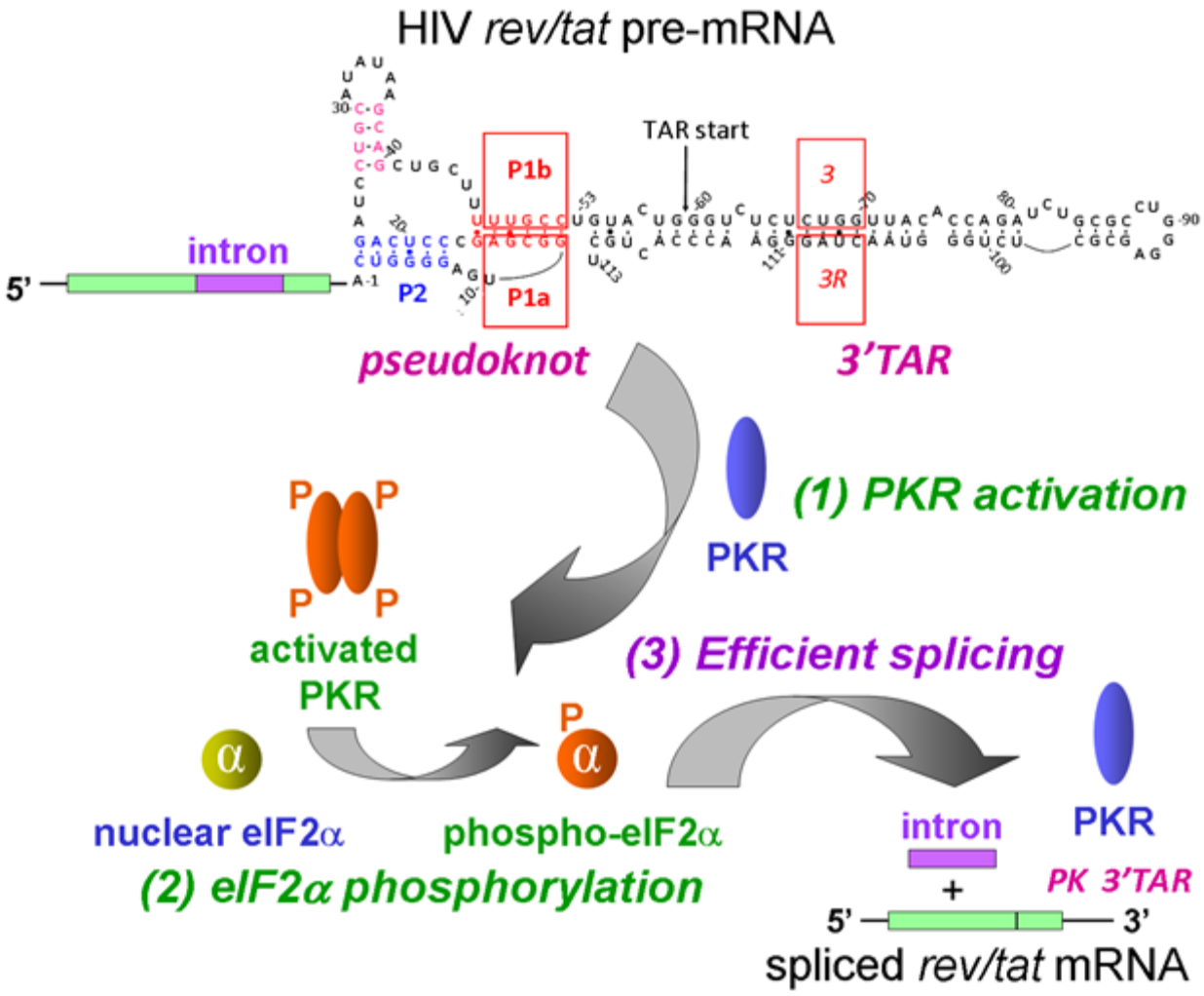
Figure. Il18tg mice are both hyperinflammatory and immunodeficient during ectromelia infection. WT and Il18tg mice were infected in the footpad with wildtype ectromelia virus (Moscow). (A) Live cells from infected (5 d.p.i) and uninfected livers were gated by CD8a and NK1.1 expression. (B) Viral DNA was quantified against 18S rRNA. Data is pooled from 2 independent experiments.

A novel concept: HIV co-opts a host antiviral mechanism, activation of IFN-inducible kinase PKR by its RNA, to enable splicing of its own mRNA

Kaempfer R[1], Namer L[1], Harwig A[2], Heynen S[2], Das A[2], Berkhout B[2]
[1]The Hebrew University, Faculty of Medicine, [2]Experimental Virology, Medical Microbiology, Amsterdam UMC

Symposium 14: Cytokines in HIV and novel targeted therapies

RNA-dependent stress kinase PKR plays a key role in the IFN-mediated antiviral response. Once activated by double-stranded RNA, especially viral dsRNA, PKR phosphorylates eIF2α, inhibiting thereby viral mRNA translation, blocking virus spread and inducing apoptosis of infected cells. We report that this negative control mechanism, a cornerstone of the host antiviral response, positively regulates splicing of a viral mRNA. Excision of the large HIV rev/tat intron depends strictly on PKR activation by the viral RNA and on eIF2α phosphorylation. Rev/tat mRNA splicing is blocked by viral PKR antagonists Vaccinia E3L and Ebola VP35, as well as by dominant-negative mutant PKR, yet enhanced significantly by overexpressing PKR. We reveal an indispensable role for eIF2α phosphorylation in HIV rev/tat mRNA splicing that accounts for the need for PKR activation. Expression of non-phosphorylatable mutant eIF2αS51A, yet not of wild type eIF2α, abrogates efficient splicing of rev/tat mRNA. By contrast, expression of phosphomimetic mutant eIF2αS51D leaves rev/tat mRNA splicing intact. Unlike eIF2αS51A, eIF2αS51D does not inhibit eIF2α phosphorylation by activated PKR. All HIV mRNAs contain terminal trans-activation response (TAR) stem-loop sequences that potentially could activate PKR, yet even upon TAR deletion, HIV mRNA production remained sensitive to inhibitors of PKR activation. We discovered a novel, compact RNA pseudoknot located upstream of 3'-terminal TAR that promotes splicing by activating PKR. This pseudoknot is conserved amongst diverse HIV and nonhuman primate SIVcpz isolates, supporting its essential role in control of splicing. The pseudoknot and 3'-terminal TAR collaborate in mediating PKR-regulated splicing of rev/tat intron, the pseudoknot being dominant. Our results on HIV provide the first example of a virus co-opting activation of PKR by its RNA, a host antiviral mechanism, to promote splicing. We are examining if other viruses may use local activation of host kinase PKR through intragenic RNA elements to achieve efficient mRNA splicing.


HIV rev/tat pre-mRNA
TAR start
P1b
P1a
P2
3
3R
intron
5'
pseudoknot
3'TAR
(1) PKR activation
PKR
P
activated
PKR
(3) Efficient splicing
α
nuclear eIF2α
phospho-eIF2α
(2) eIF2α phosphorylation
intron
PKR
PK 3'TAR
+
5'
3'
spliced rev/tat mRNA

Inflammation and immune activation are correlated with specific gut microbes in people living with HIV

Basting C[1], Escandon K[2], Anderson J[2], Cromarty R[3], Schroeder T[1], Velez A[1], Wieking G[2], Reichel J[2], Guerrero C[1,4], Briceño O[5], Torres-Ruiz F[5], Avila S[5], Salgado G[5], Schacker T[2], Klatt N[1]
[1]University of Minnesota, Department of Surgery, Division of Surgical Outcomes and Precision Medicine Research, [2]University of Minnesota, Department of Medicine, Division of Infectious Disease and International Medicine, [3]University of Minnesota, Masonic Cancer Center, [4]University of Minnesota, Center for Metabolomics and Proteomics, [5]National Institute for Respiratory Diseases

Symposium 14: Cytokines in HIV and novel targeted therapies

For people living with HIV (PLWH), antiretroviral therapy (ART) has been shown to control viral replication resulting in undetectable plasma viral load levels. However, despite ART, chronic inflammation and immune activation (IA) are continued predictors for mortality in PLWH due to increased risk of inflammation-mediated conditions. Damage to the gut mucosa from HIV infection, and subsequent translocation of microbial products such as lipopolysaccharides, have implicated the gut microbiome as a potential mediator of chronic inflammation and IA in PLWH. In addition, specific strains of gut bacteria have been shown to activate CD4+ T cells, which is a potential mechanism for chronic IA. To investigate the role of the gut microbiome in IA, we are enrolling 70 patients infected with HIV that are currently on ART from 3 study sites (located in Minnesota and Mexico City) and are collecting gut biopsies, stool, plasma, and clinical lab measures (i.e., CD4+ and CD8+ T cell counts). In a preliminary analysis, we performed 16S rRNA sequencing on ileum, rectum and stool samples from 12 patients in Minnesota categorized as either immunological “Responders” or “Nonresponders” based on their CD4+/CD8+ T cell ratios (<0.52 for Nonresponders, >1.08 for Responders). Differential abundance analysis showed that Nonresponders had significantly different relative abundance of several microbes, including higher Subdoligranulum in the rectum and ileum biopsies (p=0.06 and p=0.005 respectively). Furthermore, the relative abundance of Subdoligranulum in rectum and ileum biopsies negatively correlated with CD4+/CD8+ ratios (Spearman correlation, p=0.001 and p=0.048 respectively). In addition to this analysis, we are currently quantifying concentrations of inflammatory cytokine and biomarker concentrations (e.g., IL-6, TNF, LBP, CRP) in matching plasma samples and sequencing gut biopsies from 40 additional patients to better integrate microbiome and inflammation data in PLWH. These results highlight the important role of the gut microbiome in IA in PLWH.

POSTER PRESENTATIONS

P1-001 Distinct baseline immune characteristics associated with responses to conjugated and unconjugated pneumococcal polysaccharide vaccines in older adults

Ucar D[1], Ravi S, Banchereau J, Nahm M, Kuchel G
[1]Jackson Laboratory

Pneumococcal infections cause serious illness and death among older adults. A capsular polysaccharide vaccine PPSV23 (Pneumovax®) and a conjugated polysaccharide vaccine PCV13 (Prevnar®) are used to prevent these infections, yet underlying responses, and baseline predictors remain unknown. We recruited and vaccinated 39 older adults (>60 years) with PPSV23 or PCV13. Both vaccines induced strong antibody responses at day 28 and similar plasmablast transcriptional signatures at day 10, however, their baseline predictors were distinct. Analyses of baseline flow cytometry and RNA-seq data (bulk and single cell) revealed a novel baseline phenotype that is specifically associated with weaker PCV13 responses, characterized by i) increased expression of cytotoxicity-associated genes and increased CD16+ NK frequency; ii) increased Th17 and decreased Th1 cell frequency. Men were more likely to display this cytotoxic phenotype and mounted weaker responses to PCV13 than women. Baseline expression levels of a distinct gene set was predictive of PPSV23 responses. This first precision vaccinology study for pneumococcal vaccine responses of older adults uncovered novel and distinct baseline predictors that might transform vaccination strategies and initiate novel interventions.

P1-002 An Emerging Paradigm of Cxcl12/Cxcr4 Involvement in Breast Cancer Metastasis

Anastasiadou D[1], Sanchez L, Duran C, Chen M, Lin Y, Eddy R, Entenberg D, Condeelis J, Oktay M, Karagiannis G[1]
[1]Albert Einstein College of Medicine

The Cxcl12/Cxcr4 signaling axis promotes metastasis in multiple mouse models of breast carcinoma. The mechanism via which Cxcr4+ breast cancer cells escape the primary tumors, which highly express Cxcl12, remains poorly understood. By using a novel methodology for quantifying chemotactic gradients using fixed tissue multichannel immunofluorescence (mIF), we demonstrate in mouse primary breast tumors that Cxcl12 gradients are expressed around cancer cell intravasation doorways, known as Tumor Microenvironment of Metastasis (TMEM) doorways. Through distance analysis morphometry, we demonstrate that TMEM-mediated Cxcl12 gradients contextually associate with Cxcr4+ breast cancer cells migrating towards the underlying TMEM doorways. Pharmacological inhibition of the Cxcl12/Cxcr4 pathway abrogates translocation of Cxcr4+ cancer cells to TMEM doorways, suppressing TMEM-mediated metastatic dissemination. However, targeted elimination of Cxcr4 gene from breast cancer cells, results in a suboptimal response, thus suggesting the existence of a bypass or compensatory mechanism. Previously, it was shown that Cxcr4+ tumor-associated macrophages (TAMs) support the invasive and migratory properties of tumor cells utilizing TMEM doorways. We thus theorized that, in the absence of Cxcr4 expression in tumor cells, the accompanying Cxcr4+ TAMs still “read” TMEM-generated Cxcl12 chemotactic gradients. Indeed, clodronate-mediated TAM depletion results in the suppression of Cxcr4+ cancer cell translocation to TMEM doorways and their subsequent dissemination to peripheral circulation and future metastatic sites. Finally, we used stromal and immune cell lineage markers to identify the source of TMEM-generated Cxcl12 gradients in mouse primary breast cancers. Despite that blood vessels (irrespective of presence of TMEM doorways) were primarily lined by Pdgfrb+ stromal cells with basal Cxcl12 expression, other sources of Cxcl12 could be perivascular TAMs and/or the blood circulation itself. Overall, our data support a new paradigm for the implication of the Cxcl12/Cxcr4 axis during the early stages of the metastatic cascade, and propose a new avenue for rationalized antimetastatic treatments for breast cancer.

P1-003 Localized but not Systemic Type I Interferon Therapy Improves Immune Infiltration and PD-blockade in a Mouse Melanoma Model

Harari D[1], Rotkopf R[1], Reich-Zeliger S[1], Dov A[1], Volchinski V[1], Schreiber G[1]
[1]Weizmann Institute Of Science

Transcriptomic analysis of tumor biopsies from metastatic melanoma patients (SKCM-TCGA) demonstrates a profound survival advantage for approximately one-third of patients who exhibit the highest levels of intratumoral type I Interferon (IFN-I) signaling (Hazard Ratio 0.36; Pr: 4E-06), these which coincide with a sharp increase in transcripts indicating infiltration of CD4-T, CD8-T, B-cells and Macrophages to the tumors. Pathway analysis furthermore demonstrates that these patients exhibit T- and B-cell activation and a Th-1 response. To test if IFN-I signaling is central to and not simply correlating with these associated factors, we employed an adeno-associated virus (AAV) delivery system, locally expressing mouse IFNβ in B16F10 cells grafted into congenic C57BL/6 mice, this a known cold tumor model particularly hard to treat. Whereas anti-PDL1 monotherapy had no response in this model, combination therapy slowed, and in some cases cleared the mice of tumors. AAV-IFNβ monotherapy alone can slow but will not cure the mice. In sharp contrast to localized IFN delivery, systemic IFN therapy showed no beneficial effects in slowing tumor growth. To examine this more deeply, we injected the mice bilaterally with B16F10 tumors, where one tumor received AAV-IFNβ and the contralateral tumor received control. Both the injected and contralateral tumors nevertheless demonstrated a large reduction in tumor size, this effect lost when repeated using IFNAR2 knockout mice. Furthermore both the IFN-treated and contralateral tumors exhibited a large increase in CD3+CD4+ and CD3+CD4- lymphocytes. We submit that enforcing localized IFN-I signaling to a tumor in melanoma can drive immune cell infiltration, with the potential to elicit a systemic immune response, and possibly even cure, particularly when used in combination with immune checkpoint inhibitors.

P1-004 ISG15 modulates lipid metabolism in macrophages during Vaccinia virus infection.

Albert M[1], Falcón J[2], Vázquez J[3], Guerra S[1]
[1]Universidad Autónoma De Madrid, [2]CIC bioGUNE, [3]3Centro Nacional de Investigaciones Cardiovasculares

The interferon-stimulated gene 15 (ISG15) is a well-known antiviral molecule against a wide range of viruses. ISG15 exerts its antiviral function directly, modifying viral proteins, or indirectly, through the regulation of numerous cellular pathways, ranging from genome replication to energy metabolism. Previous work from our group identified ISG15 as a modulator of mitochondrial metabolism and dynamics in IFN-stimulated bone marrow-derived macrophages (BMDM). Our recent work presents ISG15 as a regulator of lipid metabolism during infection. We reported a dysregulation of the macrophage lipid profile in the absence of ISG15, highlighting a significant reduction in neutral lipids (NL), in line with reduced lipid droplet number and size. These observations were consistent with upregulation of proteins involved in fatty acid oxidation (FAO) and lipolysis, what correlated with increased expression of PGC-1α and PPARγ. Vaccinia virus (VACV) infection altered the lipid profile of BMDM, increasing NL, mainly cholesterol esters. Interestingly, such alterations in the lipid content were exacerbated in Isg15-/- BMDM, suggesting a role of ISG15 restraining the effects of VACV on lipid metabolism. Altogether, our results broaden the functions of ISG15 and highlight its relevance as an immunometabolic regulator during viral infections. This work was funded by the Spanish State Research Agency (Agencia Estatal de Investigación, AEI).

P1-005 An MVA-based vector expressing cell-free ISG15 increases IFN-I production and improves HIV-1-specific CD8 T cell immune responses.

Falqui M[1], Perdiguero B[2], Coloma R[1], Albert M[1], Marcos L[2], McGraill J[1], Solorzano C[2], Esteban M[2], Gómez C[2], Guerra S[1]
[1]Universidad Autónoma De Madrid, [2]Centro Nacional de Biotecnología

Human immunodeficiency virus (HIV), responsible of the Acquired Immune Deficiency Syndrome (AIDS), continues to be a major global public health issue with any cure or vaccine available. Interferon-stimulated gene 15 (ISG15) encodes a ubiquitin-like protein that is induced by interferons and plays a critical role in the immune response. ISG15 is a modifier protein that covalently binds to its targets via a reversible bond, a process known as ISGylation, which is the best-characterized activity of this protein to date. However, ISG15 can also interact with intracellular proteins via non-covalent binding or act as a cytokine in the extracellular space after secretion. In previous studies we proved the adjuvant effect of ISG15 when delivered by a DNA-vector in heterologous prime-boost combination with a Modified Vaccinia virus Ankara (MVA)-based recombinant virus expressing HIV-1 antigens Env/Gag-Pol-Nef (MVA-B). Here we extended these results evaluating the adjuvant effect of ISG15 when expressed by an MVA vector. For this, we generated and characterized two novel MVA recombinants expressing different forms of ISG15, the wild-type ISG15GG (able to perform ISGylation) or the mutated ISG15AA (unable to perform ISGylation).. In mice immunized with the heterologous DNA prime/MVA boost regimen, the expression of the mutant ISG15AA from MVA-Δ3-ISG15AA vector in combination with MVA-B induced an increase in the magnitude and quality of HIV-1-specific CD8 T cells as well as in the levels of IFN-□ released, providing a better immunostimulatory activity than the wild-type ISG15GG. Our results confirm the importance of ISG15 as an immune adjuvant in the vaccine field.

P1-006 PYHIN proteins regulate multiple aspects of the host innate immune response.

Baran M[1], Feriotti C[2], Jiang Z[3], Calderon-Gonzalez R[2], Dumigan A[2], Sa-Pessoa J[2], Fitzgerald K[3], Bengeochea J[2], Bowie A[1]
[1]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, [2]Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, [3]Division of Innate Immunity, University of Massachusetts Chan Medical School

PYHIN (pyrin and HIN domain) proteins such as AIM2, IFI16 and IFI204 were originally implicated in the host innate immune response as DNA sensors, but now their role has expanded to include restriction of DNA and RNA viruses, and transcriptional regulation of immune genes. Here we sought to understand in more detail how PYHIN proteins regulate gene transcription, and in what disease contexts this might be of importance.

We found mouse PYHIN IFI207, which as well as a pyrin and HIN domain has a large undefined middle domain, had a role in regulation of cytokine promoters such as Tnf. IFI207 co-localized with both active RNA polymerase II (RNA Pol II) and IRF7, while ectopic expression of IFI207 enhanced IRF7-dependent gene promoter induction in a Pyrin and HIN domain-independent manner. Generation of an Ifi207-/- mouse showed that IFI207 regulates the host response to Klebsiella pneumoniae. Surprisingly, as well as being required for immune gene induction by Klebsiella, IFI207 was required for the establishment of a lung infection, and for macrophage phagocytosis of the bacteria.

Next, we found a role for human IFI16 in regulation of CCL20 in keratinocytes. CCL20 is a chemokine produced by keratinocytes that drives inflammation in psoriatic lesions. Compared to WT cells, stimulation of IFI16-/- keratinocytes with a cytokine mix relevant to psoriasis (TNFα, IL17a, IFNγ and IL22) showed impaired CCL20 induction. Domain mapping showed the importance of the IFI16 pyrin domain for CCL20 promoter induction. IFI16 required both NFκB and C/EBP, but not SP1, CCL20 promoter elements for gene induction, while the lack of an effect of IFI16 absence on cytosolic signalling strongly suggested a nuclear, promoter-proximal role for IFI16 in these cells.

Overall, our work demonstrates the importance of PYHINs as proximal regulators of cytokine gene transcription within specific infection and disease contexts.

P1-007 Structures of complete extracellular receptor assemblies mediated by IL-12 and IL-23

Bloch Y[1,2,6,7], Felix J[1,2,7,8], Merceron R[1,2,3], Provost M[1,2], Alipour Symakani R[1,2,4], De Backer R[1,2], Lambert E[1,2,5], Savvides S[1,2,8]
[1]Unit for Structural Biology, Department of Biochemistry and Microbiology, [2]Unit for Structural Biology, VIB-UGent Center for Inflammation Research, [3]Eurofins DiscoverX Products, [4]VIB Center for Medical Biotechnology,

The interleukin 12 (IL-12) family cytokines are key immunological playmakers that coordinate both innate and adaptive immune responses mainly through regulation of T-cell populations. Arguably, the IL-12 cytokine family is the most unique and intriguing cytokine family because it is defined by an array of heterodimeric cytokines that signal via a puzzling network of unique and shared receptors. IL-12 and IL-23 are key members of the IL-12 family and serve as pro-inflammatory and pro-stimulatory cytokines in the development of TH1 and TH17 subsets of helper T-cells respectively. Although extensively studied on both a functional and structural level, detailed insights into the complete architecture of IL-12/IL-23 in complex with the extracellular parts of their cognate receptors has eluded the field.
Here, we present structures of complete extracellular IL-12 and IL-23 ligand:receptor complexes, elucidated via cryo-electron microscopy. A key methodological advance included the use of a DAPK1-Calmodulin heterodimerization tag fused to the C-termini of the extracellular parts of the IL-12/IL-23 receptors simplifying purification of the complexes and to reduce the conformational heterogeneity and inherent flexible character of IL-12/IL-23 ligand:receptor assemblies.
Specifically, the IL-12:IL-12R extracellular assembly provides novel structural insights into the binding of the IL-12A (p35) subunit to its receptor, IL-12Rβ2. The structures reveal important commonalities but also surprisingly diverse features. Both IL-12 and IL-23 utilize a conspicuously presented aromatic residue on their α-subunit as a hot-spot to interact with the N-terminal Ig-domain of their high affinity receptors. Contrarily, only the IL-12 complex structure reveals previously uncharacterized receptor-receptor contacts between the membrane proximal domains of IL-12Rβ1 and IL-12Rβ2, opening up new avenues that enable cytokine-specific interrogation of IL-12 and IL-23 signaling in physiology and disease.

P1-008 A Family-wide assessment of latent STAT transcription factor interactions reveals divergent dimer repertoires

Begitt A[1]
[1]University Of Nottingham

The conversion of signal transducer and activator of transcription (STAT) proteins from latent to active transcription factors is central to cytokine signaling. Triggered by their signal-induced tyrosine phosphorylation, it is the assembly of a range of cytokine-specific STAT homo- and heterodimers that marks a key step in the transition of hitherto latent proteins to transcription activators. In contrast, the constitutive self-assembly of latent STATs and how it relates to the functioning of activated STATs is understood less well. To provide a more complete picture, we developed a co-localization-based assay and tested all 28 possible combinations of the seven unphosphorylated STAT (U-STAT) proteins in living cells. We identified five U-STAT homodimers-STAT1, STAT3, STAT4, STAT5A, and STAT5B-and two heterodimers-STAT1:STAT2 and STAT5A:STAT5B-and performed semi-quantitative assessments of the forces and characterizations of binding interfaces that support them. One STAT protein-STAT6-was found to be monomeric. This comprehensive analysis of latent STAT self-assembly lays bare considerable structural and functional diversity in the ways that link STAT dimerization before and after activation.

P1-009 Itaconate increases type-I interferon responses via SDH – dependent mtRNA release.

O'carroll S[1], Peace C[1], Day E[1], Escher-Toller J[1], Zotta A[1], Hooftman A[1], F. McGettrick A[1], O'Neill L[1]
[1]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College

Metabolic reprogramming in immune cells, such as macrophages, alters immune cell function and reveals roles for specific metabolites in health and disease. In LPS-activated macrophages metabolic rewiring occurs, resulting in itaconate to be among the most upregulated metabolites. Itaconate is derived from the Krebs cycle intermediate aconitate by the enzyme ACOD1 (encoded by Irg-1). Itaconate is an anti-inflammatory, anti-viral, and anti-bacterial metabolite that functions in a number of mechanisms, primarily, by covalent modification of proteins involved in inflammatory signaling. Other mechanisms include epigenetic modification of immune cells and by GPCR receptor binding. The list of protein modifications with functional ramifications is ever-growing, including SDH, FH, TFEB, KEAP1, and NLRP3. Previous literature demonstrates that itaconate increases type-I interferon responses. However, the mechanism by which this regulation occurs is yet unclear. We confirm previous literature that itaconate increases interferon-β in-vitro. We show that genetic ablation of Acod1 reduces interferon-β in THP1s and PBMCs. Furthermore, itaconic acid boosts interferon-β in-vivo. Itaconate has been shown to block SDH activity. We show that pharmacological SDH inhibition increases interferon-β production. We demonstrate that the SDH-mediated increase in interferon-β is due to cytosolic mtRNA release. Specifically, SDH inhibition increases cytosolic mtRNA and genetic ablation of pattern recognition receptors revealing the effect of SDH inhibition on interferon-β is VDAC, RIG-I, and MDA5 dependant. These results show a new mechanism for the anti-inflammatory role of itaconate and strengthens the role of the citric acid cycle in inflammation.

P1-010 The Src-ZNRF1 axis controls TLR3 trafficking and interferon responses to restrict lung barrier damage and prevent bacterial superinfection

Lin Y[1], Chang Y[1], Hsu L[1]
[1]National Taiwan University

Type I interferons are important antiviral cytokines but prolonged interferon production is detrimental to the host. The TLR3-driven immune response is crucial for mammalian anti-viral immunity and its intracellular localization determines induction of type I interferons; however, the mechanism terminating TLR3 signaling remains obscure. Here, we show that the E3 ubiquitin ligase ZNRF1 controls TLR3 sorting into multivesicular bodies/lysosomes to terminate signaling and type I interferon production. Mechanistically, c-Src kinase activated by TLR3 engagement phosphorylates ZNRF1 at tyrosine 103, which mediates K63-linked ubiquitination of TLR3 at lysine 813 and promotes TLR3 lysosomal trafficking and degradation. ZNRF1-deficient mice and cells are resistant to infection by encephalomyocarditis virus and SARS-CoV-2 because of enhanced type I interferon production. However, Znrf1-/- mice have exacerbated lung barrier damage triggered by antiviral immunity, leading to enhanced susceptibility to respiratory bacterial superinfections. Our study highlights the c-Src-ZNRF1 axis as a negative feedback mechanism controlling TLR3 trafficking and the termination of TLR3 signaling.

P1-011 Ethanol Induces Neuroinflammation in a Chronic Plus Binge Mouse Model of Alcohol Use Disorder via TLR4 and MyD88-Dependent Signaling

Drew P[1], Holloway K[1], Douglas J[1], Rafferty T[1], Kane C[1]
[1]University Of Arkansas For Medical Sciences

Ethanol induces neuroinflammation which is believed to contribute to the pathogenesis of alcohol use disorder (AUD). Toll-like receptors (TLRs) are a group of pattern recognition receptors (PRRs) expressed on both immune cells including microglia and astrocytes as well as non-immune cells in the central nervous system (CNS). Studies have shown that alcohol activates TLR4 signaling resulting in the induction of pro-inflammatory cytokines and chemokines in the CNS. However, the effect of alcohol on signaling pathways downstream of TLR4 such as MyD88 and TRIF (TICAM) signaling have not been evaluated. In the current studies, we treated Wild-type, TLR4-, MyD88-, and TRIF-deficient mice using a chronic plus binge mouse model of AUD. Evaluation of mRNA expression by qRT-PCR revealed that ethanol increased IL-1β, TNF-α, CCL2, COX2, FosB, and JunB in the cerebellum in Wild-type and TRIF-deficient mice, while ethanol generally did not increase the expression of these molecules in TLR4- and MyD88-deficient mice. Furthermore, IRF3, IRF7, and IFN-β1, which are associated with the TRIF-dependent signaling cascade, were largely unaffected by alcohol. Collectively, these studies suggest that the TLR4 and downstream MyD88-dependent signaling pathways are essential in ethanol induced neuroinflammation in this mouse model of AUD

P1-012 Identification of the in vivo functions of Interleukin-26 in gut homeostasis

Salloum Y[2], Gros G[1], Rabahi S[1], Medena-Yanez I[1], Baudino-Garcia C[1], Morales R[3], Villablanca E[3], Hernandez-Cerda P[1]
[1]Curie Institute, [2]PSL, [3]Karolinska Institutet

The gastrointestinal tract represents a complex system housing numerous populations of immune cells and beneficial microorganisms and serves as an entry point for various pathogens. In this environment, a dynamic crosstalk between immune cells, epithelial cells, and the microbiota is essential to ensure proper organ homeostasis. This crosstalk is largely mediated by cytokines and its dysregulation can lead to inflammatory bowel disease (IBD).

Human genome-wide association studies have identified interleukin-26 (IL-26) as a risk locus for IBD. Moreover, IL-26 has been found to be over-expressed in IBD lesions. However, The in vivo functions of this cytokine have not yet been deciphered, largely due to its absence in rodents. Interestingly, the zebrafish possesses a unique homolog of the human IL-26 gene.
In this project, we exploit the zebrafish to test whether and how IL-26 plays a role in maintaining gut homeostasis.

During the early stages of IBD, the primary drivers of inflammation are the bacteria that overcome the mucosal defence mechanisms and inflict damage to the intestinal epithelium. Intriguingly, human IL-26 has intrinsic bactericidal activity through pore formation in vitro. However, the in vivo consequences of this activity remain unclear. We found that the zebrafish IL-26 also kills bacteria in vitro, and we are currently determining how this activity impacts gut homeostasis in vivo.

In the course of IBD, prolonged tissue damage could lead to the accumulation of DNA damage, eventually resulting in the proliferation of abnormal cells and cancer. Therefore, it is essential to identify agents that control DNA repair and proliferation in the gut. Interestingly, we generated il26-/- zebrafish and found that IL-26 modulates pathways related to cell cycle, DNA replication, and DNA repair. We are currently analysing the consequences of IL-26 loss on these aspect in epithelial progenitors at steady state and upon inflammation.

P1-013 Systemically Administered Flt3L Therapeutics Enhance Healing and Prevent Incidence in Two Murine Models of Non-healing Wounds

Alpar A[1], Lauterbach A[1], Bugno J[1], Weichselbaum R[1], Hubbell J[1]
[1]University Of Chicago

Chronic, non-healing wounds, including diabetic foot ulcers and treatment related ulcerations, represent a major challenge for the medical industry with few currently approved therapeutics on the market. Previous research has looked to generate growth factors related to improving vascularization and stromal infiltration into the wound bed; however, these cytokine treatments tend to come with treatment related toxicities and minimal clinical benefit. More recent research has acknowledged the involvement of the immune system in perpetuating the wound environment and seeks to modulate cells such as macrophages to enhance healing. Herein, we show that, rather than macrophages, we are capable of enhancing dendritic cells (DC) in the wound-bed using systemic treatments with the cytokine Flt3L to promote wound closure in a mouse model of Type 2 diabetic wounds, increasing the average closure of the wound by 30% with a single dose. Furthermore, we characterize the pharmacokinetics of a novel, engineered Flt3L, fused to Serum Albumin (Flt3L-SA), which increases the half-life of the cytokine by over five-fold (from 10 hours in the native form to over 55 hours as a fusion), and demonstrate the capability of this engineered Flt3L to reduce the necessary dose for healing by twenty-fold. Finally, we also demonstrate the utility of Flt3L-SA in a prophylactic context to prevent onset of oral ulcerations related to head irradiation, a major dose-limiting toxicity related to cancer treatment known as radiation-induced oral mucositis (RIOM). Overall, we show the benefit of DCs in two very different contexts of healing-resistant wounds and address challenges related to these major unmet needs in regenerative medicine. Future work with this cytokine will further establish Flt3L proteins as a therapeutic option for wound regeneration and prevention as well as elucidating exact mechanisms by which DCs are promoting a healing milieu.

P1-014 In Situ Interaction of TNF with their Receptors

Nikovics K[1], Cousin A[1], Favier A[1], Holy X[2], Riccobono D[3,4]
[1]Imagery Unit, Department of Platforms and Technology ResearchFrench Armed Forces Biomedical Research Institute, [2]Imagery Unit, Department of Platforms and Technology Research, French Armed Forces Biomedical Research Institute, [3]Department of Platforms and Technology Research, French Armed Forces Biomedical Research Institute, [4]Department of radiation bioeffects, French Armed Forces Biomedical Research Institute, [5]Imagery Unit, Department of Platforms and Technology Research, French Armed Forces Biomedical Research Institute

Cytokines are very important messengers during cell communication. They mostly act as immunomodulatory agents and play a crucial role in homeostasis, inflammation, tissue regeneration, and programmed cell death (apoptosis). Tumor necrosis factor (TNF) is a pro-inflammatory cytokine that binds to the TNF receptor (TNFR) on the cell surface and leads to the activation of a signaling cascade inside the cell. There are two types of TNF receptors, TNFR1 and TNFR2. TNFR1 is expressed in almost all cells and is involved in many biological processes, including inflammation and apoptosis. TNFR2 is expressed mainly in immune cells and is involved in the regulation of immune responses. TNF can also bind to both TNFR1 and TNFR2 and induce various functions. In addition, TNF can also interact with the receptor CD163, which is expressed mainly in macrophages. The binding of TNF to CD163 can result in the activation of anti-inflammatory pathways and suppression of pro-inflammatory cytokine production. Determining the in situ interactions between cytokines and their receptors is essential for a better understanding of inflammation and tissue regeneration. To this end, we have investigated the interactions of TNF/TNFR1, TNF/TNFR2, and TNF/CD163 using an in situ proximity ligation assay in skin, muscle, and lung regeneration models.

P1-015 Inferring metabolic pathway activity levels of bone formation from RNA-Seq data of TgA86 mouse model of Spondyloarthritis

Lee S[1], Kim M[1], Kollias G[2], Kim K[1]
[1]Department of Rheumatology, Kyung Hee University, Hospital at Gangdong, [2]Department of Physiology, School of Medicine, National Kapodistrian University

Background: Assessing pathway activity levels from RNA-Seq datasets is a plausible way to quantify metabolic differences between various conditions. TgA86 mice overexpress mouse transmembrane TNF from a Δ1-12 mTNF-globin transgene. They develop peripheral and axial joint pathology accompanied by new bone formation features, all characteristics of human spondyloarthritis (SpA) pathology.

Purposes: To infer the critical underlying mechanism leading to bone formation in spine of spondyloarthritis (SpA) using RNA-seq datasets of TgA86 model mice

Methods: RNA-seq datasets were obtained from the vertebrae tissue of TgA86 and negative control mice at two different time points (i.e., 4 month and 10 month). For transcriptome level, differential analyses using either whole gene list or immune-associated gene list were carried out. From metabolome perspective, comprehensive pathway analyses including pathway enrichment analyses and metabolic flux simulation were performed.

Results: The whole analyses showed significant enrichment of lipid-associated metabolisms such as phospholipid metabolism, as well as nucleotide metabolism on TgA86. Mitochondria-associated pathways such as oxidative phosphorylation and NAD metabolism, however, showed down-regulation on TgA86, while carnitine shuttle and amino acid metabolism exhibited significant changes between the two time points. Also, pathogenicity-associated gene clusters/modules inferred through our custom-made pattern recognition algorithm turned out to be dependent on the metabolic alterations.

Conclusions: The underlying mechanisms of bone formation-related metabolisms may specifically correlate with the findings from this study, and enhanced activities on target modules may provide clues explaining the pathogenesis of SpA.

P1-016 Understanding the Potential of Human IL-12 and Chimeric IL-15 Combination for Cancer Immunotherapy

Sharma P[1], Patel D[1], Dalai S[1]
[1]Nirma University

Autologous cell-based therapy is a preferred approach for Cancer immunotherapy. However, generating potent NK cells and antigen experienced T cells has been a challenge. To address this issue, we have developed a strategy of using the combination of IL-12 and IL-15 to activate CD4+ T cells, CD8+ T cells and NK cells. IL-12 and IL-15 possess distinct roles but both are potent inflammatory cytokines. IL-12 is a vital promoter of Th1 differentiation via activation of signal transducer and activator of transcription 4 (STAT4), while IL-15 is essential for developing innate immune response as well as contributing to the maintenance of memory CD8+ T cells. Substantial in vivo and in vitro data have been reported to support the role of IL-15 as a T cell growth factor. These activities of IL-12 and IL-15 make them ideal for combinatorial immunotherapy of cancer. However, the short half-life (~ 1hr) and poor bioavailability limits the therapeutic use of IL-15. To overcome these limitations, we have designed chimeric IL-15-IgG2 (Indian Patent Application No. 201721010096A) with an objective of augmenting the generation of memory T cells as well as reactivating them during the antigenic challenge. Our stable chimeric IL-15 has a half-life of >40 Hrs. In humans, IL-12 is needed for DCs to provoke rapid IFN-g production by NK cells, IL-15 seems essential for the NK cell proliferation. In the present study, we are testing the potential of chimeric IL-15 individually and in combination with native hIL-12 as an activator of CD4+ T cells, CD8+ T cells and NK cells. The combination of both cytokines shows potent release of IFN-g as well as activation of the T-cell subsets and NK cells. In our future studies we will use these pre-activated cells for adoptive transfer in cancer immunotherapy. Detailed findings will be reported during the conference.

P1-017 Potential of Chimeric IL-15 in Treatment and Prevention of Cancer

Patel D[1], Sharma P[1], Dalai S[1]
[1]Nirma University

Effective therapeutic and preventive strategies are essential for battling infectious diseases and cancer. Adjuvants are used in treatment and prevention because they play a vital role in triggering the immune response. Cytokines, in particular Interleukin-15, is effective candidate for adjuvant usage in cancer immunotherapy. IL-15, a pleiotropic cytokine, is widely used for immunotherapy as it enhances the differentiation and functional activity of T, B and NK cells that participate in anti-tumor immunity. IL-15 also been identified by the NCI (NIH, USA) as a top candidate, for cancer immunotherapy. Moreover, IL-15 was known to generate memory T cells as well as reactivate the memory T cells during the antigenic challenge. Thus, IL-15 could be used as an effective adjuvant to help augment the generation of memory T cells by vaccines. However, the limitation of the therapeutic use of IL-15 is its short half-life (~1hr) and lower bioavailability. To overcome these limitations, we have made a stable chimeric IL-15 whose half-life is >40 hr (Indian Patent:201721010096A). We carried out research involving both therapeutic and preventative approaches using this chimeric IL-15. We used the chimeric IL-15 protein to induce an anti-tumor immune response in a mouse model of melanoma for therapeutic purposes. The administration of chimeric IL-15 has resulted in tumor regression and activation of cytotoxic CD8+T cells. We used Plasmid-OVA and our chimeric IL-15-plasmid DNA construct for preventive purposes. The mice were immunized twice with pOVA-Ag, with or without plasmid chimeric IL-15, and subsequently challenged with the B16F10-OVA cell line. The presence of chimeric IL-15 demonstrated delayed tumor growth and development of effector and memory CD8+T cells. We concluded that our chimeric IL-15 may have great adjuvant potential for cancer immunotherapy based on the outcomes of both therapeutic and preventative approaches. The details of the findings will be presented at the conference.

P1-018 A resource for the development of immunological reagents for biomarker identification in New World Monkeys.

Callery J[1], Lambrou E[1], Turbow B[1], Hodara V[2], King J[1], Simmons J[3], Ross C[2,4], Makower B[5], Ahlborg N[5], Giavedoni L[1]
[1]Trinity University-Department of Biology, [2]Texas Biomedical Research Institute, [3]The University of Texas MD Anderson Cancer Center, [4]Southwest National Primate Research Center, [5]Mabtech AB

Marmosets (MAR), squirrel monkeys, and owl monkeys are New World Monkeys (NWM) studied in a broad array of research areas. One critical shortcoming of the NWM animal models is the limited number of immunological reagents that are available due to more than 45 million years of evolutionary divergence with humans. This scarcity of research tools reduces the translational value of these nonhuman primates (NHPs). The goal of this NIH/ORIP-funded scientific resource is to remedy this deficiency by generating antibodies and validating immunological assays that identify important NWM biomarkers of inflammation and metabolism, for which there is currently lack of reagents. Inflammation is a common process involved in aging, obesity, and pathologies such as infectious diseases, multiple sclerosis, and age-related neurodegenerative disorders, all of which are being modeled in NWM. The biomarkers targeted include C-reactive protein (CRP), interleukin-4 (IL-4), IL-6, IL-10, interferon gamma-inducible protein 10 (CXCL10, IP-10), granzyme B, and the glycoprotein CD69. Gastrointestinal hormones of interest, critical for validation of studies of metabolic syndrome, include insulin, adiponectin, and leptin. The resource includes scientists from academic and research institutions and a biotechnology company specialized in the production of reagents for veterinary and human use. We produce MAR proteins in eukaryotic cells, generate monoclonal antibodies (mAbs) in mice, identify mAb epitope specificity, and establish optimal antibody pairs for techniques such as ELISA, ELISPOT, Luminex, and intracellular cytokine staining assays. Finally, these immunological assays are validated with samples obtained from plasma aliquots from NWM exposed to transient metabolic and inflammatory challenges, and from ex vivo stimulated cells. This resource provides scientists working in different NWM models of human disease access to research tools for improved validation of their preclinical models. A resource website disseminates the availability of reagents, tracks the impact of these reagents, and distributes protocols and SOPs.

P1-019 Blood monocyte-derived CD169+ macrophages contribute to antitumor immunity against glioblastoma

Kim H[1], Park J[1], Kim H[1], Kim C[1], Kang I[1], Lee H[1]
[1]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST)

Infiltrating tumor-associated macrophages (TAM) are known to impede immunotherapy against glioblastoma (GBM), however, TAMs are heterogeneous, and there are no clear markers to distinguish immunosuppressive and potentially immune-activating populations. Here we identify a subset of CD169+ macrophages promoting an anti-tumoral microenvironment in GBM. Using single-cell transcriptome analysis, we find that CD169+ macrophages in human and mouse gliomas produce pro-inflammatory chemokines, leading to the accumulation of T cells and NK cells. CD169 expression on macrophages facilitates phagocytosis of apoptotic glioma cells and hence tumor-specific T cell responses. Depletion of CD169+ macrophages leads to functionally impaired antitumor lymphocytes and poorer survival of glioma-bearing mice. We show that NK-cell-derived IFN-γ is critical for the accumulation of blood monocyte-derived CD169+ macrophages in gliomas. Our work thus identifies a well-distinguished TAM subset promoting antitumor immunity against GBM, and identifies key factors that might shift the balance from immunosuppressive to anti-tumor TAM.

P1-020 Induction of Long-Term Mucosal Immunity against SARS-CoV-2 by an Intranasal Adenovirus-Vector Vaccine Expressing a Modified Spike Protein and Genetic Adjuvant

Jung H[1], Ku K[1,2], Kang B[1], Park J[1], Kim H[1], Kim K[2], Lee H[1]
[1]Korea Advanced Institute of Science and Technology (KAIST), [2]Department of Convergent Research of Emerging Virus Infection, Korea Research Institute of Chemical Technology (KRICT)

The COVID-19 pandemic caused by SARS-CoV-2 has had a profound global impact, highlighting the urgent need for effective vaccines. Despite the development of several intramuscular COVID-19 vaccines, challenges persist concerning the longevity of vaccine-induced immunity and the lack of mucosal-specific immune responses. In this study, we engineered Ad5-S.Mod, an adenoviral-based vaccine expressing a modified spike antigen and the genetic adjuvant human CXCL9. Through intranasal administration, Ad5-S.Mod elicited robust humoral and T-cell responses in the lung, conferring protection against lethal SARS-CoV-2 infection. Our investigations revealed the crucial role of cDC1 cells in generating antigen-specific CD8+ T-cell responses and the establishment of tissue-resident memory CD8+ T cells. Furthermore, we confirmed the effectiveness of intranasal Ad5-S.Mod vaccine through transcriptional analysis and identified lung macrophages as key supporters in maintaining lung-resident memory T and B cells. Our study demonstrates the potential of Ad5-S.Mod in providing protective immunity against SARS-CoV-2 and highlights the supportive role of lung macrophages in sustaining vaccine-induced tissue-resident memory lymphocytes.

P1-021 Innate lymphoid cells regulate inflammation in acute fungal lung infection

Tsymala I[1], Penninger P[1], Nogueira F[2,3], Jenull S[4], Arzani H[1], Gotthardt D[5], Sexl V[5], Kuchler K[1]
[1]Department for Medical Biochemistry, Max Perutz Labs, Campus Vienna Biocenter, Medical University, [2]CCRI—St. Anna Children's Cancer Research Institute, [3]ABF Pharmaceutical Services GmbH, [4]Institute of Microbiology, Department of Pathobiology, University of Veterinary Medicine, [5]Institute of Pharmacology and Toxicology, Department of Biomedical Sciences, University of Veterinary Medicine

Innate Lymphoid Cells (ILCs) are part of the first-line immune defense protecting mucosal barriers from pathogens. ILCs support barrier integrity, but can also promote inflammation by contributing to tissue hyperreactivity and allergy. However, factors guiding their pro-inflammatory or protective functions in acute fungal infections are poorly understood. Thus, we explored how pulmonary ILCs respond to fungal pathogens in vitro and in the context of acute infections by two of the most clinically relevant fungal pathogens, Candida albicans and Aspergillus fumigatus.
By analyzing the secretory activity, downstream pathways and short-term plasticity of ex vivo isolated ILCs, we found a predominant role of a regulatory ILC subset. Consequently, we compared the activation and subset polarization of ILCs in mouse models of acute systemic and lung infection. Flow cytometry analysis revealed a pathogen-dependent plasticity of ILCs as reflected by elevated IL-4, IL-9 and concurrent IL-10 expression. As a result, adoptive transfer of ex vivo expanded ILC lineages into lymphocyte-deficient Rag2-/-γc-/- mice reduced inflammation and tissue damage at the cost of an increased fungal burden in acute lung infection.
Overall, our data indicates that pulmonary ILCs are essential for maintaining tissue homeostasis during fungal infection. Furthermore, our findings suggest that ILCs are early responders to fungal pathogens and are able to attenuate acute inflammation.

P1-022 Movement disorder dystonia caused by mutations in interferon induced protein kinase R (PKR) and its activator protein PACT.

Patel R[1], Vaughn L[1], Burnett S[1]
[1]University Of South Carolina

DYT-PRKRA (dystonia 16 or DYT-PRKRA) is caused by mutations in PRKRA gene that encodes PACT, the protein activator of interferon (IFN)-induced double-stranded (ds) RNA-activated protein kinase (PKR). Recently, PKR mutations have also been reported in early-onset dystonia in a worldwide occurrence. PACT participates in several cellular pathways, of which its role as a PKR activator protein during integrated stress response (ISR) is the best characterized. Previously, we have established that the DYT-PRKRA mutations cause enhanced activation of PKR during ISR to sensitize DYT-PRKRA cells to apoptosis. We also evaluated if the most prevalent substitution mutation reported in DYT-PRKRA patients alters PACT's functional role in induction of type I IFNs via the retinoic acid-inducible gene I (RIG-I) signaling. Our results indicated that the P222L mutation augments PACT's ability to induce IFN β in response to dsRNA and the basal expression of IFN β and IFN-stimulated genes (ISGs) is higher in DYT-PRKRA patient cells compared to cells from the unaffected controls. Additionally, IFN β and ISGs are also induced at higher levels in DYT-PRKRA cells in response to dsRNA. In a mouse model of DYT-PRKRA that contains a frameshift mutation and produces a truncated PACT protein in brain, IFN β is produced at higher levels thus indicating a possible connection between IFN overproduction and dystonia. Additionally, several PKR mutations are reported in childhood-onset dystonia patients that developed dystonia after a febrile illness. These results offer a new avenue for investigations directed towards understanding the underlying molecular pathomechanisms in DYT-PRKRA as well as PKR in early onset dystonia.

P1-023 Stefin B increased IL-10 expression , downregulated caspase 11 expression and inflammasome activation in macrophages

Trstenjak-Prebanda M[1], Biasizzo M[1], Dolinar K[2], Pirkmajer S[2], Turk B[1,3], Kopitar-Jerala N[1]
[1]Jozef Stefan Institute, [2]Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, [3]Faculty of Chemistry and Chemical Technology, University of Ljubljana

Stefin B (cystatin B) is an inhibitor of nuclear and lysosomal cysteine cathepsins. The gene encoding stefin B is found on human chromosome 21 and loss-of-function mutations in the gene encoding stefin B are associated with a neurodegenerative condition called Unverricht-Lundborg disease (EPM1), which is characterised by progressive myoclonus epilepsy and ataxia. We previously reported that stefin B-deficient mice are significantly more sensitive to lethal lipopolysaccharide (LPS)-induced sepsis and have increased NLRP3 inflammasome activation. Here, we found lower caspase-11 gene expression higher IL-10 expression in bone marrow-derived macrophages of stefin B trisomic mice — mice with an additional copy of stefin B gene. Stefin B trisomy prevented mitochondrial reactive oxygen species (ROS) formation and impaired the NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in macrophages. Additionally, increased AMP-activated kinase activation and suppressed mTOR activity were observed in stefin B trisomic macrophages and cells with increased stefin B expression. Our study demonstrated that increased stefin B expression induced autophagy and downregulated mitochondrial ROS generation. Our findings reveal the basis of the anti-inflammatory properties of stefin B and highlight the role of stefin B in the interplay between inflammasome activation and autophagy in macrophages.

P1-024 ELUCIDATING THE ANTI-INFLAMMATORY ROLE OF SCFAs IN JEV MEDIATED NEUROINFLAMMATION IN MICE

Siva Venkatesh I[1]
[1]National Brain Research Centre

Background-Short chain fatty acids (SCFAs), majorly, acetate, propionate and butyrate are metabolites generated by anaerobic fermentation of complex carbohydrates by the bacteria in the gastrointestinal tract. Japanese Encephalitis Virus (JEV), a neurotropic flavivirus, causes severe neuro-inflammation and neuronal death leading to various neurological symptoms and eventually death. Emerging evidence suggests anti-inflammatory nature of SCFAs in multiple neurodegenerative disorders. Whereas there is a lacunae of evidence suggesting if dietary SCFAs can be used to ameliorate viral infection. In this study we aim to elucidate the possible role of SCFAs in JEV induced neuro-inflammation.
Methods-Post-natal day 10 BALB/c mice were randomly assigned to groups. Each group was intraperitoneally injected either with SCFA mixture (Acetate:propionate:butyrate = 35mM:15mM:10mM) or PBS for a period of 7 days, followed by JEV infection. All mice were observed for onset and progression of symptoms. Upon reaching terminal illness, mice were sacrificed by transcardial perfusion and the brain tissue was collected for further analysis.
Reults-SCFA treated JEV infected mice (SCFA+JEV) showed delayed onset of symptoms, lower General Locomotory Score and decreases weight loss upon infection as opposed to the PBS treated JEV infected animal (JEV), thereby increasing the survival by 3 days. Significant downregulation of inflammatory cytokines TNF-α, MCP-1, IL-6, IFN-Y in SCFA+JEV group relative to JEV infected control group was observed. Tissue section analysis exhibited reduced glial activation in SCFA treated animals with respect to the JEV animals as seen from the astrocytic and microglial morphometric analysis. TUNEL assay showed reduced incorporation of fluorescently tagged deoxy uridine nucleotide in SCFA+JEV conditions with respect to JEV conditions. Nissl staining showed higher incidents of chromatolysis in JEV condition as compared to SCFA+JEV condition.
Conclusion-Through our study, we establish that circulating SCFA can possible be used as a supplementary intervention in attenuating JEV mediated neuro-inflammation.

P1-025 Vitamin D metabolism, inflammation and colorectal cancer progression

Hamza Z[1], Feki S[1], Abida O[1], Fakhfakh R[1], Hachicha H[1], Ben amar M[2], Masmoudi H[1]
[1]Immunology Departement, Habib bourguiba HU, [2]Surgery Department; Habib Bourguiba UH

Background
The active metabolite of vitamin D (VitD) seems to inhibit proliferation and promote differentiation of colorectal cancer (CRC) cells which express vitamin D receptor (VDR). The VitD metabolism seems also to regulate inflammatory processes involved in CRC development and progression, including CD4+ T cell differentiation and cytokine production.
In this study, we investigated in Tunisian CRC patients in comparison with controls the expression of VitD-related genes and Th17-related parameters.
Methods
This is a case-control study including 43 untreated CRC patients and 43 healthy controls. The expression levels of VDR, CYP27B1 (encoding 1α-hydroxylase) and IL23R genes in PBMC were studied using a qPCR. The measurement of circulating levels of VitD (25(OH)D) and IL17A was performed using ELISA kits.
Results
The mean level of 25(OH)D was significantly lower in CRC patients than in controls(p=0.001). A significant association was observed between VitD deficiency (25(OH)D <20 ng/mL) and risk of CRC ([OR]=4.86; IC95%=1.8 et 13.11; p=0.001). In contrary, the mean levels of VDR and CYP27B1 gene expression were significantly higher in patients than in controls (p<0.001; p=0.003, respectively). Regarding Th17 axis, the mean level of IL17A was significantly higher in patients than in controls (p=0.004). The mean level of IL23R gene expression was higher in patients than in controls. The levels of IL17A were inversely correlated with 25(OH)D levels (r=-0.33; p=0.033) and positively correlated with VDR and CYP27B1 gene expression (r=0.33; p=0.035; r=0.39; p=0.012, respectively).
Conclusion
The vitD, by binding to its receptor VDR, seems to regulate the expression of a high number of genes involved in CRC cell proliferation as well as T helper cell differentiation. Further clinical studies are required to confirm the close interplay between vitD, anti-tumor immunity and CRC.

P1-026 Conserved transcriptomic signatures and protein markers in cellular senescence models

Tsolakos N[1], Fotis C[1], Tsolakos N[2], Pliaka V[2], Verrou K[3], Alexopoulos L[1]
[1]National Technical University of Athens, [2]Protavio Ltd, [3]Center of New Biotechnologies & Precision Medicine, Medical School, National and Kapodistrian University of Athens

Cellular senescence is described as an irreversible cell cycle arrest induced in response to various stresses. Senescent cells are characterised by heterogeneous signalling alterations, complex secretory phenotype, known as senescence-associated secretory phenotype (SASP), and diverse transcriptomic profile.
With the aim to investigate senescence heterogeneity and identify conserved transctiptomic alterations and senescence markers, we performed RNA-seq and multiplex proteomic analysis in proteasome inhibition-induced and stress-induced premature senescence models of HFL1 and BJ human fibroblasts.
Our data revealed diverse transcriptomic signatures, but also, 231 common differentially expressed genes related to cell division and ECM remodelling, and enriched pathways that remained conserved among the different models with senescence onset. Moreover, we identified a subset of conserved protein senescence markers and validated them in replicative senescent models. These proteins are involved in cell cycle arrest and promote a pro-inflammatory environment in premature and replicative senescence models.
We suggest that the simultaneous analysis of p21, p-c-JUN, BCL-xL and survivin in cellular lysates, and IL-8, GM-CSF, GDF-15 and GROa in culture supernatants can provide a powerful tool for the identification and monitoring of senescent cells and can support the assessment of the efficacy of potential senotherapeutic approaches.

P1-027 NRF2-independent inhibition of human coronaviruses by “NRF2 activators”

Waqas F[1], Zapatero F[2], Elgaher W[3], Lacour A[3], KURMASHEVA N[4], Dahlmann J[5], Carriqui B[2], Gerold G[2], Olagnier D[4], Olmer R[5], Pessler F[1]
[1]Biomarkers for Infectious Diseases, TWINCORE GmbH, [2]Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, [3]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), [4]Department of Biomedicine, Aarhus University, [5]Research Center for Translational and Regenerative Medicine, Hannover Medical School

Background. The KEAP1/NRF2 signaling pathway contributes to antiviral defenses of eukaryotic cells. Consequently, small molecules that activate NRF2-signaling constitute promising host-directed antivirals. We have previously found that NRF2-activators 4-octyl itaconate, bardoxolone, and sulforaphane reduced release of IAV progeny from transformed and primary human cells, but this effect was independent of NRF2 and was, rather, due to interference of the compounds with XPO1-mediated nuclear export of viral ribonucleoprotein.
Results. To assess the potential of these compounds as broadly active antivirals, we have now tested their efficacy against human coronaviruses. All three compounds markedly reduced viral copies of SARS-CoV-2 in supernatants and lysates of Calu3 cells and luminescence of a luc-labeled HCoV-229E strain in human-iPSC-derived vascular endothelial cells (EC). CRISPR/Cas9 mediated NRF2-KO in ECs led to increased 229E infectivity and replication, thus confirming an innate antiviral function of NRF2-signaling. However, the antiviral effects of the compounds were essentially unimpeded in NRF2-KO cells. We then evaluated XPO1 as an alternate target. Indeed, siRNA-mediated knock-down of XPO1 in A549 cells and treatment of wild-type cells with the selective XPO1 inhibitor selinexor reduced 229E infectivity to a similar extent. Treating XPO1 knock-down cells with 4-octyl itaconate or sulforaphane lead to a minor further reduction of 229E infectivity. In contrast, bardoxolone abrogated 229E infectivity independent of XPO1 expression. Pull-down assays with an alkynated 4-octyl itaconate click-chemistry probe revealed specific binding of 4-octyl itaconate to XPO1. Moreover, ligand-target modelling predicted binding of 4-octyl itaconate to the nuclear-export-signal binding site with similar binding energy score as selinexor.
Conclusion. These results suggest that (i)“NRF2-activators” can inhibit infectivity of human coronaviruses by interfering with XPO1 function and (ii)BARD blocks HCoV-229E replication via a third, as yet unknown, target. Taken together, the results underscore that these compounds merit further evaluation as scaffolds for the development of broadly active host-directed antivirals.

P1-028 Contribution of CCL17 as an inflammatory mediator in rheumatoid arthritis

Eivazi M[1]
[1]University of Melbourne

Rheumatoid arthritis (RA) is an inflammatory, progressive, and destructive autoimmune disease. During RA, intra-articular as well as extra-articular manifestations result in morbidity and mortality. Currently available RA therapies are costly and accompanied by significant adverse side effects, as well as limited effectiveness in some patients, highlighting the need for new therapies. Preclinical studies in my laboratory identified chemokine CCL17 as a potential target for treating RA. During my PhD, I screened a panel of FDA-approved drugs and identified six candidate drugs that inhibit CCL17 expression so that they can be repurposed. The identified CCL17-inhibiting drugs were tested in a preclinical model of arthritis and their efficacy in ameliorating arthritic pain and disease were evaluated. In the second part of my PhD, I developed a method to identify CCL17-producing and -responding cells in the RA synovium, utilising multiplex immunohistochemistry (OPAL). The optimised method enables selection of RA patients with high expression of CCL17, while identified CCL17-inhibiting drugs accelerates potentially repurposing the drugs in future clinical trials.

P1-029 SLC5A3 regulates gemcitabine resistance by modulating cytokine-mediated signaling pathway in pancreatic cancer

Kim M[2], Kang H[2], KIM H[1], Park J[1]
[1]Department of Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine,
[2]Department of Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine; Brain Korea 21 PLUS Project for Medical Science, Yonsei University

Pancreatic cancer is the most aggressive cancer characterized with high mortality and poor prognosis rates worldwide. Gemcitabine is considered a standard treatment for pancreatic cancer, but developing drug resistance greatly limits the effectiveness of chemotherapy and increases the rate of recurrence. Therefore, new therapeutic targets are urgently needed to improve pancreatic cancer treatment. SLC5A3 (sodium/myo-inositol co-transporter) is highly expressed in pancreatic cancer and regulates EMT. However, studies on the role of SLC5A3 in gemcitabine resistance are limited. Here, we investigated the mechanism of SLC5A3 induction and the effect of SLC5A3 on apoptosis in gemcitabine-resistant pancreatic cancer. Here, we found SLC5A3, a glutamine transporter, is highly overexpressed in gemcitabine-resistant than sensitive patients and cell lines in pancreatic cancer. Furthermore, deletion of SLC5A3 decreased the ability of cell proliferation and increased apoptosis in gemcitabine-resistant pancreatic cancer cell lines. We also found inhibition of SLC5A3 regulates the JAK-STAT3 signaling pathway through RNA sequencing data. In addition, inhibition of SLC5A3 resulted in reduced cytokine genes (IL-6, TNF-α and CXCL2) expression via NF-κB inactivation, indicating that SLC5A3 is a potential therapeutic target related to cytokine-mediated signaling pathway in gemcitabine resistance. Therefore, our study provides insight into the role of SLC5A3 in gemcitabine resistant pancreatic cancer and suggests that targeting SLC5A3 may be a potential strategy for overcoming gemcitabine resistance.

P1-030 Serum CXCL10 and GDF15 levels offer valuable information for diagnosis and subtyping of autoimmune myositis

De Paepe B[1], Bracke K[2], De Bleecker J[1]
[1]Ghent University Hospital, Neuromuscular Reference Center, [2]Ghent University Department of Pneumology

Autoimmune myositis is further classified into distinct disorders including dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (IBM), immune-mediated necrotizing myopathy (IMNM), and the anti-synthetase syndrome (ASS). Definite diagnosis often requires a combination of clinical, pathological and serological analyses, prolonging the diagnostic delay. The need persists for novel blood-based biomarkers and quantifying cytokines with key pathologic roles represents an attractive prospect.
This retrospective study focused on C-X-C motif chemokine ligand 10 (CXCL10) and stress-related growth differentiation factor 15 (GDF15). Sera and muscle biopsies were selected from fully characterized IMNM (n=21), IBM (n=18), PM (n=3), DM (n=2) and ASS (n=1), from healthy individuals (n=10), and patients with a hereditary neuromuscular disorder (n=14). Enzyme-linked immunosorbent assays were performed and reported as mean±sd of duplicates of two dilutions. Myopathological changes were investigated using standard histology stains and immunohistochemical stains with antibodies directed against immune cell markers, CXCL10 and GDF15.
In myositis, serum CXCL10 levels significantly increased 10-fold compared to healthy controls and 4-fold compared to hereditary neuromuscular disorders. With the threshold set to 180pg/ml, myositis patients could be differentiated from healthy and disease controls with 0.80 sensitivity and 0.71 specificity. Mean CXCL10 levels were significantly higher in IBM (929±658 pg/ml) compared to IMNM (425±324 pg/ml). Incorporating a threshold of 300pg/ml serum GDF15 reduced false negatives to two IMNM patients only. In myositis biopsies, subsets of infiltrating immune cells were CXCL10 positive, and circulating levels increased with inflammation grade. GDF15 localized mostly to regenerating and vacuolated muscle fibers.
We propose adding CXCL10 quantification to the diagnostic toolkit for myositis could represent a valuable patient-friendly approach. Combined with GDF15 and established diagnostic testing of blood creatine kinase levels and autoantibody profiling, the need for taking a diagnostic muscle biopsy might be further reduced, and therapeutic responses and disease prognosis might be better predicted.

P1-031 IL-6 Dependent Expansion of Inflammatory MDSCs (CD11b+ Gr-1+) Promote Th-17 Mediated Immune Response During Experimental Cerebral Malaria

Bhattacharyya A[1]
[1]University of Calcutta

Background:Myeloid derived suppressor cells (MDSCs) are a group of heterogeneous cell population which are able to suppress T cell responses. Various aspects of MDSCs in regulating immune responses in several cancer and infectious diseases have been reported till date. This study depicts the phenotypic and functional characteristics of splenic MDSCs and how they regulate Th-17 mediated immune response during Experimental Cerebral Malaria (ECM).
Methods:Flow cytometry, Histology, Immunohistochemistry
Results:Flow cytometric analysis reveals that MDSCs in spleen and bone marrow expands at 8 dpi during ECM. Among subtypes of MDSCs, PMN-MDSCs show significant expansion in spleen but M-MDSCs remain unaltered. Functional analysis of sorted MDSCs from spleens of Plasmodium berghei ANKA (PbA) infected mice show suppressive nature of these cells and high production of Nitric oxide (NO). Besides, MDSCs were also found to express various inflammatory markers during ECM suggesting M1 type phenotype of these cells. Invivo depletion of MDSCs by the use of Anti Gr-1 increases mice survival but doesn't significantly alter the parasitemia. Previously, it has been reported that Treg/Th-17 balance in spleen is skewed towards Th-17 during ECM. Depletion of MDSCs was found to regulate Th-17 percentages to homeostatic levels and subvert various inflammatory changes in spleen.
Conclusion:Among different factors, IL-6 was found to play an important role in expansion of MDSCs and expression of inflammatory markers on MDSCs in a STAT3 dependent manner. These findings provide a unique insight into the role of IL-6 in expansion of MDSC population which causes inflammatory changes and increased Th-17 responses during ECM.

P1-032 Correlation of IL-6 with other parameters of inflammation and clinical data in patients infected with SARS-Cov-2 virus

Jurisic V[1], Sarić A[1], Obradovic J[2], Djokic Lišanin M[3], Stanijevic Pirkovic M[1]
[1]Faculty of Medical Sciences, University of Kragujevac,, [2]Institute for Information Technologies University of Kragujevac, [3]University Clinical Center Kragujevac

Backgound:
IL-6 is a pro-inflammatory cytokine that plays a role in many immune reactions, is the main initiator of the cytokine storm, and is a negative prognostic marker in inflammation. In this paper, serum IL-6 and other pro-inflammatory parameters including C reactive protein (CRP), procalcitonin (PCT) were analyzed in patients infected with the SARS-Cov-2 virus. The study was conducted in 134 persons affected by Covid-19 in the adult population, including both sexes.
Methods: CRP, PCT, IL-6 were analyzed in serum using standard kits on a UniCel DCX-800 biochemical analyzer (Beckman Coulter) for clinical diagnostics. Hematological and other biochemical parameters were determined by routine analysis. All patients had a confirmed diagnosis of viral infection using a rapid throat and nose swab test or a positive result using the PCR test for SARS-Cov2 virus, which were a condition for hospitalization.
Results:
The results showed that statistically significant correlation was found between CRP and IL-6 variables ($p<0.001$; Spearman's rho correlation coefficient was 0.531). Also, statistically significant results were found for PCT and IL-6 ($p<0.001$; Spearman's rho correlation coefficient was 0.285). In addition, IL-6 was correlated with blood count values, neutrophil lymphocyte ratio, D dimer, clinical data as well as respiratory parameters that included peripheral oxygen saturation (SpO2), partial pressure of oxygen in arterial blood (PaO2) and hemoglobine level. IL-6 also showed significant differences in treatment outcomes over time.
Conclusion: Based on the obtained results, it was shown that the determination of IL-6 can be useful for monitoring the outcome of patients in real clinical conditions. We recommend measuring this cytokine in other infections as well.

P1-033 Application of Lactobacillus reuteri B1/1 (Limosilactobacillus reuteri) and Lactobacillus fermentum 2i3 CCM 7158 modulates cytokine gene expression in a 3D porcine intestinal model

Karaffová V[1], Štofilová J[2], Kiššová Z[3], Mudroňová D[3], Hudec E[1]
[1]Department of morphological disciplines, University Of Veterinary Medicine And Pharmacy In Košice, [2]MEDIPARK Clinical and Preclinical Research Center, Faculty of Medicine, P.J. Šafárik University in Košice, [3]Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice

Background: In recent years, there has been a growing interest in the use of lactobacillus isolates in the form of probiotics in the veterinary field as well. This is facilitated by the extensive breeding of farm animals as well as consumer demands for the quality of animal products. However, the development of each new probiotic preparation is preceded by a phase of microbiological testing of the selected probiotic strain as well as in vitro and in vivo conditions. Therefore the aim of this study was to observe the influence of L. reuteri B1/1 (LR) isolated from pheasant intestine and chicken isolate L. fermentum 2i3 (LF) on the gene expression of selected pro-inflammatory (IL-1β, IL-6, IL-8, IL-18) and anti-inflammatory (IL-4, IL-10) interleukins on porcine 3D gut model after their stimulation with lipopolysaccharide (LPS) obtained from E. coli serotype 055:B5 in a concentration of 10 µg/ml.
Methods: The effect of the lactobacilli on the 3D intestinal model was analysed by measuring TEER with the STX4 electrode using the EVOM2 device. After 24 h, total RNA was isolated from the cells using the RNEasy mini kit. Isolated RNA was transcribed into cDNA using the iScript cDNA Synthesis Kit and oligo DT-primers. The relative gene expression of cytokines was evaluated after 24 hours of incubation using the quantitative Real-Time PCR method on a LightCycler 480 II Instrument according to a predefined temperature program. Obtained Cq values of genes were normalized to the average Cq value of reference gene (glyceraldehyde-3-phosphate dehydrogenase).
Results: Our results revealed that both lactobacilli isolates significantly suppressed the gene expression of pro-inflammatory interleukins in the combined groups (LR+LPS; LF+LPS) compared to the infected groups ($P < 0.05$, $P < 0.01$, $P < 0.001$).
Conclusion: Both probiotic strains showed an anti-inflammatory effect during LPS infection.

P1-034 Suppressing osteoclastogenesis by anti-CD64 inhibitor and PAD2 inhibitor in rheumatoid arthritis

Min H[1]
[1]Konkuk University Medical Center

Objective : Osteoclastogenesis is main pathologic process of joint destruction in rheumatoid arthritis (RA). Anti-citrullinated protein antibody (ACPA) is specific autoantibody of RA, and known to augment osteoclastogenesis. In present study, we evaluated role of anti-CD32, anti-CD64 (receptor for ACPA), and PAD2/PAD4 (ACPA producing enzyme) inhibitor on ACPA induced osteoclastogenesis of RA.

Method: Peripheral blood mononuclear cells (PBMCs) was collected from RA patient, and stimulated with ACPA-stimulated RA patients driven fibroblast like synoviocyte (RA-FLS). These were cultured for 14 days, then TRAP stain was used to quantify osteoclast formation. We also stimulated RA-FLS with ACPA (100 ng/mL) with or without anti CD32 Ab, anti CD64 Ab, PAD2 inhibitor, and PAD4 inhibitor. Flow cytometry and ELISA was performed.

Results: The RANKL+ FLS decreased dose-dependently in 5 and 10 ug/mL anti-CD64 inhibitor added group, whereas addition of anti-CD32 inhibitor did not suppressed RANKL+ FLS counts. In PAD inhibitor experiments, the proportion of RANKL+ FLS decreased in 200 and 500 nM PAD2 inhibitor added condition, whereas PAD4 inhibitor did not decreased RNAKL+ FLS. The level of IL-6 and IL-1b in culture media was decreased by anti-CD64 inhibitor and PAD2 inhibitor added condition. Finally, TRAP+ multinucleated osteoclast count was decreased in anti-CD64 inhibitor and PAD2 inhibitor added condition of monocyte and ACPA stimulated RA-FLS coculture experiment.

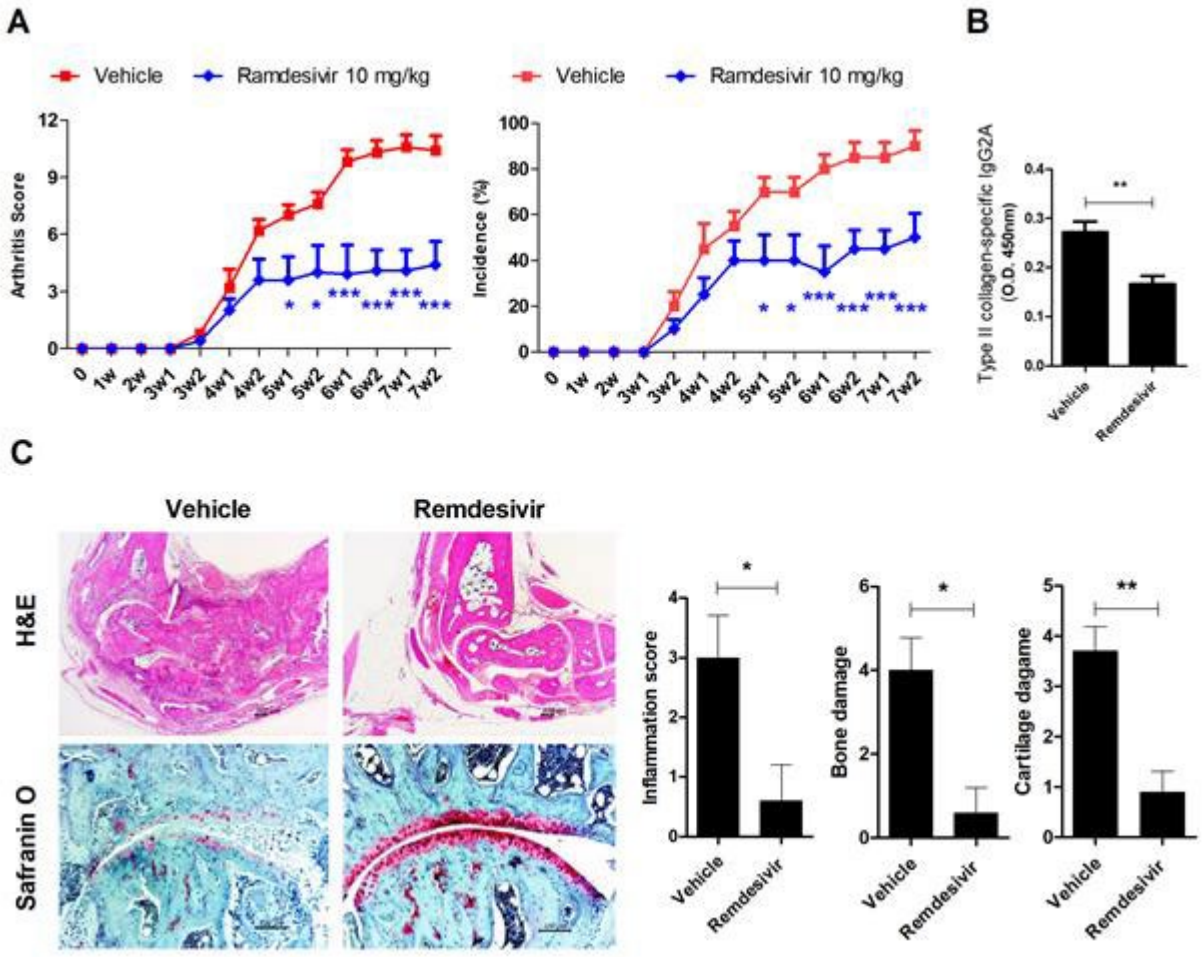
Conclusions: The present study showed RANKL and pro-inflammatory cytokines expression in RA-FLS by anti-CD64 inhibitors and PAD2 inhibitors. Also, anti-CD64 inhibitors and PAD2 inhibitors reduced osteoclastogenesis of RA. These implies that CD64 mediated signal and PAD2 induced pathway may have certain role on ACPA induced osteoclastogenesis in RA, and regulating CD64 and PAD2 pathway may have beneficial role on osteoclastogenesis of RA.

2023 cytokine figures.pptx (could not be inserted)

P1-035 Remdesivir alleviates joint damage in collagen-induced arthritis and inhibits inflammatory cell death of rheumatoid arthritis synovial fibroblasts

Min H[1]
[1]Konkuk University Medical Center

The antiviral agent, remdesivir, is adenosine analogue which is currently also used as anti-coronavirus disease 2019. Remdesivir also had anti-inflammatory effect which reduced pro-inflammatory cytokine production, and inhibition of the cyclic GMP-AMP synthase–STING pathway. We evaluated the antiarthritic effects of remdesivir in a mouse model of High-fat diet (HFD) collagen-induced arthritis (CIA) and in fibroblast-like synoviocytes from patients with RA.Type II collagen was administered to DBA/1J mice to induce CIA. Vehicle or remdesivir was injected subcutaneously three times a week. During 7 weeks of treatment, the arthritis score and incidence were evaluated twice a week. Flow cytometry and confocal imaging were used to evaluate CD4 + T cells in the spleen. FLSs from patients with RA were stimulated in vitro with remdesivir and tumor necrosis factor (TNF)-α, and western blotting was used to measure the expression of STING and necroptosis-related markers such as receptor-interacting serine/threonine-protein kinase (RIP) K3 and mixed lineage kinase domain-like protein (MLKL). Remdesivir administration suppressed the incidence and progression of arthritis in mice with CIA. Histological analysis revealed lower inflammation and cartilage damage scores in remdesivir-treated than in vehicle groups. Interleukin (IL)-17 + CD4 + T-cell differentiation was inhibited in the remdesivir-treated group. Furthermore, IL-17/-6/-1β, monocyte chemoattractant protein -1, and TNF-α expression was reduced in the remdesivir group. In vitro, remdesivir suppressed the expression of STING, nuclear factor-κB, RIPK3, and phosphorylated MLKL in RA–FLSs under TNF-α stimulation. The antiviral agent remdesivir suppressed arthritis by regulating Th cell differentiation, pro-inflammatory cytokine expression, the STING pathway, and necroptosis.


A
Vehicle
Ramdesivir 10 mg/kg
Arthritis Score
Incidence (%)
0 1w 2w 3w1 3w2 4w1 4w2 5w1 5w2 6w1 6w2 7w1 7w2
B
Type II collagen-specific IgG2A (O.D. 450nm)
Vehicle
Remdesivir
C
Vehicle
Remdesivir
H&E
Safranin O
Inflammation score
Bone damage
Cartilage dagame

P1-036 Progenitor and differentiated macrophages exhibit distinct responses to STING activation

Van Dis E[1]
[1]University of Washington

Type I diabetes (T1D) is an autoimmune disease caused by T cell destruction of insulin-producing β cells in the pancreas. Daily insulin injections remain the only treatment option, and the underlying innate immune trigger of autoreactivity remains unknown. MDA5, an innate immune sensor, detects cytosolic dsRNA and signals through MAVS to drive type I IFN production and an antiviral response. This upregulates the kinase PKR which initiates the integrated stress response (ISR), inhibits protein translation, and can lead to cell death. MDA5 can be aberrantly activated by host nucleic acids causing autoimmune activation, and hyperactivate human MDA5 alleles are associated with the development of T1D. We hypothesize that T1D pathogenesis begins with MDA5 activation by a self-RNA ligand followed by PKR-dependent activation of the ISR and pancreas immunopathology. To test this, we have ablated PKR and genes along the MDA5 signaling pathway in the NOD mouse model of T1D, and have tested whether ISR inhibition prevents disease onset in NOD mice. These studies help define the connections between nucleic acid regulation, innate immune signaling, and β cell destruction and, by defining the underlying cause of β cell autoimmunity, will open the door to new and innovative therapies for T1D.

P1-037 Regulation of Inflammatory Cell Death by Caspase-10 Impacts the Pathogenesis of Primary Biliary Cholangitis

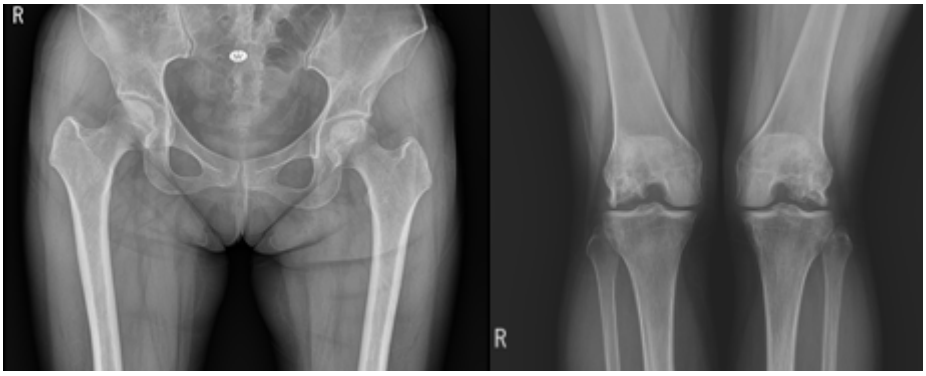
Dho S[1], Cho M[2], Shin S[1], Lee Y[2], Kim Y[1], Lee J[2], Yu S[3], Woo W[2], Park S[4], Lee K[1], Kim L[1]
[1]Yonsei University College Of Medicine, [2]BK21 PLUS Project for Medical Science, Yonsei University College of Medicine, [3]Seoul National University College of Medicine, [4]Hallym University College of Medicine

Background: Primary biliary cholangitis (PBC) is a chronic autoimmune disease that causes inflammation and injury to the biliary epithelial cells.
Methods: To identify critical genetic factors in PBC patients, we performed whole-exome sequencing of five female siblings from a multi-PBC family. The siblings included one unaffected sister and four affected sisters.
Results: We identified 61 rare heterozygote variants that segregated only within the affected sisters. Among them, we were particularly interested in caspase-10. Although several caspases are involved in cell death, inflammation and autoimmunity, caspase-10 is little known from this perspective. We generated caspase-10 knockout macrophages and compared the obtained phenotypes with those of its structurally similar protein, caspase-8. Unlike caspase-8, caspase-10 does not play a role during differentiation into macrophages. However, after differentiation, it regulates the process of inflammatory cell deaths such as necroptosis and pyroptosis more strongly. Interestingly, Caspase-10 displays higher protease activity than Caspase-8 in the process of RIPK1 cleavage, as well as an enhanced ability to form a complex with RIPK1 and FADD in human macrophages. Inflammatory cell death had a greater effect on the fibrotic response of hepatic stellate cells, but this effect could be mitigated by treatment with UDCA and OCA, which are currently approved for PBC patients.
Conclusion: Our findings suggest that defective Caspase-10 activity in macrophages contributes to the pathogenesis of PBC, and therefore may represent a promising target for future therapeutic interventions.

P1-038 Avascular necrosis of bone in a patient with systemic lupus erythematosus without corticosteroid use: A case report

Noh J[1], Han S[1]
[1]Department of Internal Medicine, Hankook Hospital

Background: Avascular necrosis of bone [AVN] can cause significant disability and limitation of mobility in systemic lupus erythematosus [SLE] patients. Risk factors of AVN include a longer disease duration, high LDL-C, positive aCL IgG and anti-dsDNA, cushingoid body habitus, and the use of corticosteroid. In the absence of steroid use, AVN is extremely rare. Herein, we present an experience of AVN in an SLE patient without use of corticosteroid.
Methods: The patient was a 39-year old female, diagnosed at 17 with SLE. She had taken medicines irregularly but her disease status had been relatively stable. She visited our hospital with left hip joint pain, taking hydroxychlorquine 200mg/day, losartan 50mg/day, aspirin 100mcg/day and NSAID from another hospital. She denied a history of steroid use. On laboratory testing, ANA was 1:320, anti-ds-DNA Ab, anti-Ro/La Ab, and anti-Smith Ab were negative. CBC, liver, and kidney function test were in normal range. LDL-C was 79 mg/dL, ESR was 36 mm/hr, CRP was 0.79 mg/dL. Anti-phospohlipid antibodies were positive (aCL IgG 79.0 GPL, anti-β2 glycoprotein I IgG 142.0 G units, confirmative lupus anticoagulant 1.42). X-ray of hip joint demonstrated marginal irregularity and sclerotic change with central lucency in the head of left femur. We started conservative management of joint pain. After 10 months, she newly complained of bilateral knee pain. X-ray of knee joint demonstrated joint space narrowing and severe bony sclerotic changes in both lateral condyles of femur.
Results: The risk factors for AVN in SLE have been reported by several studies. There is a strong causal relationship between corticosteroid intake and AVN development in SLE patients. However, in this case, the patient had never taken corticosteroid since diagnosis of SLE.
Conclusions: The pathophysiology of AVN is not clear yet, however SLE itself should be considered an important risk factor of AVN.



P1-039 Varicella zoster virus increases lymphocyte adhesion and virus spread through manipulation of type II interferon

Jürgens C[1], Ssebyatika G[1,2], Beyer S[1], Plückebaum N[1], Kropp K[1], González-Motos V[1,3], Ritter B[1], Böning H[1], Nikolouli E[4,5,6,7], Kinchington P[8], Lachmann N[4,5,6,7], Depledge D[1,7,9], Krey T[1,2,7,10,11], Viejo-Borbolla A[1]
[1]Institute of Virology, Hannover Medical School, [2]Institute of Biochemistry, University of Lübeck, [3]University of Veterinary Medicine Hannover, Foundation, [4]Department for Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School , [5]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), [6]Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), German Center for Lung Research (DZL), [7]Excellence Cluster 2155 RESIST, Hannover Medical School, [8]Department of Ophthalmology and of Molecular Microbiology and Genetics, University of Pittsburgh, [9]German Center for Infection Research (DZIF), partner site Hannover-Braunschweig, [10]Centre for Structural Systems Biology (CSSB) , [11]German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems

During primary infection, varicella zoster virus (VZV) infects epithelial cells in the respiratory lymphoid organs and mucosa. Subsequent infection of lymphocytes, T cells in particular, causes primary viremia allowing systemic spread throughout the host, including the skin. This results in the expression of cytokines, including interferons (IFNs) which partly limit primary infection. VZV also spreads from skin keratinocytes to lymphocytes prior to secondary viremia. How VZV infects lymphocytes from epithelial cells and evades their antiviral activity has not been fully established. Here, we show that VZV glycoprotein C (gC) binds IFN γ and modifies its activity. Transcriptomic analysis revealed that gC increased the expression of a small subset of IFN-stimulated genes (ISGs), including intercellular adhesion molecule 1 (ICAM1), as well as several chemokines and immunomodulatory genes. The higher ICAM1 protein level at the plasma membrane of epithelial cells resulted in lymphocyte function-associated antigen 1 (LFA-1)-dependent T cell adhesion. This gC activity required a stable interaction with IFN-γ and signalling through the IFN-γ receptor. Finally, the presence of gC during infection facilitated VZV spread from epithelial cells to peripheral blood mononuclear cells. This constitutes the discovery of a novel strategy to modulate the activity of IFN-γ, inducing the expression of a subset of ISGs, leading to enhanced T cell adhesion and virus spread.

P1-040 B-cell ablation Therapy Improves Lung Function Decline in Patients with Idiopathic Inflammatory Myopathy-associated Interstitial Lung Disease

Kim H[2], Hur J[1], Jang Y[2], Yoon H[2], KIM S[3], Hong S[4]
[1]Division of Rheumatology, Department of internal medicine, Nowon Eulji Medical Center, [2]Soonchunhyang University Seoul Hospital, [3]Division of Rheumatology, Department of internal medicine, Gangneung Asan Hospital, Ulsan University College of Medicine, [4]Division of Rheumatology, Department of Internal Medicine, Kyung Hee University Medical Center

Background: Idiopathic inflammatory myopathy (IIM)-associated interstitial lung disease (ILD) is often rapidly progressive with a poor prognosis. Glucocorticoid has long been considered first-line therapy, but a combination with immunosuppressant is required for refractory cases. B- cell ablation therapy, showed favorable outcomes in several recent studies in patients with refractory IIM-ILD. This study investigates the efficacy and safety of rituximab in refractory IIM-ILD.
Method: We retrospectively reviewed who were administered rituximab for IIM-ILD at least once between August 2016 and November 2021. Using the Wilcoxon signed-rank test, we compared forced vital capacity (FVC) at 6-month intervals one year before and after the initiation of rituximab. Disease progression, defined as a greater than 10% relative decline in FVC compared to the baseline, was also compared before and after treatment. Adverse events after treatment were recorded for safety analysis.
Results: Five IIM-ILD patients (median age: 40 years, female: 80%), including dermatomyositis (60%) and polymyositis (40%), received eight cycles. The median FVC and diffusing capacity of the lung for carbon monoxide at baseline were 48.5% predicted and 34.0% predicted, respectively. FVC predicted values significantly decreased from 6 months before rituximab administration to those at the baseline (54.1% indicated [pre six months] vs. 48.5% predicted [baseline], P = 0.043). Measured FVC also decreased for six months before rituximab administration (1.8 L [pre six months] vs. 1.6 L [baseline], P = 0.046). The rate of disease progression before rituximab administration showed a tendency to decrease after treatment (75% [before] vs. 12.5% [post six months, P = 0.059] vs. 14.3% [post 12 months, P = 0.102]). Three adverse events developed after treatment, including two cases of infection and one of ileus, but none resulted in death.
Conclusions: Our data suggest that B-cell ablation therapy, stabilizes lung function decline with tolerable safety in IIM patients with refractory ILD.

P1-041 Outcomes of percutaneous coronary intervention in elderly patients with rheumatoid arthritis: A nationwide population-based cohort study

Kim H[1], Kim B[2], KIM S[2]
[1]Soonchunhyang University Seoul Hospital, [2]Division of Rheumatology, Department of Internal Medicine, Gangneung Asan Hospital, University of Ulsan College of Medicine

Background: Rheumatoid arthritis (RA) increases the risk of cardiovascular disease. This study aimed to evaluate the clinical outcomes of elderly patients with and without RA who underwent percutaneous coronary intervention (PCI).
Method: The Korean National Health Insurance Service claims database extracted data on 74,623 patients (14,074 with RA and 60,549 without RA) aged ≥65 years diagnosed with acute coronary syndrome and underwent PCI between 2008 and 2019. The primary outcome was survival between elderly patients with and without RA. The secondary outcome was survival in the RA subgroup.
Results:During a 10-year follow-up, the all-cause mortality survival rate was lower in patients with RA than in patients without (53.7% vs. 58.3%, respectively, log-rank: $p<0.001$). Patients with elderly-onset RA have poor survival outcomes in the all-cause mortality RA subgroup. In contrast, patients with young-onset RA have better survival outcomes than those without RA (48.1% vs. 73.7% vs. 58.3%, respectively, log-rank: $p<0.001$).
Conclusion: Elderly patients with RA who underwent PCI had an increased mortality risk, particularly those with elderly-onset RA, than young-onset RA.

P1-042 Reduced DNase1L3 activity and increased anti-NET protective antibodies contributes to accumulation of NETs in plasma of pediatric SLE patients.

Matta B[1]
[1]The Feinstein Institutes for Medical Research, Northwell Health, Northwell Health, [2]Amity University of Biotechnology, Amity University Rajasthan

Background/Purpose: Pediatric systemic lupus erythematosus (pSLE) is a multisystemic chronic autoimmune disease with high renal involvement. In SLE, neutrophil extracellular traps (NETs) are considered a potential source of antigen, leading to autoantibody production. NETs activate plasmacytoid dendritic cells to produce high levels of interferon-α, a known driver of lupus pathogenesis. NETs have also been shown to play a role in kidney pathology leading to lupus nephritis (LN). Also, low DNase activity and mutations in DNaseIL3 have been associated with lupus. This study was undertaken to investigate levels of NETs in pSLE patients compared to healthy children (pHC). Further, to understand the contributions of levels and function of DNase1L3 and protective anti-NET antibodies leading to the accumulation of NETs in pSLE patients.
Methods. Plasma was obtained from 13 pSLE patients and 12 pHC. ELISA and Smear assay were used to detect NETs in plasma samples. DNASE1L3 concentration was measured using ELISA and DNASE1L3 enzymatic activity was assayed by nuclei digest. The ability to degrade NETs was measured in plasma samples using NET degradation assay. Lupus disease activity was measured using SELENA-SLEDAI scoring.
Results. 10/13 of pSLE patients were female, with a mean of 13±4.4years, and a mean SLEDAI score of 13.8±8.1. 9/13 (69%) patients had either proven or suspected LN, out of those 7/13 had biopsy-proven proliferative LN. Significantly higher levels of circulating NETs were found in pSLE plasma which positively correlated with disease activity and anti-ds DNA titers. Plasma from pSLE failed to degrade NETs efficiently. Although DNASE1L3 levels were higher in pSLE patients, DNASE1L3 activity was deficient, as compared to healthy children. Moreover, higher anti-NET protective antibodies were found in pSLE plasma.
Conclusions. These data suggest that defective functional DNase1L3 activity and decreased anti-NET protective antibodies could lead to delayed clearance and accumulation of NETs in pSLE plasma.

P1-043 Th9 cells via IL-9 cytokine promote RANKL-mediated osteoclastogenesis and aggravate bone loss in Osteoporosis

Srivastava R[1]
[1]All India Institute of Medical Sciences (AIIMS)

Th9 cells have an important role in several pathological and auto-immune diseases including rheumatoid arthritis (RA), T1D, etc. due to the pleiotropic nature of its IL-9 cytokine. However, the role of Th9 cells in the progression and development of osteoporosis has not been defined yet. To investigate the role of Th9 cells in the development of osteoporosis, we evaluated the frequencies of Th9 cells in a preclinical mice model of osteoporosis. Remarkably, we observed enhanced frequencies of CD4+IL-9+ Th9 cells in bone marrow (BM: prime site of osteoclastogenesis), spleen, and mesenteric lymph nodes (MLN), suggesting the inflammatory role of Th9 cells in osteoporosis. Moreover, our serum cytokine data suggest that levels of IL-9 cytokine were found to be profoundly enhanced in the case of osteoporosis. Additionally, our Pearson correlation analysis revealed that IL-9 cytokine levels are observed to be negatively correlated ($r = -0.6$ & $p < 0.01$) with bone mineral density (BMD). Moving ahead, we determined the percentage of CD4+IL-9+ Th9 cells in PBMCs of osteoporotic patients and our flow cytometry data demonstrated significant enhancement in the number of Th9 cells in the case of post-menopausal osteoporotic patients, and Th9 cells were observed to be positively correlated with bone resorbing CTX-1 biochemical marker. Thus, our pre-clinical and clinical data clearly suggest towards the osteoporotic role of Th9 cells in osteoporotic conditions. Furthermore, our in vitro data suggested that 17-β estradiol significantly reduced the differentiation of CD4+ naïve T cells to Th9 cells thus indicating towards potent role of estrogen in regulating the percentage of Th9 cells and IL-9 levels in pre-menopausal women. Together, the present study for the first time demonstrated the role of Th9 cells and its associated IL-9 cytokine in inflammatory bone loss observed in the case of osteoporosis.

P1-044 Differential responses of microglia and macrophages to T-helper cells-derived inflammatory cytokines

Chan L[1], Yongliang Z[1]
[1]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore

Introduction:
Multiple sclerosis (MS) is a disabling autoimmune disease that afflicts the myelin sheath of neurons. Current consensus recognises MS pathology to be driven by autoreactive CD4+ helper T (TH) cells. Notably, IFNγ-secreting TH 1 and IL17-secreting TH 17 are TH subsets frequently associated with MS. Recent identification of GM-CSF secreting TH GM has been demonstrated to exert potent inflammatory capacity during disease. These TH subsets cytokines does not directly cause demyelination but orchestrate the pathology through effector immune cells macrophages and microglia. However, there are limited studies that investigate the relative inflammatory potential of TH subset cytokines have on macrophages and microglia.

Methods:
Macrophages (RAW 164.7) and microglia (BV2) cell lines were stimulated by IFNγ, IL-17, and GM-CSF individually. The cytokine expression profile of the two effector immune cells were evaluated and contrasted by quantitative PCR and ELISA. JAK/STAT and MAPK pathway activities were assessed to reveal possible differences in activation between macrophages and microglia towards stimulation.

Results:
Across IFNγ, IL-17, and GM-CSF stimulation, microglia consistently reflected higher pro-inflammatory capacity compared to macrophage, particularly elevated IL-1β and MCP-1 expression. Mechanistically, microglia exhibited distinct JNK activation under all stimulation. Furthermore, GM-CSF stimulated microglia revealed exceptional STAT-3 phosphorylation, not observed in macrophage under the same stimulant.

Discussion:
With significantly elevated pro-inflammatory cytokines and chemokines displayed by microglia compared to macrophages, results suggest that microglia may have a higher contribution to disease progression in response to autoreactive TH cells over peripheral macrophages. Moreover, preferential activation of JNK and STAT3 unique to GM-CSF stimulated microglia revealed a potentially novel disease-mediating pathway. These insights highlight the unexplored mechanism and phenotype surrounding displayed by effector immune cells during MS.

P1-045 Crucial role of CX3CR1-CX3CL1 axis during thrombus resolution on stasis-induced murine deep vein thrombosis model

Nosaka M[1], Ishida Y[1], Ishigami A[1], Kuninaka Y[1], Yamamoto H[1], Shimada E[1], Kawaguchi M[1], Kimura A[1], Mukaida N[1], Kondo T[1]
[1]Department of Forensic Medicine, Wakayama Medical University

Background: During the process of venous thrombus formation and resolution, several biological events are often observed, including intra-thrombus accumulation of neutrophils and macrophages and remodeling of the extracellular matrix. We have previously investigated the pathophysiological roles of several cytokines and chemokines in the process of thrombus formation and dissolution.
Methods: Male C57BL/6 (WT) and CX3CR1 knockout (KO) mice underwent anesthesia and, the inferior vena cava (IVC) was ligated with a silk suture. At selected time points after ligation, the blood flow in thrombosed IVCs were measured and the thrombi were harvested. The morphological study, immunohistochemical analyses, double-color immunofluorescence analyses were performed, and the intrathrombotic gene expressions were obtained by real-time RT-PCR.
Results: In WT mice, intrathrombotic CX3CR1-positive cells and these gene expression were detected. When KO mice were treated in the same manner, thrombus size was much larger than WT mice. Intrthrombotic CX3CL1-positive cells and gene expressions were found in both WT and KO mice, but were more abundant in WT mice than KO mice. Double color immunofluorescence analyses of WT mice thrombi, detected the intrathrombotic CX3CR1- or CX3CL1-positive cells in the F4/80-positive macrophages. The gene expressions of Mmp2, Plau, Plat and Vegf were significantly reduced in KO mice than in WT. The results of bone marrow transplantation experiments showed that bone marrow transplanted from WT mice enhanced thrombus resolution in KO mice. We also confirmed that administration of murine recombinant CX3CL1 reduced thrombus size and recovered blood flow.
Conclusion: Consistently, these results indicated the thrombus mass of KO mice were larger than WT mice. CX3CR1-CX3CL1 axis can have benefit roles the thrombus resolution by inducing MMP-2, uPA, tPA and VEGF expression. CX3CR1-CX3CL1 can be a good therapeutic target for the DVT.

P1-046 BCAT1-mediated leucine metabolism regulates Th17 responses via the mTORC1-HIF1α pathway

Kang Y[1], Song W[1], Choi S[1], Kim H[1,2], Lee W[1,2]
[1]Laboratory of Autoimmunity and Inflammation (LAI), Dept. of Biomedical Sciences and Dept. of Microbiology and Immunology, Seoul National University College of Medicine, [2]Institute of Infectious Diseases, Seoul National University College of Medicine

Background: Branched-chain amino acids (BCAAs), such as leucine (Leu), are essential amino acids that play a modulatory role in immune responses by participating in metabolic rewiring. However, the molecular mechanism underlying this phenomenon remains unclear.
Methods: We investigated the effects of cytosolic leucine metabolism on the immune responses of human CD4+ T cells by analyzing the cytokine production profile and the signaling activities. Experimental autoimmune encephalomyelitis (EAE) was used for examining in vivo effect of the inhibition of cytosolic leucine metabolism on inflammatory responses.
Results: TCR stimulation induces the expression of BCAT1, a cytosolic BCAA catabolic enzyme, and SLC7A5, a major transporter of BCAAs, in human CD4+ T cells. SLC7A5-mediated Leu influx and BCAT1-mediated Leu catabolism are important for Th17 responses. RNA-Seq data indicate that the expression of cytosolic Leu catabolism-related genes for the synthesis of β-hydroxy β-methylbutyrate (HMB) is upregulated in TCR-activated CD4+ T cells, implying an important role of BCAT1-mediated Leu metabolites for IL-17 production. In CD4+ T cells, HMB supplementation ameliorated the suppression of IL-17 production by the BCAT1 inhibitor. Mechanistically, HMB contributes to the regulation of the mTORC1-HIF1α pathway, a major signaling pathway for IL-17 production, through enhanced mRNA expression of HIF1α. This was further supported by the finding that treatment with L-β-homoleucine (LβhL), a Leu analogue and competitive inhibitor of BCAT1, diminished IL-17 production by TCR-activated CD4+ T cells. An in vivo EAE model demonstrated that the blockade of BCAT1-mediated Leu catabolism by a BCAT1 inhibitor or LβhL treatment ameliorates EAE severity through decreasing HIF1α expression and IL-17 production in spinal cord mononuclear cells.
Conclusion: Our findings suggest a possible role of SLC7A5-mediated influx and BCAT1-mediated catabolism of Leu in modulating CD4+ T-cell responses, especially IL-17 production, via the regulation of HIF1α. Moreover, this mechanism might be related to a broad range of inflammatory conditions.

P1-047 CTLA-4 fusion protein suppresses inflammatory cytokine secretion and increases serum procollagen type 1 amino-terminal propeptide in patients with rheumatoid arthritis

Chen M[1], Leu C[2]
[1]Division of Allergy, Immunology & Rheumatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [2]Institute of Microbiology & Immunology, and Infection and Immunity Center, National Yang-Ming University

Background: Cytotoxic T lymphocyte antigen-4 (CTLA-4) competes CD28 binding their ligands CD80/CD86 to prevent T cell activation but its role in B cells is not well known. Rheumatoid arthritis (RA) is the most common form of chronic inflammatory arthri¬tis and a risk factor of osteoporosis. The aim of the study was to investigate the influence of CTLA-4 on B cell function and bone metabolism in RA patients.
Methods: We assayed the effect of CTLA-4 on human B cells in vitro. Purified human B cells were treated with CTLA-4 and cytokine production were measured. Next, bone turnover markers, including receptor activator of nuclear factor κB ligand (RANKL), Osteoprotegerin (OPG), Dickkopf-related protein 1 (DKK1), C-terminal crosslinking telopeptide of type I collagen (CTX), N-terminal telopeptides of type I collagen (NTX), and procollagen type 1 amino-terminal propeptide (P1NP) were quantified by enzyme linked immunosorben assay from RA patients before and after CTLA-4 fusion protein (abatacept) treatment. The change of bone mineral density (BMD) in patients was measured by Dual-energy x-ray absorptiometry scan.
Results: In the in vitro assays, abatacept reduced Staphylococcus aureus Cowan strain-induced pro-inflammatory cytokine production from B cells, including TNF-α and IL-6. An increase in serum P1NP values, a bone formation marker, was observed in patients receiving abatacept treatment for 6 months ($p < 0.001$). In contrast, there were no significant changes in the serum levels of OPG, RANKL, DKK1, NTX, and CTX (all $p > 0.005$). Interestingly, abatacet increased BMD at femur neck, total hip, and lumbar vertebra 1–4 significantly in RA patients when comparing the values before and 3 years of treatment ($p = 0.025$, 0.010, and 0.001, respectively).
Conclusion: CTLA-4 may suppress human B cell function and long-term abatacept treatment for 3 years may have a bone loss protection effect in patients with RA.

P1-048 SARS-CoV-2 membrane protein induces a robust in vitro and in vivo cytokine storm

Scarneo S[1], Haystead T[1]
[1]EydisBio Inc, [2]Duke University School of Medicine

The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, has been shown to induce moderate to severe pulmonary inflammation in patients. Although inflammation in response to viral infection is critical for normal immune-pathogen response, inflammation greater than that needed to mount the appropriate immune response is considered maladaptive and can cause long term tissue damage. However, to date, very little is known about the immunogenicity of individual SARS-CoV-2 proteins. The SARS-CoV-2 virus consists of 29 proteins, categorized either as nonstructural proteins (NSP's), structural proteins (SP's) or accessory proteins. Here we sought to evaluate the immunogenicity of NSP 1,7,8,9,10,12,14,16 and the SP's spike protein (full length, S1, S2 and receptor binding domain subunits), nucleocapsid and membrane SARS-CoV-2 proteins against THP-1 and human peripheral mononuclear cells (PBMCs). Our work identified that several individual proteins such as membrane, NSP's 8,14, 16 induced a dose dependent increase in inflammatory cytokines. In particular, we have observed that the membrane protein of SARS-CoV-2 induces a large TNF response in vitro. Further evaluation of intranasal membrane protein challenge (15µg) in male and female BALB/c mice show increases in BALF (bronchalveolar lavage fluid) proinflammatory cytokine expression, elevated cellularity, predominantly neutrophilic, and concomitant peribronchiolar and perivascular lymphomononuclear and neutrophilic inflammation. Our results suggest that individual membrane associated SARS-CoV-2 proteins induce a robust immune response that may contribute to viral induced cytokine release syndrome (CRS) in the lungs of moderate to severe COVID-19 patients.

P1-049 Novel pathogenetic mechanisms mediated by dysregulation of TRIM21-STING-type I interferon axis in lupus

Park Y[1], Jeon H[2], Kim D[3,4], Cho M[3], Kwok S[1,3]
[1]Division of Rheumatology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, [2]Division of Rheumatology, Department of Internal Medicine, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, [3]The Rheumatism Research Center, Catholic Research Institute of Medical Science, The Catholic University of Korea, [4]Department of Biomedicine & Health Sciences, College of Medicine, The Catholic University of Korea

Background: Tripartite motif-containing protein (TRIM) 21 is an E3 ubiquitin-protein ligase, involved in the ubiquitin-dependent proteolysis pathway of various proteins including factors related to type I interferon pathways. Although the presence of autoantibodies against TRIM21 in various autoimmune diseases suggests potential pathogenetic roles, no studies have clarified its exact implications especially in lupus. We aimed to elucidate the functions of TRIM21 in dysregulation of type I interferon signals in lupus.

Methods: To investigate effects of TRIM21 dysfunction in lupus pathogenesis, two independent lupus animal models, the R848-induced model and the B6/lpr mice model were performed using TRIM21 knockout mice and their phenotypes and immunological profiles were determined. In addition, we investigated the degree of TRIM21 dysfunction and therapeutic effects of in vivo delivery of TRIM21 in MRL/lpr mice. To evaluate the E3 ubiquitin ligase activity of TRIM21 for targeted proteins in type I interferon pathways, we performed specialized immunoblot assay.

Results: The R848 induced model and the B6/lpr model both presented with more severe lupus-like phenotypes such as nephritis, lymphadenopathies, and inflammatory immune cell profiles in TRIM21 knockout mice than in control mice. TRIM21 deficiency resulted in activation of intracellular factors related to type I interferon pathways such as STING, TBK1, and IRF3 in both models. MRL/lpr mice presented with activation of type I interferon pathways including STING, TBK1, and IRF3, and decreased expressions of TRIM21. Overexpression of TRIM21 attenuated the disease phenotypes in MRL/lpr mice. Using immunoblot assay, we observed E3 ubiquitin ligase activity of TRIM21 directly targeting STING via the proteasome pathway.

Conclusion: TRIM21 dysfunction induces dysregulation of STING-type I interferon pathways and exacerbates the disease in lupus animal models. Targeting TRIM21-STING-type I interferon axis can be a novel therapeutic strategy in lupus treatment.

P1-050 B cell class switching through CD40L expression on platelets under influence of IL-9 in Rheumatoid arthritis.

Chowdhury K[1]
[1]All India Institute Of Medical Sciences.

Title: B cell class switching through CD40L expression on platelets under influence of IL-9 in Rheumatoid arthritis.
Kaustav Chowdhury1,2, D.K. Mitra1, Uma Kumar2, Prabin Kumar1,Soumabha Das1,3,Maumita Kanjilal1.
1 Dept of Transplant Immunology & Immunogenetics, 2Dept. of Rheumatology, All India Institute of Medical Sciences, New Delhi, India.
3Comanche County Memorial Hospital, Lawton, Oklahoma, USA

Rheumatoid arthritis (RA) is a systemic autoimmune disease of bone joints. T cell participation and their secreted cytokines like TNF-α, IL-17 are known to be detrimental for bone and cartilage in RA. IL-9 is reported as pivotal cytokine in RA research recently due to their role in disease progression. It has been shown that IL-9 is tightly correlated with RA disease activity score (DAS-28). IL-9 has been shown to up regulate function if Th1 and Th17 cells and to down regulate Treg cell function in RA. In the current study we have identified a new role of IL-9 for the first time.IL-9 itself up regulates CD40L on platelets from RA patients. Synovial fluid of RA patients (cell free) also up regulates CD40L on platelets from healthy individuals as like recombinant IL-9 (rIL-9) addition ex vivo. CD40L expression on platelets helps plasma B cells to class switch to produce more IgG and IgM based rheumatoid factor (RF) in RA. Blocking of IL-9 with anti Human -IL-9 and its receptor CD129 (IL-9Rα) in Ex vivo coculture of platelet and B cells reduces RF autoantibody production in RA. Therefore, IL-9 blocker could be a potential novel therapy in RA.

P1-051 Gender-dependent association of interferon lambda genetic variants with peripheral blood profiles in healthy individuals

Guha Roy D[1], Chinnaswamy S[2]
[1]National Institute Of Biomedical Genomics, [2]National Institute Of Biomedical Genomics

Interferon Lambda (IFNL) locus got into the limelight after the HCV GWAS. Within a decade, studies showed its genetic association with different infectious and inflammatory diseases, however, there is a lack of studies investigating the effect of the polymorphisms on human health. Herein, we try to investigate the association of IFNL gene polymorphisms with multiple phenotypes in the peripheral blood of healthy individuals, considering gender as a possible effect modifier. 552 healthy individuals from West Bengal were selected and tested for thirty metabolic, hematological and biochemical parameters. They were genotyped for: IFNL-4-related SNPs rs12979860 and rs117648444 and IFN-L3-related SNPs rs28416813, and rs4803217. We tested the association between the genotypes and the clinical parameters in dominant and recessive models. We found a significant difference in distribution of the variance between the groups in the two models involving phenotypes like Monocyte levels, SGOT, VLDL, Triglycerides, TSH etc. Stratification according to gender, indicated that the differences are mostly in males. Next, we tested for association of the SNPs with median levels of the phenotypes. We saw a significant association ($p<0.05$) with rs4803217 and uric acid levels in the combined cohort under the recessive model; the association was lost in females but was strong ($p<0.001$) in males. Only in males under the dominant model we saw significant association ($p<0.05$) with all three SNPs and SGOT or monocyte levels. When we tested the IFN-λ4 activity modifying SNP rs117648444 within groupings based on absence or presence of one or two copies of IFN-λ4 and on different activity level of IFN-λ4, we found several significant ($p<0.05$) associations in males with monocyte, triglyceride, VLDL, ALP and uric acid levels, whereas only eosinophils showed association in females. Overall, we found that the IFNL genetic variants showed a significantly ($p<0.05$) higher number of associations in males than in females.

P1-052 Immune profiles differentiate mycobacterial and KSHV triggered secondary hemophagocytic lymphohistiocytosis in people with advanced HIV

Rocco J[1], Manion M[1], Laidlaw E[1], Galindo F[1], Lage S[1], Rupert A[2], Lisco A[1], Sereti I[1]
[1]National Institute Of Allergy And Infectious Diseases, [2]Frederick National Laboratory for Cancer

Research
People with advanced HIV (PWH) are at risk of Kaposi-sarcoma herpesvirus (KSHV) and mycobacterial infections. Both can cause inflammatory disease including secondary hemophagocytic lymphohistiocytosis (sHLH). These hyperinflammatory syndromes can occur after starting antiretroviral therapy (ART) due to immune reconstitution inflammatory syndrome (IRIS). Severe mycobacterial-IRIS resulting in sHLH (myco-HLH-IRIS) has overlapping pathophysiology with genetic HLH and involves the IFNγ-IL18 axis. We evaluated immune profiles in patients with sHLH triggered by mycobacterial-IRIS or KSHV-associated diseases to identify biomarkers that differentiate these etiologies.

Overall, 48-PWH were included (median age 38.5-years [IQR:34-43]) with 35-men. Twenty-PWH had myco-HLH-IRIS, 7-KSHV-triggered HLH, and 19-PWH without infections were included as controls. All participants were enrolled on NIH-IRB approved protocols and were taking ART. Clinical data and 10-biomarkers associated with HLH were measured. Imagestream flow cytometry was performed for activated and regulatory T-cells and inflammasome activation in monocytes. Principal component (PCA) and decision tree analyses were performed.

There was no difference in CRP, IL6, or HIV viral load. PCA of IFNγ-IL18 axis markers revealed distinct clustering of the two sHLH etiologies (Fig1). There was greater IFNγ and CXCL9 in myco-HLH-IRIS (IFNγ 351.2pg/mL [IQR:190-616]; CXCL9 1869.7pg/mL [IQR 1103-2573]) vs KSHV-HLH (IFNγ 30.9pg/mL [IQR 21-211]; CXCL9 272pg/mL [IQR:164-587])($p<0.001$). IL18 was increased in KSHV-HLH (IL18 4364pg/mL [IQR:2796-6124]) compared to myco-IRIS (IL18 1836.8pg/mL [IQR:1164-2477])($p<0.001$). Soluble CD25 was increased in both groups, but T-cell phenotypes showed increased CD4+CD38+DR+ and CD8+CD38+DR+ activated T-cells in myco-HLH-IRIS compared to KSHV-HLH. However, KSHV-HLH had greater inflammasome activation providing pathophysiologic support of the unique biomarker profiles. The IL18/CXCL9 ratio showed the greatest distinction between the sHLH triggers.

Although clinically indistinguishable, sHLH driven by mycobacterial-IRIS or KSHV demonstrate unique immune profiles involving the IFNγ-IL18 axis and differential T-cell and inflammasome activation. The IL18/CXCL9 ratio may provide a novel marker to differentiate them and targeting these pathways could improve clinical outcomes.

P1-053 Application of RNAi against RORγt for alleviation of disease severity in animal model of psoriasis

Chen W[1], Wu P[1], Chen Y[1], Chiang B[1]
[1]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University

Background
Psoriasis is a chronic, immune-mediated disorder, which is characterized by epidermal hyperplasia and dermal infiltration of immune cells. Th17 cells have been reported to be abundantly expressed in patients with psoriasis and have a positive correlation with the severity of the disease. Given that pathogenic Th17 cells play such a pivotal role in the pathogenesis of psoriasis, targeting Th17 cells, especially via blocking RORγt, might be a good option for treating psoriasis, such as digoxin, ursolic acid. Therefore, we want to develop siRNA drugs for topical treatment. This study was to investigate whether the delivery of siRNA against RORγt via the lentiviral vector system could be applied in a mouse model of IMQ-induced psoriasis-like skin inflammation.

Methods
For in vitro, thymocytes and Th17 cells from BALB/c mice were infected with mixture including polybrene and lentivirus containing siRORγt-2 or siRORγt-3, or mock respectively (MOI = 30) for 22 hours. BALB/c mice between 8-10 weeks of age were used for IMQ-induced psoriasis experiments. Lentivirus (1x107 IFU) mixed with hydrogel and were treated to skin on day 1, day 3, and day 5, then mice were sacrificed on day 6.

Results
Lentivirus expressing siRNA against RORγt reduced RORγt expression in thymocytes. In addition, it can reduce the differentiation of Th17 cells and the production of pro-inflammatory cytokines such as IL-17 and IL-22 to achieve therapeutic effects.

Conclusion
Our study demonstrated that the functionality of lentivirus expressing siRNA against RORγt reduced RORγt expression and proinflammatory cytokines level in vitro. The results demonstrated that lentivirus expressing siRNA against RORγt could be used for the treatment of the imiquimod (IMQ) induced psoriasis model.

P1-054 Characterization of CD8+ regulatory T cells induced by B cells and their modulatory effect in murine model of inflammatory bowel disease

Fan J[1], Chiang B[1]
[1]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taiwan

Background
Inflammatory bowel disease (IBD) is an autoimmune disease. The pathophysiology of IBD is multifactorial, including genetic, environmental factors, and dysregulated immune responses. Regulatory T cells have be found to play an important role in maintaining immune homeostasis. Previous studies showed that naïve B cells can convert CD4+CD25- T cells into CD25+Foxp3- regulatory T cells, named Treg-of-B cells by our group. Hence, we further induced CD8+ Treg-of-B cells and study their immunomodulatory functions. Our study aimed to characterize CD8+ regulatory T cell induced by B cells, and investigate their modulatory effect in murine model of inflammatory bowel disease.

Methods
We isolate CD8+CD25- T cells and cultured with B cells to induce CD8+ Treg-of-B cells, and analyzing their phenotype, immunosuppressive function and cytokines secretion. In addition, we have also established the animal model of inflammatory bowel disease with DSS-induced colitis, and adoptive transfer CD8+ Treg-of-B cells to investigate their immunomodulatory function in vivo.

Results
The CD8+ Treg-of-B cells expressed ICOS, LAG3, OX40, GITR, PD1 and CTLA4, but did not express Foxp3. Activated-CD8+ Treg-of-B cell can secret higher levels of IL-10, IFN-γ, and TNF-α compared to activated CD8+ T cell. In addition, CD8+ Treg-of-B cell exert the suppressive ability on both CD4+ and CD8+ T cells. In DSS-induced colitis model, the result showed that CD8+ Treg-of-B cells can not alleviate the body weight loss and colon length shortening, but slightly decrease pro-inflammatory cytokine TNF-α production. Moreover, CD8 Treg-of-B cells treatment exhibited milder histopathology in colon section.

Conclusion
We provide a new insight on the characterization of CD8+ Treg. CD8+ Treg-of-B is a novel Foxp3- CD8+ regulatory T cell, which can exert the suppressive function in vitro and have the potential to alleviate inflammatory bowel disease.

P1-055 The GM-CSF/CCL17 pathway in obesity-associated osteoarthritic pain and disease in mice

Shin H[1], Prasad V[1], Lupancu T[1], Malik S[1], Achuthan A[1], Biondo M[2], Kingwell B[2], Thiem M[3], Gottschalk M[3], Weighardt H[3], Förster I[3], de Steiger R[4], Hamilton J[1], Lee K[1]
[1]University Of Melbourne, [2]CSL Ltd, Bio21 Institute, [3]Immunology and Environment, Life and Medical Sciences Institute, University of Bonn, [4]Department of Surgery, Epworth Healthcare, University of Melbourne

Background
We have previously identified a granulocyte macrophage-colony stimulating factor (GM-CSF)/C-C motif ligand 17 (CCL17) pathway in monocytes/macrophages, in which GM-CSF regulates the formation of CCL17 and it is important for an experimental osteoarthritis (OA) model. We explore here in additional OA models, including in the presence of obesity, such a requirement for this pathway.
Methods
The roles of GM-CSF, CCL17, CCR4 and CCL22 in various experimental OA models, including those incorporating obesity (8-week high-fat diet), were investigated using gene-deficient male mice. Pain-like behaviour and arthritis were assessed by relative static weight distribution and histology, respectively. Cell populations (flow cytometry) and cytokine mRNA expression (qPCR) in knee infrapatellar fat pad (IPFP) were analyzed. Human OA sera were collected for circulating CCL17 levels (ELISA) and OA knee synovial tissue for gene expression (qPCR).
Results
We present evidence that i) GM-CSF, CCL17 and CCR4, but not CCL22, are required for the development of pain-like behaviour and optimal disease in three experimental OA models, as well as for exacerbated OA development due to obesity, ii) obesity alone leads to spontaneous knee joint damage in a GM-CSF- and CCL17-dependent manner, and iii) in knee OA patients, early indications are that BMI correlates with a lower Oxford Knee Score ($r=-0.458$, $p=0.0096$), with elevated circulating CCL17 levels ($r=0.2108$, $p=0.0153$) and with elevated GM-CSF and CCL17 gene expression in OA synovial tissue.
Conclusions
The above findings indicate that GM-CSF, CCL17 and CCR4 are involved in obesity-associated OA development, broadening their potential as targets for possible treatments for OA.

P1-056 IL-33 overexpression in mice induces an extrafollicular B cell response inducing plasma cell expansion in the spleen and break of tolerance

Conde Garcia E[1], Asrat S[1], Kaur N[1], Ben L[1], Murphy A[1], Sleeman M[1], Orengo J[1], Limnander A[1]
[1]Regeneron Pharmaceuticals

Cytokines, such as IL-4, IL-5, IL-13 and IL-33 play critical roles in driving pathogenic pathways in asthma, COPD and autoimmune disorders. IL-33 has been shown to drive both type 1 and type 2 inflammation by impacting different cell types. Recently, IL-33 has also been implicated in promoting a break in tolerance leading to different autoimmune disorders. The role of IL-33 on B cell maturation and tolerance, however, is poorly understood. In our study, we overexpressed IL- 33 by hydrodynamic DNA delivery (HDD) and analyzed the B cell response. Overexpression of IL-33 induced the differentiation and accumulation of plasma cells of all isotypes in the spleen leading to subsequent increase in antibody production. Our data showed that the effect is not mediated by a direct effect of IL-33 on B cells, but rather by induction of distinct pathways that drive an associated humoral response. Specifically, we show that IL-33 overexpression leads to IgM/IgG2a production in an IL-5 dependent way and IgG1/IgE in a T-cell dependent way. In a NP-KLH immunization, a T cell-dependent mouse model, we showed that overexpression of IL- 33 inhibits germinal centers and promotes a rapid differentiation of marginal zone B cells to plasma cells. This extrafollicular response causes a break of tolerance and induces an increase of autoantibodies in the serum. Taken together, our data demonstrates that IL-33 impacts antibody responses by multiple mechanisms with potential implications in autoimmunity, vaccination, and infection.

P1-057 Role of the IFIH1-A946T risk variant in lupus nephritis

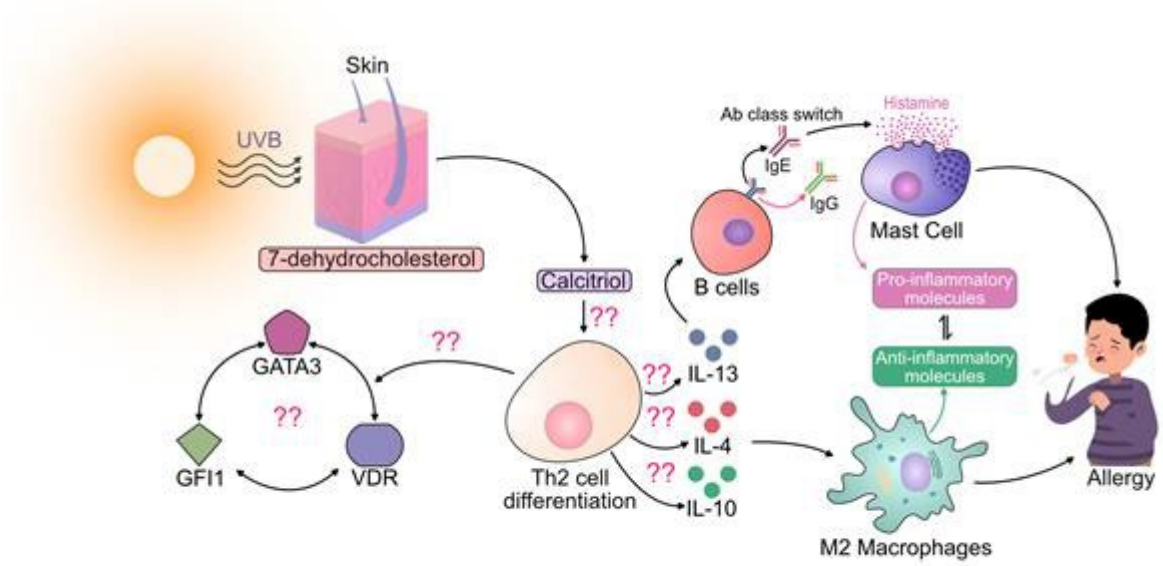
Stock A[1], Gonzalez Paredes P[1], Previato de Almeida L[1], Budde H[1], Kaur A[1], Strecker B[1,2], Deshmukh U[1,2], Bagavant H[1], Gorman J[1,2]
[1]Arthritis and Clinical Immunology, Oklahoma Research Medical Foundation, [2]Microbiology and Immunology, University of Oklahoma Health Sciences Center

Single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) have revealed a link between anti-viral immunity and autoimmune diseases. The common SNP, rs1990760, is in the gene encoding for a cytosolic dsRNA viral sensor, interferon-induced helicase C domain-containing protein 1 (IFIH1), and results in an alanine to threonine substitution at amino acid 946 (IFIH1-A946T). This variant is strongly associated with an increased risk for multiple autoimmune diseases including systemic lupus erythematosus (SLE). Our previous work has shown this risk allele to be associated with a basal interferon-stimulated gene (ISG) signature in both healthy human subjects and in a knock-in mouse model mimicking the human allele (Ifih1-T946). Furthermore, Ifih1-T946 mice exhibited increased basal expression of type I interferon (IFN I). Autoantibodies were elevated in mice expressing the risk compared to non-risk Ifih1 allele in an inducible murine lupus model. However, the role of IFIH1-T946 in SLE pathogenesis remains to be defined. Lupus nephritis (LN) affects a large portion of SLE patients, leading to high-cost medical complications. Elevated IFN I is associated with LN in SLE patients. Understanding how a genetic variant like IFIH1-T946 may be involved in LN pathogenesis is poorly understood. We hypothesize that IFIH1-T946 contributes to lupus nephritis by augmenting the inflammatory environment and promoting altered immune cell activation and infiltration into the kidney. Using an inducible lupus nephritis murine model, we show Ifih1-T946 variant expressing mice exhibit modified disease pathogenesis. Moreover, Ifih1-T946 immune cell activation is altered most likely due to the inflammatory environment within the risk mice. These findings unveil important mechanisms linking IFIH1-T946 to the pathogenesis of SLE and LN.

P1-058 Vitamin D downregulates IL-4 and IL-13 expression in Th2 cells by modulating type 2 transcription factors via a common regulatory element

Biswas B[1], Goswami R[1]
[1]IIT Kharagpur

Calcitriol, the active form of vitamin D, is one of the critical micronutrients required to maintain calcium homeostasis in our body. Apart from its usual physiological roles, the function of calcitriol as an immunomodulator has been under investigation for some time. The relevance of calcitriol as a regulator of peripheral T cell responses has really come into spotlight in recent times. Th2 cells are known to orchestrate protective immune responses against helminths. However, dysregulation of Th2 cells may cause allergic inflammation. Type 2 cytokines including IL-4 and IL-13 are the key contributors to the pathophysiology of allergic asthma. Interestingly, IL-4 and IL-13 share common receptor complexes and a conserved location in the vertebrate genome. Calcitriol is a well-known anti-inflammatory micro-nutrient; however, its regulation of Th2 cell differentiation is still obscure. In this study, we observed calcitriol to be a negative regulator of Th2 cell differentiation. Calcitriol significantly reduced Il4 and Il13 expression in murine Th2 cells. Interestingly, IL-10 production was increased in the presence of calcitriol. The expression of GATA3, the master regulator of Th2 cells, was significantly attenuated post calcitriol treatment. Interestingly, GFI1, a well-known transcriptional repressor and a crucial regulator of hematopoiesis, was also downregulated post calcitriol treatment. GFI1 impaired the transactivation of both Il4 and Il13 gene promoters. We also for the first time report a distally located conserved regulatory element (cECR) as a positive regulator of both Il4 and Il13, and a crucial element for calcitriol-mediated regulation of Il4 and Il13 genes in Th2 cells.



P1-059 Transcriptome analysis to stratify systemic lupus erythematosus patients

Haljasmägi L[1], Meisalu S[2], Vanker M[1], Kisand K[1]
[1]Institute of Biomedicine and Translational Medicine, Department of Biomedicine, Molecular Pathology Research Group, University of Tartu, [2]East Tallinn Central Hospital, Centre of Rheumatology

Background. Systemic Lupus Erythematosus (SLE) is a very heterogenous autoimmune disease which can affect almost every major organ system, making the treatment of this disease very challenging. One of the most common characteristics of SLE is upregulation of type I interferons (IFN), which causes interferon-stimulated gene signature (ISG), thus making them of interest in the diagnosis and treatment of SLE. There is a need for more personalized treatment and for that it is important to know the endotype of the disease, which could be based on many different factors (e.g ISG signature, IFNα levels, transcription patterns) to provide more precise treament to every single patient. The aim of our study is to describe the molecular profile of SLE patients.
Methods. We analyzed 70 Estonian SLE patients and 20 controls (age- and sex-matched). IFNα levels were measured by Single Molecul Array. RNA was isolated from whole blood using Tempus Spin Isolation kit and cDNA was synthesized. We performed qPCR (Quantitative Polymerase Chain Reaction) using ISGs and RNA-sequencing.
Results. When comparing patients to controls, we discovered 404 differentially expressed genes which were upregulated in patients. Most of the top upregulated genes were ISGs. We also compared patients based on IFNα concentration (high vs low) and discovered 118 upregulated genes, but interestingly very few of them were interferon-stimulated. We tested 9 ISGs with qPCR and calculated IFN score based on the results. We compared IFN score to IFNα levels and discovered that they were highly correlated.
Conclusions. Based on our results we suggest that there is a subset of patients who harbor interferon-stimulated gene signature. However, there is also another subset of patients, non-dependent of ISGs, whose pathogenicity may be affected by other immune pathways. Our results from qPCR suggest that IFN score could be used to predict IFNα concentration in SLE patients.

P1-060 Low level of Deltex1 in T cells may be associated with high disease activity of Sjögren's syndrome through enhancing IFN-γ secretion

Wang C[1], Chen M[2]
[1]Division of Allergy, Immunology & Rheumatology, Department of Medicine, Taoyuan General Hospital, Ministry of Health and Welfare, [2]Division of Allergy, Immunology & Rheumatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; 3 Department of Medicine, National Yang-Ming University

Background: Deltex1 is a transcription target of nuclear factor of activated T cells (NFAT) that promotes T cell anergy. We have reported that silencing of Deltex1 expression enhanced IFN-γ secretion by human T cells after stimulation with anti-CD3 and anti-CD28. Sjögren's syndrome (SjS) is a common autoimmune disease and mainly impairs the function of the exocrine glands with a focal lymphocytic infiltration, and usually presents clinically as persistent dryness of the mouth and eyes. The aim of this study was to investigate the gene expression of Deltex1 on T cells obtained from patients with SjS and healthy controls (HCs).
Methods: Blood samples were collected from SjS patients and HCs. T cells were purified from peripheral blood mononuclear cells of study subjects by negative selection for CD14, CD19, CD235A, CD11b with immunomagnetic beads. Quantitative real-time polymerase chain reaction (q-RT-PCR) was used to measure Deltex1 expression of T cells. European League Against Rheumatism (EULAR) SjS outcome measures, the disease activity index (ESSDAI), and the patient reported index (ESSPRI) were used to evaluate systemic activity and patients' symptoms in SjS, respectively.
Results: Forty-two subjects, including 15 patients with primary SjS, 13 secondary SjS, and 14 HCs were enrolled. Deltex1 expression in T cells was significantly lower in the primary SjS patients than in the secondary SjS ($p = 0.004$), while it was tent to be lower in the primary SjS patients than in HCs ($p = 0.067$). In addition, Deltex1 expression in T cells was negatively correlated with the visual analogue scale (VAS) for dryness, VAS for fatigue, ESSPRI, and ESSDAI ($p < 0.001$, $= 0.003$, 0.017, and 0.003, respectively), but not with VAS for pain ($p = 0.495$).
Conclusion: Low level of Deltex1 in T cells may be associated with high disease activity of SjS through enhancing IFN-γ secretion.

P1-061 CYTOKINE PROFILE OF DISEASE SITE IN PATIENTS WITH SARCOIDOSIS AND TUBERCULOSIS

Jain R[1], Kumari R[2], Mitra D[2], Guleria R[1]
[1]Department of Pulmonary Critical Care and Sleep Medicine, All India Institute Of Medical Sciences, New Delhi, [2]Department of Transplant Immunology and Immunogenetics, All India Institute of medical Sciences

Background:
Sarcoidosis is a multisystem inflammatory disorder of unknown cause that manifests as non-necrotizing granuloma. This disease has high clinical, radiological, and histopathological similarities with tuberculosis. In this study, our aim was to compare lung-specific cytokine profiles in patients with pulmonary sarcoidosis (PSAR) and pulmonary tuberculosis (PTB).

Methods:
Ten age and sex-matched patients with PSAR and PTB were recruited. Clinical history, Chest X-ray, Chest CT scan, and Pulmonary Function Test data were recorded for all patients. Bronchoalveolar lavage (BAL) was collected through flexible bronchoscopy. The BAL was immediately centrifuged and filtered. The cells were pelleted down and stored in a lysis buffer at -80□c for future investigations. Expression of Interferon (IFN) - γ, Tumour Necrosis Factor (TNF)-α [Th1 cytokines]; Interleukin (IL)-10 [Th2 cytokine]; IL-17 [Th17 cytokine]; IL-9 [Th9 cytokine] in BAL derived cells was checked by Quantitative Real-Time Polymerase Chain Reaction.

Results:
Gene expression of IFN- γ was significantly higher in patients with PSAR as compared to patients with PTB (p =0.02). IL-17 expression was also significantly higher in patients with PSAR as compared to PTB (p=0.02). In contrast to IFN- γ and IL-17, BAL IL-10 expression was lower in PSAR than PTB (p= 0.03). The BAL cytokine gene expression of IL-9, and TNF-α was similar between the patient groups (p= 0.55, p= 0.94 respectively) (Figure 1).

Conclusion:
In comparison to PTB, significantly higher expression of pro-inflammatory cytokines (IFN-γ and IL-17) and significantly lower expression of regulatory cytokines IL-10 explains exaggerated immune responses in the lungs of patients with PSAR. Differential expression of these cytokines in between pulmonary sarcoidosis and pulmonary tuberculosis could be used to develop a model that can aid in their diagnosis.

P1-062 Diesel exhaust particles (DEP) exacerbate CNS autoimmune disease by increasing IL-23 production through the AhR signaling pathway in macrophages/microglia

Lee S[1], Choi S[1], Kang Y[1], Kim H[1,2], Lee W[1,2]
[1]Laboratory of Inflammation and Autoimmunity (LAI), Dept. of Biomedical Sciences and Dept. of Microbiology and Immunology, Seoul National University College of Medicine, [2]Institute of Infectious Diseases, Seoul National University College of Medicine

Background
Airborne Particulate Matter (PM) including diesel exhaust particles (DEP), is one of the major environmental risk factors due to its harmful effects on human health, particularly on the respiratory and cardiovascular systems. Accumulating epidemiological has implicated the pathogenic roles of PM in several CNS disorders such as Parkinson's disease, stroke, dementia, depression, and multiple sclerosis (MS), however, the mechanism of how PM affects neuroinflammation in the CNS remains unclear.
Methods
Experimental autoimmune encephalomyelitis (EAE) was induced in mice, which were intranasally exposed with or without DEP. The severity of symptoms was compared between the two groups. Immune cells infiltrated into the spinal cord of EAE mice and their cytokine production profiles were analyzed by flow cytometry. Human monocyte-derived macrophages (HMDM) and microglia-like cells (HMDMi) were used for investigating whether DEP induces the production of proinflammatory cytokines via the aryl hydrocarbon receptors (AhR) signaling pathway.
Results
We demonstrate that repeated intranasal DEP exposure aggravates EAE severity, showing an increased frequency of inflammatory macrophages and pathogenic IL-17A+GM-CSF+ CD4+ T cells among infiltrating immune cells in the spinal cord and an enhanced production of Th17-polarizing IL-23 but not Th1-polarizing IL-12 in the spinal cord compared with control mice. Using in vitro assay, we found that DEP acts as an exogenous ligand for AhR in HMDM and HMDMi. Inhibition of AhR by GNF351 shows that DEP-activated AhR contributes to an augmented production of IL-23 by LPS-stimulated HMDM.
Conclusion
These results provide insight into the pathogenic role of DEP in microglia/macrophages, which is critical in inflammatory responses in the CNS.

P1-063 Selectivity of Cytokine Receptor Cleavage by the Rhomboid Protease RHBDL2

Garbers C[1], Schumertl T[1], Kespohl B[1], Lokau J[1]
[1]Otto-von-Guericke University Magdeburg

The cytokines interleukin-6 (IL-6) and IL-11 activate their target cells by binding to specific IL-6 and IL-11 receptors (IL-6R/IL-11R) before inducing intracellular signaling cascades via homodimers of gp130. Thus, the expression profile of IL-6R/IL-11R determines which cells are responsive to these cytokines, as gp130 is expressed ubiquitously. However, there are also soluble agonistic forms of both receptors (sIL-6R/sIL-11R), and so-called trans-signaling via sIL-6R/sIL-11R widens the spectrum of cells that can be stimulated by both cytokines. This has important consequences, because IL-6 is already used as a therapeutic target in the clinic to treat inflammatory diseases, and antibodies targeting IL-11 signaling are in pre-clinical development. How these soluble cytokine receptors are generated is therefore of utmost importance. We have recently identified the rhomboid protease RHBDL2 as a so far unrecognized sheddase that can efficiently trigger soluble IL-11 receptor (sIL-11R) secretion. We determined the cleavage site used by RHBDL2, which is located in the extracellular part of the receptor in close proximity to the plasma membrane, between Ala-370 and Ser-371. Furthermore, we identified critical amino acid residues within the transmembrane helix that are required for IL-11R proteolysis. We also showed that ectopically expressed RHBDL2 is able to cleave the IL-11R within the early secretory pathway and not only at the plasma membrane, indicating that the subcellular localization of RHBDL2 plays a central role in controlling its activity. Moreover, RHBDL2-derived sIL-11R is biologically active and able to induce IL-11 trans-signaling. In contrast, the IL-6R is a rather bad RHBDL2 substrate compared to the IL-11R. Using a domain swapping approach and site-directed mutagenesis, we were able to identify molecular determinants that regulate cytokine receptor proteolysis by RHBDL2. Understanding the proteolytic generation of soluble cytokine receptors will help to design selective therapeutics targeting trans-signaling pathways and unravel how RHBDL2 recognizes its substrates.

P1-064 Determining lncRNA GAPLINC's Mode of Regulation in Sepsis

Escalona D[1], Carpenter S
[1]University of California, Santa Cruz

Sepsis occurs as a result of hyper activation of the immune system, producing a systemic cytokine storm that can lead to death. Sepsis is challenging to treat and the CDC indicates that 1 in 3 patients who die in a hospital setting have sepsis. Individuals who survive sepsis can have long-lasting cognitive and physical impairments, therefore there is a critical need to better understand the molecular mechanisms driving inflammation during endotoxic shock if we are to identify new therapeutic targets. Our main focus is on the role that long noncoding RNAs (lncRNAs) play in controlling inflammation. Here we describe the role for a lncRNA called GAPLINC (gastric adenocarcinoma predicted long intergenic noncoding RNA) in controlling the immune response during sepsis.
GAPLINC is highly expressed in macrophages averaging about 300 copies per cell. A knock down model of GAPLINC in primary human macrophages resulted in upregulation of a number of inflammatory genes even in the absence of stimulation. GAPLINC is dramatically downregulated upon inflammatory activation. We identified a conserved GAPLINC transcript in mice and generated a knockout using CRISPR technology. GAPLINC KOs were resistant to LPS induced shock and had altered cytokine production, even at baseline, consistent with findings in human macrophages. We recently generated a transgenic mouse line overexpressing GAPLINC which we subsequently crossed to our GAPLINC KO mice. We aim to determine the impacts that gain of GAPLINC has on the immune response and whether we can reverse the KO phenotype by crossing with our transgenic animals. Our ultimate goal is to understand the molecular mechanisms by which GAPLINC regulates the immune response during sepsis.

P1-065 Patients in remission in SLE have abundant activity and number of NK cells

Bennett T[1], Tang T[1], Kandane-Rathnayake R[1], Schuster I[2], Morand E[1], Delgi-Esposti M[2], Jones S[1]
[1]Centre for Inflammatory Diseases, School of Clinical Sciences, Monash University, [2]Department of Microbiology, Biomedicine Discovery Institute, Monash University

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that has suffered from an astonishing lack of treatment approaches and patients lack safe therapeutic options. While achieving clinical remission is the ultimate treatment goal in SLE, studies to date have mainly focused on the transcriptional programs that underlie active disease, such as the Type 1 IFN signature. We are interested in the molecular pathways that enforce remission, inspired by our knowledge that immunological quiescence is a state that is actively maintained. Therefore, to understand the molecular processes that enforce clinical quiescence, genes whose expression was upregulated in PBMCs from SLE patients in remission were compared to patients with active disease. Interestingly, we discovered enrichment of an NK cell signature in SLE patients in remission. The chemokine receptor CX3CR1 was strongly expressed in these patients, implicating this chemotactic pathway in remission. These findings were supported by the observation that there were increased numbers of circulating NK cells in patients in remission compared to those in active disease which negatively correlated with titers of anti-double stranded DNA antibodies. NK cell activation and function are regulated by cytokines including IL-15 and IL-2 and PBMCs from patients in remission displayed increased expression of IL2Rβ (also known as CD122), a core component of the IL-2/IL-15 receptor complex. Low-dose IL-2 is in clinical development for SLE treatment, and our findings may implicate a role for this therapy on NK cell function. While NK cell counts have previously been associated with lower disease activity in SLE, we have demonstrated for the first time that they are abundant specifically in patients in remission. This research has uncovered a novel biological pathway, and reveals potential biomarkers and therapeutic targets, associated with remission in autoimmune disease.

P1-066 INCREASED ANTIGEN PRESENTATION AND TUMOR IMMUNOGENICITY BY HDAC INHIBITOR, ABEXINOSTAT, VIA JAK/STAT PATHWAY IN NON-SMALL CELL LUNG CANCER

Shin E[1,2], Shin D[1]
[1]National Cancer Center, [2]Department of Cancer Biomedical Science, National Cancer Center Graduate school of Cancer Science and Policy

Histone deacetylase(HDAC) is an enzyme that represses antigen presenting by inducing heterochromatin structure. Antigen presenting plays a vital role in recognition of self/non-self antigen and cancer cells reduce the tumor antigen by interfering the recognition from other immune cells. Thus, in our studies, we investigated the effects of AXS, which is most effective HDAC inhibitor in our cell lines. AXS upregulated antigen presenting machinery(APM)-related genes and co stimulatory molecules as well as the level of histone acetylation. Among the 11 HDAC enzymes, HDAC1 is representative target of AXS and is a key factor of antigen presenting.

Furthermore, in the perspectives of tumor microenvironment, CCL2/MCP1 is dramatically secreted after treating AXS. Since CCL2 is known as monocyte/macrophage marker, we observed the increase of monocytes/macrophage and M1/M2 ratios.

All these mentioned effects are related to IFNγ, which is secreted by treating AXS. Using STAT1 siRNA, we observed the decreased expression level of protein related to the mentioned effects such as antigen presenting, APM, costimulatory molecules and M1 differentiation.

Taken together, AXS is a potential anti-cancer drug in NSCLC, increasing immunogenicity and regulating tumor microenvironment by IFNγ.

P1-067 HiChIP analysis revealed novel distal STAT5-bound enhancers in primary CD8+ T cells

Li P[1], Spolski R[1], Chandra V[2], Vijayanand P[2], Leonard W[1]
[1]National Institutes of Health (NIH), [2]La Jolla Institute for Immunology

Transcriptomic profiles determine the phenotype and function of cells, and this process is tightly controlled by various transcription factors (TFs), epigenetics, and chromatin interactions to define transcriptional patterns in response to cellular signals. Elucidation of the chromatin interactome provides a distinctive window into the complex nature of gene regulation. Here, we have investigated gene regulation by interleukin-2 (IL-2), which activate the JAK kinases and transcription factor STAT5, to elucidate the mechanism of how distal regulatory elements bound by STAT5 can control differential gene expression in T cell receptor activated mouse and human CD8+ T cells. By combining global RNA-Seq gene expression data with histone and transcription factor ChIP-Seq DNA binding patterns and linking these results to genome-wide chromatin interaction analysis with H3K27ac HiChIP (mouse) and RNA Pol II ChIA-PET (human), we provide the detailed analysis of IL-2-induced STAT5 in mediating long-distance chromatin interactions in primary mouse and human CD8+ T cells. These results reveal the new role of IL-2-activated STAT5 binds at distal enhancers to regulate target genes expression from novel and distal regulatory elements via DNA looping.

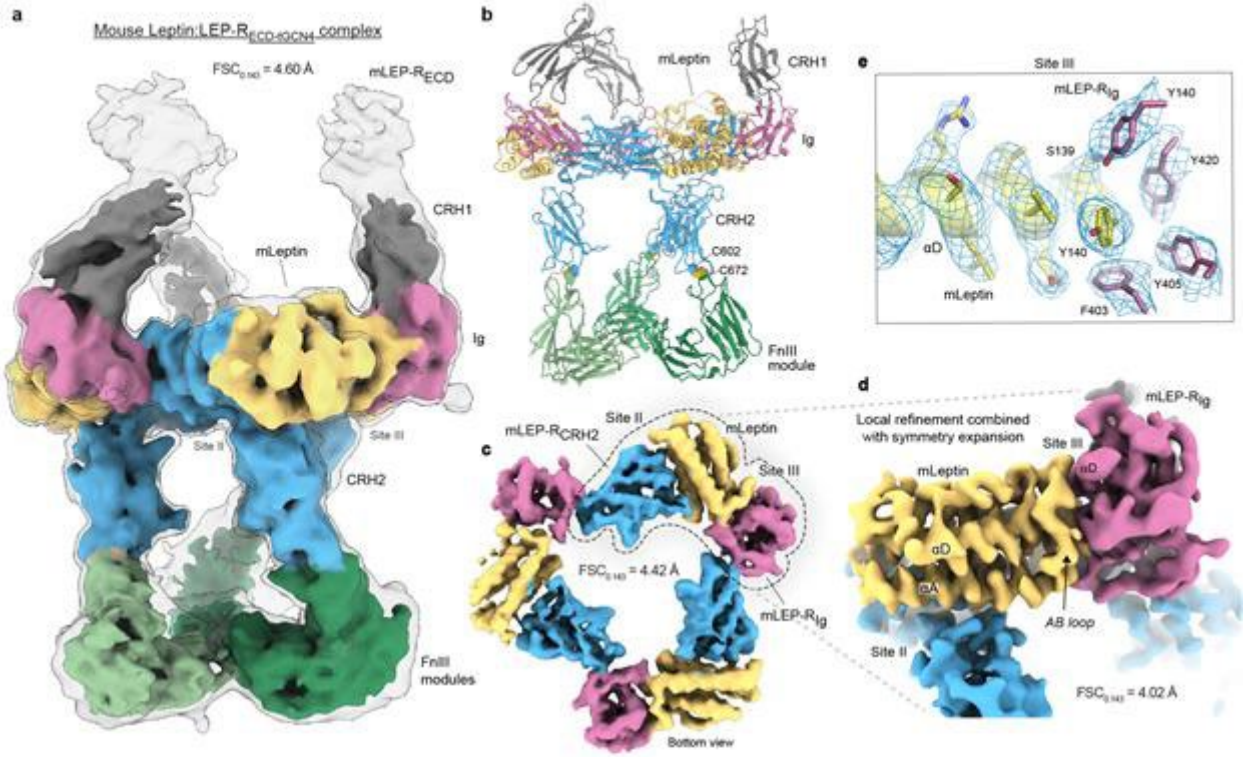
P1-068 Mechanism of receptor assembly via the pleiotropic adipokine Leptin

Tsirigotaki A[1], Dansercoer A[1], Verschueren K[1], Markovic I[1], Hafer M[2], Pollmann C[2], Felix J[1], BIRCK C[3], Van Putte W[4], Catteeuw D[5], Tavernier J[6], Bazan F[1], Piehler J[2], Savvides S[1], Verstraete K[1]
[1]Flemish Institute for Biotechnology and Ghent University, [2]Osnabrück University, [3]University of Strasbourg, [4]Puxano, [5]Ghent University, [6]Orionis Biosciences

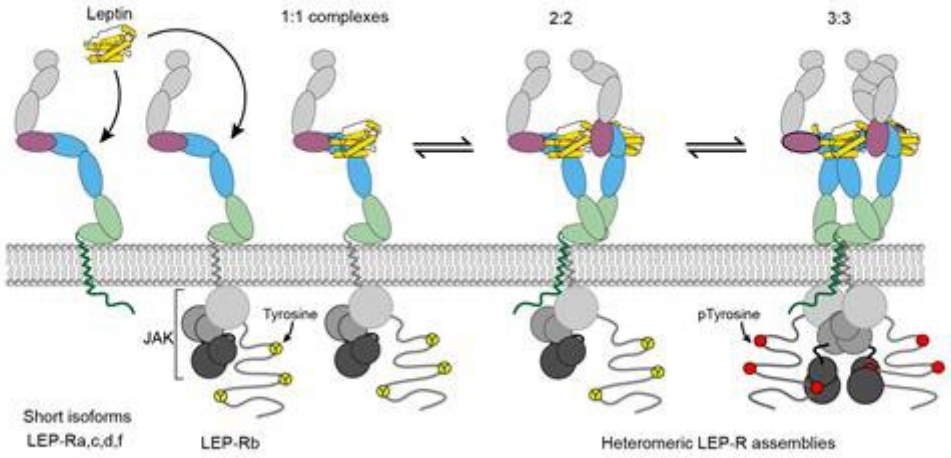
The adipokine Leptin activates its receptor LEP-R in the hypothalamus to regulate body weight and exerts additional pleiotropic functions in immunity, fertility, and cancer. However, the structure and mechanism of Leptin-mediated LEP-R assemblies has remained unclear. Intriguingly, the signaling-competent isoform of LEP-R is only lowly abundant amid several inactive short LEP-R isoforms contributing to a mechanistic conundrum. Here, we show by X-ray crystallography and cryo-EM that Leptin induces unprecedented type I cytokine receptor assemblies featuring 3:3 stoichiometry and demonstrate such Leptin-induced trimerization of LEP-R on living cells via single molecule microscopy. In mediating these assemblies, Leptin undergoes drastic restructuring that activates its site III for binding to the Ig-domain of an adjacent LEP-R. These interactions are abolished by mutations linked to obesity. Collectively, our study provides the structural and mechanistic framework for how the 3:3 stoichiometry of the evolutionarily conserved Leptin:LEP-R complex might support signaling-competent complexes comprising distinct LEP-R isoforms.

Reference:
Tsirigotaki A, Dansercoer A, Verschueren KHG, Marković I, Pollmann C, Hafer M, Felix J, Birck C, Van Putte W, Catteeuw D, Tavernier J, Fernando Bazan J, Piehler J, Savvides SN, Verstraete K. Mechanism of receptor assembly via the pleiotropic adipokine Leptin. Nat Struct Mol Biol. 2023 Apr;30(4):551-563. doi: 10.1038/s41594-023-00941-9. Epub 2023 Mar 23. PMID: 36959263.

Structural basis of trimerized LEP-R mediated by Leptin



Possible Leptin-mediated LEP-R assemblies at the cell surface comprising different stoichiometries of LEP-R isoforms.



Nat Struct Mol Biol. 2023 Apr;30(4):551-563. doi: 10.1038/s41594-023-00941-9.

P1-069 IL-23/IL-17 axis function differs according to disease stage in the patients with axial spondyloarthritis/ankylosing spondylitis

Yeo J[1], Lee H[1], Seo M[1], Choi H[1], Kim M[2], Kwon H[2], Lee E[2], Baek H[1]
[1]Gachon University College of Medicine Gil Medical Center, [2]Seoul National University College of Medicine

Background
: Interleukin (IL)-23 induces the differentiation of naive CD4+ T cells into highly pathogenic helper T cells producing IL-17. This IL-23/IL-17 axis might play a central role in pathogenesis of immune mediated inflammatory diseases including ankylosing spondylitis (AS). However, IL-23 blockade failed to show a significant effect, whilst IL-17 inhibition has a good effect, in the patients with AS. This study aimed to evaluate the function of the IL-23/IL-17 axis in terms of disease stages of axial spondyloarthritis (axSpA)/AS
Methods
: We assessed the levels of IL-17 and IL-23 in serum and the fraction of CD4+ IL-17+ cells, monocytes (Mo, CD14+ HLR-DR+), dendritic cells (DC, CD11c+ HLA-DR+), and type 1 regulatory T cells (Tr1, CD4+CD49b+LAG3+FoxP3-IL-10+) of peripheral bloods obtained from the patients with axSpA using enzyme-linked immunosorbent assay and flow cytometry. The comparison was made between early disease (pre-radiographic [pr-] axSpA) and advanced disease (radiographic axSpA/AS). We also assessed the production of IL-17 from T cells following co-culture with Mo-derived DC from the patient of pr-axSpA compared with from the patient with AS.
Results
: Serum levels of IL-17 and the percentages of CD4+ IL-17+ cells were similar between pr-axSpA and AS. However, serum levels of IL-17 and the percentages Mo and DC were significantly higher in pr-axSpA than in AS. Production of IL-17 from T cells following co-culture with monocyte-derived DC from the patient of pr-axSpA was increased compared with AS. The fraction of Tr1 was elevated in AS compared with in pr-axSpA
Conclusions
: IL-23 might play a pathogenic role only in early disease of axSpA and IL-17 might be dissociated from IL-23 in advanced AS with enhanced regulatory counterpart, which requires further elucidation.

P1-070 In vivo responses in the peritoneal cavity to LPS and their modulation by type I IFN

Alvarez-Martinez M[1], Slawinski H[2], Wu X[1], O'Garra A[1,3]
[1]Immunoregulation and Infection Laboratory, The Francis Crick Institute, [2]Advanced Sequencing Facility, The Francis Crick Institute, [3]National Heart and Lung Institute, Imperial College London

It is well documented that the peritoneal cavity is mostly composed of large peritoneal macrophages (LPM) and B cells at the steady state, while LPS challenge induces resident LPM and B cells to adhere to the mesothelium and efflux to the omentum. LPS challenge also results in the recruitment of monocytes and neutrophils into the peritoneal cavity. We have recapitulated these cellular changes using flow cytometry and single-cell RNA-sequencing (scRNA-Seq) at the steady state and during LPS challenge, which allowed for in-depth analysis of the cellular changes and heterogeneity observed. Type I IFN induced by various pathogens and their products, for example LPS, has complex effects, including modulation of recruitment and/or cytokine production by immune cells. Here we report that LPS-induced recruitment of monocytes and neutrophils and their expression of proinflammatory genes is partially decreased in the absence of type I IFN signalling as seen in Ifnar1-/- mice as compared to wild-type (WT) mice. Additionally, bioinformatic analysis of ligand-receptor interactions induced by LPS showed reduced signalling to neutrophils in LPS-treated Ifnar1-/- mice, with the abrogation of G-CSF, prokineticin, CXCL2/CXCR2 and IFN-γ pathways that contribute to neutrophil differentiation, survival, migration or activation. Moreover, resident LPM and B cells were less reduced upon LPS challenge of Ifnar1-/- mice as compared to their WT counterparts, which may be explained by sustained interaction between CXCL13 from LPM to CXCR5 on B cells in LPS-treated Ifnar1-/- mice which was not observed in LPS-treated WT controls. Collectively our findings from the scRNA-Seq data analysis demonstrate the heterogeneity of effects induced by LPS in the peritoneal cavity and the complexity of type I IFN signalling in this context, which has implications for the regulation of the immune response during inflammation and bacterial infection.

P1-071 Engineering and molecular characterisation of new human designer cytokine IL-23α/p19 and EBI3

Bergmann C[1], Feige M[1], Zacharias M[1]
[1]Technical University Munich

The human interleukin-12 (IL-12) cytokine family comprises a group of four heterodimeric members: IL-12, IL-23, IL-27, and IL-35 that play pivotal roles in mediating immune cell functions. These cytokines possess unique structural properties and exhibit diverse functions in both innate and adaptive immunity. Each family member is composed of a 4-helical bundle α-subunit (IL-23α/p19, IL-27α/p28 or IL-12α/p35) and a β-subunit either consisting of two fibronectin domains (IL-27β/EBI3) or one immunoglobulin and two fibronectin domains (IL-12β/p40). Hence, nature only uses 3 α- and 2 β-subunits to assemble all four IL-12 heterodimers and in principle, six αβ pairs should be possible.

In mice, interleukin-39 (IL-39), has recently been described as a key regulator of several immune processes, including inflammation and autoimmune diseases. IL-39 is composed of the IL-23α/p19 subunit and the Epstein-Barr virus-induced gene 3 (EBI3) subunit. Its existence in humans remains debated.

Here, we verify the absence of this pair in humans via the characteristic feature of assembly-induced secretion. All human α-subunits depend on their corresponding β-subunit to be secreted. These findings were validated by co-immunoprecipitation experiments from mammalian cells, where no interaction between human IL-23α/p19 and EBI3 could be shown.
Based on MD simulations and docking experiments the missing pair of IL-23α/p19 and EBI3 was rationally designed. Mutations were implemented in the protein interface leading to a stable novel heterodimer. This novel heterodimer is currently investigated structurally and functionally and will thereafter constitute a novel member of the human IL-12 family

Taken together, our study will provide a well-defined newly engineered IL-12 family member. This engineered cytokine may prove useful as a new biologic or bio-orthogonal cytokine to regulate engineered immune cells.

P1-072 Identification of a Novel Salivary Diagnostic Biomarker in Rheumatoid Arthritis

Lee Y[1], Kim M[1], Hong S[1], Chung S[1]
[1]Division of Rheumatology, Department of Internal Medicine, Kyung Hee University Hospital

Background:
Diagnosis of rheumatoid arthritis is based on clinical features, radiographic images and serological markers, such as rheumatoid factor (RF) and anti-citrullinated peptide antibodies (Anti-CCP). Calprotectin (S100A8/S100A9 protein) is known as a damage-associated molecular pattern (DAMP) protein and reflects mainly neutrophil activation. Recent studies have observed an increased calprotectin level in blood and synovial fluid from RA patients. This study aims to investigate whether salivary calprotectin has the potential to be utilized as a biomarker in the diagnosis or monitoring of RA.
Methods:
We performed this cohort study including 158 patients with RA and 35 Healthy Control (HC). Unstimulated whole saliva was collected by the spitting. Salivary calprotectin was assessed using the ELISA (Arigo biolaboratories, ARG82070 Taiwan). Serum Anti-CCP was measured by the ECLIA (Roche (Cobas e601, Elecsys).
Results:
Our study recruited 158 RA (82% female, mean age 59.5 years, mean RA duration 10.2 years) and 35 HC (87% female, median age 46.7 years). RA patients had a higher level of calprotectin of 22.1μg/ml than HC salivary calprotectin of 12.1μg/ml (P=0.018). There was no noticeable difference between SNRA and SPRA in saliva calprotectin (SPRA salivary calprotectin mean of 22.6 μg/mL and a mean SNRA salivary calprotectin of 21.0 μg/mL. P= 0.920). Salivary calprotectin had no correlation with ESR, CRP and RA disease activity score. But anti-CCP levels were statistically different across saliva calprotectin quartiles. (P=0.035). Salivary calprotectin levels were higher in RA patients with Joint Space Narrowing (JSN) (P=0.042) Using the ROC curve analysis, 13.9 μg/mL was determined to be cut-off value. Specificity and sensitivity for this value were reported as 68.57% and 52.53%, respectively.
Conclusion:
Salivary calprotectin can be used as a surrogate marker for diagnosis of RA

P1-073 Aging in Down syndrome is associated with inflamed cytokine milieu and with the elevation of the wound healing cytokine TGFb-3

Ten Caten F[1], Coirada F[1], Majithia M[1], Romano Bruno F[1], Del Rio P[1], Malheiros J[2,3], Covolan L[2,3], Pereira Ribeiro S[1]
[1]Emory University, [2]Department of Physiology, Federal University of Sao Paulo, [3]Center for Research, Education and Innovation (CEPI), Instituto Jo Clemente

Down syndrome (DS) is the most common chromosomal disorder as it occurs in 1:700 live births. Chromosome 21 encodes 4/6 IFN receptor subunits. The trisomy predisposes affected individuals to a wide range of comorbidities including immunological and neurological disorders, most of which are associated with inflammation. To evaluate the inflammatory milieu in these individuals, a cohort of equally distributed DS and non-DS people was recruited (n=120; age range [12-56 years old]). Forty-three cytokines were evaluated in the plasma of this cohort. DS individuals presented significantly higher plasma levels of 18/43 evaluated analytes (Fig 1) including chemokines associated with the migration of innate and adaptive cells (i.e. MCP1 and 2, MIP3b, SDF1a, ITAC, FLT3LG) and inflammatory cytokines. (i.e. IL-6, IP-10). This inflammation was associated with the upregulation of IL-10, an anti-inflammatory cytokine which promotes other anti-inflammatory cytokines such as the three isoforms of TGFb. This inflammatory milieu is reported to be associated with tissue damage and of note, the wound healing cytokine TGFb3, was significantly elevated in the plasma of DS individuals and positively associated with the elevated IL-10 plasma levels. Interestingly, none of the IFN cytokines (type I, II, III) were significantly elevated probably due to their consumption by target cells that highly express 4/6 IFN receptors in DS individuals, resulting in the intrinsic upregulation of IFN/JAK/STAT pathways. Importantly, plasma levels of IL-6, IP-10, IL-23 and TNFa and TGFb3 were upregulated at significantly higher levels in older DS individuals. These data highlight the inflammatory milieu present in DS individuals and the induction of anti-inflammatory counterplayers, important to tone down inflammation and to maintain tissue integrity. These data highlights pathways to be targeted to improve immune regulation and the quality of life in DS individuals.

P1-074 IMMUNE RESPONSES IN MULTIPLE SCLEROSIS: A NEURO-AUTOIMMUNE DISSORDER

Patil S[1], M N[1], TALY A[1], D N[1]
[1]National Institute Of Mental Health And Neurosciences, Bangalore, I N D I A

Multiple scerosis is a neuro-autoimmune disorder triggered mainly by autoreactive T cells. Chronic inflammation, demyelination and gliosis are pathological conditions encountered. Perivascular white matter changes with punched out lesions and generlised atrophy is seen in brain and spinal cord of patients with MS.
In the present study cytokines, the immune modulators were evaluated in the CSF of patients with MS in relation to oligoclonal study. The cytokines, tumor necrosis factor TNF alpha, Interferon gamma, interleukin 4, interleukin 6 and Nitric oxide were evaluated using standard protocol given by BD biosciences. NO was estimated indeginously. Oligoclonal band was detected by agar immunoelectrophoresis.
It is observed that cytokines are expressed in the CSF of patients with MS. IL-4 was the predominantly expressed cytokine to the tune of 75%, whereas the other cytokines (IL-6, TNF-alpha and IFN-gamma were expressed to the extent of 25%. Nitric oxide was seen in relatively lesser CSF samples.
Oligoclonal band was expressed to the extent of 80% in the CSF of MS.
It appears that the cytokines do play pathological role in MS. Oligoclonal band which is a synthesis of abnormal immunoglobulin is expressed more than cytokines put together. It appears that the small percentage of cytokines present in the CSF of MS might trigger a major pathological reaction in CNS.

P1-075 Serum levels of IL-1beta, IL-18 and their inhibitors in systemic autoinflammatory diseases :Preliminary results from the Immunome project consortium for AutoInflammatory Disorders (ImmunAID) cohort

Girard-Guyonvarc'h C[1,2], Rodriguez E[1], Caruso A[1], Gabay C[1,2]
[1]Department of Pathology and Immunology, University of Geneva, Faculty of Medicine, [2]Division of Rheumatology, Department of Medicine, University Hospitals

Systemic autoinflammatory diseases (SAID) are rare disorders caused by inadequate activation of innate immune responses. They are characterized by recurrent bouts of inflammation leading to significant morbidity and mortality. Diagnosis and management of SAID can be challenging. ImmunAID is a multicenter project which aims to collect multiple clinical and biological data in order to better characterize monogenic and genetically undiagnosed SAID.
IL-1β and IL-18 are proinflammatory cytokines of IL-1 family that are released out of the cells as mature and active cytokines upon activation of inflammasome-caspase-1 pathway. We measured serum levels of IL-1β and IL-18 and their natural inhibitors IL-1Ra and IL-18 binding protein (IL-18BP) in patients recruited in the ImmunAID cohort, using immunoassays. Of note, as only free IL-18BP-unbound IL-18 reflects IL-18 bioactivity, we also assessed serum levels of free IL-18.
Sera from 148 patients and 14 healthy controls were analyzed. Levels of total IL-18 were markedly elevated in adult-onset Still's disease (AOSD), systemic juvenile idiopathic arthritis (sJIA) and familial Mediterranean fever (FMF), while free IL-18 levels were only significantly increased in AOSD and sJIA patients. IL-1β serum levels were extremely low and not significantly different between groups. IL-1Ra levels were the highest in sJIA patients and, unlike IL-1β, correlated with total IL-18 levels. Overall, we observed substantial variations of cytokine levels within each group of patients. We could not demonstrate any correlation between IL-18, IL-18BP, IL-1β and IL-1Ra levels and the presence of fever, ongoing treatment, or C-reactive protein (CRP) levels. Of note, there were significant and positive correlations between both total and free IL-18 and ferritin levels.
In conclusion, our results suggest a substantial heterogeneity of SAID regarding the levels of measured mediators. Total IL-18 and especially free IL-18 were specifically elevated in AOSD and sJIA and correlated with ferritin but not with CRP levels.

P1-076 Regulation of transcription at TNF loci in mouse primary immune cells.

Cho M[1], Woo W[1], Dho S[2], Kim L[2]
[1]BK21 PLUS Project for Medical Science, Yonsei University College of Medicine, [2]Yonsei University College of Medicine

Background: Tumor Necrosis Factor (TNF) is a major cytokine secreted by multiple immune cells that can cause various diseases, including psoriasis, autoimmune disease, and chronic disease. Therefore, it is important to understand the process of controlling TNF expression, which is regulated by various regulatory elements. Enhancer RNA (eRNA) is a non-coding RNA that is transcribed from enhancer regions. In the canonical pathway, it is a feature of the enhancer activation for this region. However, recent studies have shown that not only enhancers but also eRNAs can regulate gene expression by several mechanisms, such as regulating RNA polymerase.
Methods: To investigate the role of Y enhancer in TNF expression, we generated mice with a Y enhancer deletion. We examined the expression of eRNA in the Y enhancer and confirmed its role by treating with ASOs that target the corresponding eRNA.
Results : We identified that LPS treatment in BMM induces TNF expression, activates enhancer Y, and induces eRNA transcribed from the Y enhancer region. Furthermore, we found that the deletion of the Y enhancer alleviates TNF expression in mouse bone marrow cells and derived macrophages. To investigate whether Y eRNA plays a role in TNF expression, we designed anti-sense oligos (ASOs) targeting Y eRNA and electroporated them into BMM. Surprisingly, the production of TNF-a was reduced in ASO-treated BMM.
Conclusion: Our findings indicate that Y eRNA is a potential target for regulating TNF expression.

P1-077 STAT3 activation by the type I IL-20 receptor: two ways are better than one.

Calà O[1], Courtain C[1], Dumoutier L[1]
[1]UCLouvain

The interleukin-19 (IL-19), interleukin-20 (IL-20), and interleukin-24 (IL-24) are pro-inflammatory cytokines acting on non-immune cells, such as keratinocytes. Under physiological conditions, they have a beneficial role in immune defense and tissue regeneration by promoting antimicrobial peptide production, keratinocyte proliferation, and chemokine expression. However, excessive and uncontrolled production of these cytokines is harmful and can lead to inflammatory diseases, such as psoriasis. Therefore, it seems important to block the deleterious effects of IL-19, IL-20 and IL-24 while leaving the good ones unaffected.

To elicit their biological effects, IL-19, IL-20 and IL-24 bind to the type I IL-20 receptor (IL-20R), composed of the IL-20Ra and IL-20Rb chains. IL-20 and IL-24 also bind to the type II IL-20R, consisting of the IL-22Ra and IL-20Rb chains. Upon binding to their receptor, these three cytokines activate the JAK-STAT signaling pathway.

By through studying the type I IL-20R signaling, we recently discovered that, in addition to the canonical activation pathway, STAT3 is activated via a non-canonical pathway that is entirely independent of tyrosine residues. Indeed, cells expressing a mutant tyrosine-less IL-20Ra chain still exhibit an activation of STAT3 but not of STAT1 nor STAT5. This non-canonical activation pathway depends on the pre-association of STAT3 and the IL-20R-Cter region and is similar to the one discovered in the IL-22R, suggesting that several members of the IL-20 subfamily of cytokines share a tyrosine-independent STAT3 activation. Furthermore, we are using a reconstructed human epidermis (RHE) model expressing different type I IL-20R mutants to assess the role of STAT3 and the IL-20R C-ter region on epidermal structure and differentiation.

Since STAT3 plays a significant role in the development of several inflammatory diseases like psoriasis, we believe that blocking the IL-20R-Cter region, and thereby reducing STAT3 activation, may represent a novel targeted therapy.

P1-078 Investigating the role of IL-20-related cytokines in inflammatory bowel disease

Stewardson C[1], Dumoutier L[1]
[1]UCLouvain

Inflammatory bowel disease (IBD), encompassing Crohn's disease and ulcerative colitis, is a group of complex multifactorial diseases with increasing incidence in Europe. IBD appears to be driven by many different mechanisms, including dysregulation in the Th1, Th17 and Tregs populations. Other important elements that are modulated in the context of IBD are the IL-20-related cytokines, which we have been studying in our laboratory for many years. These cytokines are all upregulated in the biopsies and sera of IBD patients. Certain polymorphisms in their genes have been associated with an increased risk of developing the disease.

The IL-20 related cytokine family comprises IL-19, IL-20, IL-22, IL-24 and IL-26, with IL-22 being the best characterized cytokine of the group. They act on epithelial cells and signal via different combinations of 4 receptor chains: IL-10Rß, IL-20Ra, IL-20Rß and IL-22R. These cytokines have been shown to play a role in various pathologies in which the mucosa is involved, such as psoriasis. In IBD, our team and others have already demonstrated that IL-22 has a protective effect: it attenuates colitis by stimulating enterocyte proliferation, mucus production and promoting wound healing. We are now investigating the role of the other cytokines of this family in IBD.

To this end, we first investigated the effect of the IL-20-related cytokines on human colorectal cancer cell lines, as well as in mice colon, intestine and esophagus. Furthermore, we have used the Citrobacter rodentium model of colitis to assess whether mice deficient for IL-19, IL-20 and IL-24 are differentially affected by the pathogen. We are also characterizing the expression pattern of the IL-20-related cytokines in immune cell subpopulations in healthy and diseased donors using scRNAseq. This will enable us to better understand the role of these cytokines in IBD and how they could potentially be targeted in patients.

P1-079 Type I interferon expression requires transcriptional activation of IRF7 by the RNA binding protein SRSF7

Scott H[1], Smith M[1], Coleman A[1], Wagner A[1], Watson R[1], Patrick K[1]
[1]Texas A&M School Of Medicine

While the signaling cascades and transcription factors that turn on expression of innate genes are well-characterized, the role of RNA binding proteins in activating and resolving this response is poorly understood. Members of the serine/arginine-rich (SR) family of mRNA processing factors play diverse roles in transcription, pre-mRNA splicing, export, and translation. Recent transcriptomics analysis revealed that knockdown of one such SR protein, SRSF7, dampened expression of type I interferon (IFN) and interferon stimulated genes (ISGs) both in resting cells and in cells infected with the gram-negative bacterial pathogen Salmonella enterica serovar Typhimurium. Srsf7 knockdown macrophages fail to control replication of vesicular stomatitis virus (VSV), demonstrating a bona fide role for SRSF7 in antiviral immunity. Based on multiple lines of evidence, we concluded that the Srsf7 knockdown ISG defect is reminiscent of loss of a master regulator of the type I IFN response. Indeed, we found that SRSF7 plays a privileged role in maintaining proper levels of the transcription factor IRF7 in macrophages. Suspecting a role for SRSF7 in Irf7 transcription, we performed chromatin immunoprecipitation and observed a profound defect in STAT1 recruitment to the Irf7 promoter in the absence of SRSF7. This was concomitant with a build-up of RNA polymerase II in the same genomic region. Consistent with a direct role for SRSF7 in coordinating STAT1 recruitment and RNAPII promoter clearance at the Irf7 gene, ChIP-qPCR and ChIP-seq both measured enrichment of SRSF7 itself at the Irf7 promoter. These data reveal an unexpected role for SRSF7, a protein best characterized as a splicing factor, in activating transcription. They also highlight a critical role for IRF7 in controlling tonic and early interferon expression in macrophages and add to growing literature that suggests Irf7 expression is subject to multiple levels of control mediated by RNA binding proteins.

P1-080 CTLA-4 signaling peptide induces tissue-regulatory T cells in response to IL-33 and ameliorates experimental autoimmune encephalomyelitis.

Kim G[1], Choi J[1]
[1]Department of Life Science, College of Natural Sciences, Hanyang University

In our previous study, we demonstrated that CTLA-4 signaling peptide induces Foxp3+ regulatory T (Treg) cells by increasing TGF-β signaling, which modulates experimental autoimmune encephalomyelitis (EAE) progression and relapse. In this study, we further investigated whether CTLA-4 signaling peptide could induce tissue-Treg cells in the spinal cord of EAE. Our findings revealed that CTLA-4 signaling peptide can induce IL-33R+ Treg cells in vitro in response to IL-33 signaling, which are involved in inducing tissue-repair Treg cells. Transcriptomic analysis showed increased expression of tissue-Treg-related genes encoding IL-33, IL-33R and CD103 proteins in Foxp3+ T cells induced by CTLA-4 signaling peptide. Furthermore, we found that the CTLA-4 signaling peptide induced CD69+CD103+ tissue-resident Treg cells in the spinal cord of both the MOG35-55 EAE model and the PLP139-151 relapse model. Mechanistically, we identified that the lysine residue of CTLA-4 physically interacts with PKC-η, and PKC-η inhibitory peptide can induce IL-33R+ CD103+ Treg cells in the spinal cord of EAE mice. Consistent with these results, PKC-η mRNA levels are lowered in tissue-Treg cells compared to circulating Treg cells. Therefore, our findings suggest that the CTLA-4 signaling peptide plays a role in inducing tissue-Treg cells in response to IL-33 signaling, and it may be a potential therapeutic target for autoimmune diseases such as multiple sclerosis.

P1-081 Primary Immune Regulatory Disorders (PIRDs) that amplify mTOR signaling have shared T cell exhaustion-like process

Conrey P[1], Campos J[1,2], Mauracher A[1], Sayed S[1], Walter J[3], Su H[4], Leiding J[5], Cooper M[6], Macfarland S[1], Ruffner M[1], Farmer J[7], Rao K[4], Henrickson S[1,2]
[1]Children's Hospital Of Philadelphia, [2]University of Pennsylvania, [3]University of South Florida, [4]NIAID, NIH, [5]Johns Hopkins University, [6]Washington University in St. Louis, [7]Lahey Health

Background: Primary Immune Regulatory Disorders (PIRDs) are a complex and challenging to treat subset of Inborn Errors of Immunity (IEI), characterized by immune dysregulation, including recurrent infections, autoimmunity and lymphoproliferation. Given that there are many ultra-rare monogenic PIRDs, it will not be feasible to design targeted therapies for each one. An alternate strategy for precision medicine in PIRDs is to identify shared aspects of T cell dysfunction and strategies to target them. We have focused our studies on PIRDs that chronically amplify T cell receptor (TCR) signaling; mimicking chronic infection. In the setting of chronic inflammation and antigen presentation (i.e., chronic infections), CD8 T cell exhaustion (Tex) can result and is characterized by increased inhibitory receptor expression, altered transcriptional networks, epigenetic poise and impaired T cell function, including cytokine production.
Methods: Deep immune phenotyping and T cell functional analysis via CyTOF and spectral flow cytometry and single cell RNA-sequencing and CITE-seq of PIRD patient and healthy control PBMCs.
Results: We identified a Tex-like process in activated PI3 kinase delta syndrome (APDS), CTLA-4 haploinsufficiency and Ras-associated Autoimmune Leukoproliferative Disease (RALD), which share increased mTOR activation. We identified altered CD8 T cell immunophenotype consistent with Tex (e.g., increased PD-1, CD39, TIGIT and TOX). We also evaluated function and, using cytokine and proliferation assays, found impaired CD8 T cell function consistent with Tex. Lastly, we have used CRISPR/Cas9 to edit CTLA-4 in healthy control CD8 T cells towards creating a model of CTLA-4 haploinsufficiency (and, in the future, APDS and RALD) for perturbation studies to evaluate best available therapies and target novel therapeutic targets in these rare disorders.
Conclusion: By identifying shared patterns of CD8 T cell dysfunction in these ultra-rare disorders, we may both identify novel therapeutic strategies and increase our understanding of control of CD8 T cell function, including cytokine production.

P1-082 Functional and molecular analysis of SKAP2 in T lymphocytes

SPINELLA M[1], Kenyon A[2], Bianchi N[1], Gualdrini F[2], Low J[1,3], Polletti S[2], Džafo E[1], Sallusto F[1,3], Natoli G[2,4], Monticelli S[1,4]
[1]Institute for Research in Biomedicine, Università della Svizzera italiana, [2]Department of Experimental Oncology, European Institute of Oncology (IEO) IRCCS, [3]Institute of Microbiology, ETH Zürich, [4]equal contribution

Background
Inflammatory bowel disease encompasses two idiopathic chronic inflammatory disorders affecting the gastrointestinal tract: Crohn's disease (CD) and ulcerative colitis. The genetic component, primarily consisting in single nucleotide polymorphisms (SNPs), appears to play a prominent role as demonstrated by the 200 risk loci identified by genome wide association studies. However, for most of them, the causal variants, genes, and pathological cell types in which these variants exert their effects have not been elucidated yet.

Methods
A combination of different datasets and information obtained by fine-mapping, sequence constraint and expression quantitative trait loci (eQTL) analyses drew our attention to the SKAP2 locus. It contains 194 non-coding SNPs in linkage disequilibrium and a single gene, encoding Src kinase-associated phosphoprotein 2 (SKAP2), whose function has been characterized in myeloid cells and linked to integrin-mediated cell adhesion/migration. Interestingly, eQTL analysis revealed an increase of SKAP2 expression in CD4+ T cells and CD risk. Additionally, Skap2-deficient mice are resistant to experimental autoimmune encephalomyelitis, a T cell-mediated disease. Thus, we hypothesized that SKAP2 expression levels as determined by genetic variation may affect TCR activation, impacting autoimmunity risk. To unveil SKAP2 role in human T cells we used a combination of functional and molecular approaches.

Results
Our data showed that SKAP2 expression is highly regulated in T cells both at the transcriptional and post-transcriptional level. We identified the ensemble of SKAP2 interactors, most of which have a known role in proximal TCR signalling and LFA-1 activation. Furthermore, we found that caspases regulate SKAP2 function by mediating its cleavage following T cell activation. Ablation of SKAP2 expression led to a reduced ability to produce IFN-γ and TNFα in primary human T cells, whereas overexpression had the opposite effect.

Conclusions
Our results point towards a role for SKAP2 in modulating T cell functions and risk of CD.

P1-083 Human heterozygous TBK1 variations lead to autoinflammation

Akalu Y[1], Richardson A[1], Taft J[1], Buta S[1], Milner J[2], Peng X[3], Meyts I[4], Bogunovic D[1]
[1]Precision Immunology Institute, Icahn School Of Medicine At Mount Sinai, [2]Division of Allergy, and Rheumatology, Department of Pediatrics, Columbia University Medical Center, [3]Department of Genetic Medicine, Johns Hopkins University School of Medicine, [4]Department of Microbiology and Immunology, Catholic University of Leuven

Background: TANK binding kinase 1 (TBK1) is a signaling intermediate that functions in the induction of both type I interferon (IFN-I) expression and nuclear factor-κB-mediated inflammatory cytokine production. Concomitantly, TBK1 is also shown to negatively regulate TNF-induced cell death through inactivation of RIPK1. Previous work from our laboratory has identified patients with biallelic loss-of-function mutations in TBK1 that exhibit early-onset autoinflammatory syndrome. While the loss of TBK1 expression results in hypomorphic induction of IFN-I, there nevertheless remains sufficient antiviral response in these patients. Notably, the absence of TBK1 expression also releases the brake on RIPK1, thus favoring a RIPK1-mediated cell death that ultimately drives autoinflammation.

Hypothesis: Our prototype TBK1 variations are gain-of-function (GoF).

Methods: The impact of TBK1 variantions on IFN-I response, NF-κB signaling, and induction of inflammatory cell death is assessed by overexpressing wild-type or variant TBK1 constructs in cells with no or WT TBK1 expression.

Preliminary Results: We have discovered five new patients, comprised of children and adults, suffering from type I interferonopathies, a distinct form of chronic autoinflammation. Genetically, they harbor four novel heterozygous mutations in TBK1. Computationally, we predict a change in protein function resulting from these variations. Ex vivo, evaluation of a panel of interferon-stimulated genes (ISGs) in the blood of these patients revealed that some variations of TBK1 can lead to increased expression of these genes. In vitro, cells carrying the prototype TBK1 variations produced TBK1 protein at comparable levels to WT and resulted in higher pTBK1 and pSTAT1 levels, at baseline.

Conclusions: Variations in TBK1 contribute to a range of inflammatory conditions in children and adults. These TBK1 variations can increase ISG expression and biochemical activity of TBK1, indicators of possible TBK1 GoF. How these TBK1 variations simultaneously regulate IFN-I, NF-κB and RIPK1 function remains to be defined.

P1-084 IL-17-induced C/EBPδ underlies inflammation in autoimmune kidney pathology

Dey I[1], Li Y[1], Biswas P[1], Sterneck E[2], Gaffen S[1]
[1]University of Pittsburgh, [2]National Cancer Institute

Autoimmune disorders are a global concern due to increasing prevalence and limited treatment options. Chronic kidney disease (CKD) affects 10-15% of the world's adult population, but our understanding of the underlying disease pathogenesis remains poorly understood. Experimental autoantibody-induced glomerulonephritis (AGN) is characterized by the deposition of autoantibodies at the glomerular basement membrane (GBM), which lead to tissue pathology. This disease is primarily associated with immune cell infiltration and elevated expression of chemokines and cytokines (IL-6), as well as kidney injury markers such as lipocalin 2 (Lcn2, also known as NGAL). IL-17 is a key driver of AGN and mediates kidney-specific inflammation selectively in renal tubular epithelial cells (RTECs). To date, the downstream signaling mechanisms that orchestrate IL-17R signaling are only partially defined. It has long been known that IL-17 promotes expression of the CCAAT/Enhancer binding protein (C/EBPδ) transcription factor, but to the full extent its contribution to the IL-17 signal transduction program is unclear. We show that C/EBPδ is expressed in RTECs human renal biopsies from glomerulonephritis patients and is consistently elevated in various pathological conditions of the kidney. In keeping with this, mice lacking C/EBPδ (Cebpd-/-) exhibited markedly reduced renal pathology compared to WT controls. Moreover, C/EBPδ functioned exclusively in the non-hematopoietic compartment to promote disease, which is in keeping with the known restricted function of IL-17RA in RTECs. Cebpd-/- cells similarly showed a reduction in inflammatory markers (Il6 and Lcn2) following IL-17 stimulation. By ChIP analysis, we observed C/EBPδ bound to the proximal promoters of these and other disease-causing genes. Understanding the in-depth molecular mechanisms of C/EBPδ-mediated inflammation may ultimately provide therapeutic strategies to treating autoimmune disorders of kidney.

P1-085 Targeting CCL17 to ameliorate inflammatory arthritis

Lupancu T[1], Lee K[1], Lim K[1], Hamilton J[1], Achuthan A[1]
[1]University Of Melbourne

Glucocorticoids (GCs) are potent anti-inflammatory agents and are broadly used in treating rheumatoid arthritis (RA) patients, albeit with adverse side effects associated with long-term usage. The negative consequences of GC therapy provide an impetus for research into gaining insights into the molecular mechanisms of GC action. We have previously reported that GM-CSF-induced CCL17 has a non-redundant role in inflammatory arthritis.

Here, we provide molecular evidence that GCs can suppress GM-CSF-mediated upregulation of IRF4 and CCL17 expression via down regulating JMJD3 expression and activity. In mouse models of inflammatory arthritis, GC treatment inhibited CCL17 expression and ameliorated arthritic pain-like behavior and disease. Significantly, GC treatment of RA patient peripheral blood mononuclear cells ex vivo resulted in decreased CCL17 production.

This delineated pathway potentially provides new therapeutic options for the treatment of many inflammatory conditions, where GCs are used as an anti-inflammatory drug but without the associated adverse side effects.

P1-086 ISG15 Deficiency as possible mediator of Lung Fibrosis

Sazeides C[1], Martin-Fernandez M[1], Buta S[1], Youssef M[2], Milner J[2], Boneparth A[2], Bogunovic D[1]
[1]Icahn School Of Medicine at Mount Sinai , [2]Columbia University Medical Center

Introduction: ISG15 is a Type I IFN-inducible intracellular protein that interacts with and stabilizes USP18, a negative regulator of IFN-I pathway, and thus acts in a negative feedback loop. Lung damage caused by pulmonary fibrosis cannot be repaired, and can require lung transplantation; therefore, a better understanding of the causes is crucial. Here, we describe the case of a 13-year-old female presented with interstitial lung disease due to a mutation in ISG15. There is an increasing number of ISG15 deficient patients who present with lung fibrosis, but the significance of ISG15 in proper wound healing and fibrosis is unknown. We aim to investigate ISG15's role in fibrosis by studying this mutant.

Methods: Whole exome sequencing and molecular cloning was performed to identify the mutation and its expressivity. INFα stimulation of cells carrying the mutation to assess differential levels of apoptosis, necroptosis and pyroptosis compared to WT cells. Scratch wound assay to assess the mutant's ability to effectively heal a wound. Fibrosis due to the over-activation of fibroblasts to myofibroblasts due to TGFβ and IFNα exposure.

Results: The mutation is a duplication of a cysteine at position 463 of ISG15, causing a frameshift mutation at the very end of the gene which generates an mRNA transcript without a stop codon. Further analysis revealed that even though the mutant ISG15 mRNA gets transcribed, it does not get translated (ISG15 deficiency). In-vitro experiments revealed no changed in apoptosis, necroptosis nor pyroptosis when mutant cells were treated with IFNα, but they were more sensitive to the antiproliferation effects of IFNα. Therefore, these data suggest a possible mechanism by which ISG15 deficiency could aid to lung fibrosis due to the inability of the fibroblasts to properly proliferate and heal a wound.

P1-087 Anti-Inflammatory Effects of the Combination of LMT-28 and Kaempferol in a Collagen-Induced Arthritis Mouse Model

Kim H[1], Kim L[1], Heo T[1]
[1]Integrated Research Institute of Pharmaceutical Sciences and BK21 FOUR Team for Advanced Program for Smart Pharma Leaders, College of Pharmacy, The Catholic University of Korea

Backgroud: Rheumatoid arthritis is one of the autoimmune diseases characterized by inflammation and swelling of the joints. Activated fibroblast-like synovial cells (FLS), one of the main causes of joint destruction, contribute to synovial inflammation by secreting inflammatory cytokines including interleukin -1β, IL-6 and tumor necrosis factor. We previously confirmed that LMT-28 inhibits the IL-6-induced signal pathway in RA-FLS. Although LMT-28 and kaempferol both possess anti-inflammatory activity, the beneficial effect of LMT-28 and kaempferol combination on a collagen-induced arthritis (CIA) model has not yet been investigated. This study aimed to investigate the inhibitory effect of RA symptoms by the combined administration of LMT-28 and kaempferol. Methods: Arthritis score, incidence rate, inflammatory cytokine, and T cell subsets were measured in CIA mice following administration of LMT-28 and kampferol combination. The level of Th17 cell differentiation from CD4+ T cells was analyzed in mouse splenocytes. In MH7A and C28/12 cells, cell proliferation, migration, invasion and the IL-6-mediated signaling pathway following administration of LMT-28 and kaempferol combination was analyzed through CCK-8 , wound-healing, matrigel invasion assay and western blotting. Results: Co-administration of LMT-28 and kaempferol reduced arthritis scores and secretion of inflammatory cytokines in collagen-induced arthritis (CIA) mice, and it was confirmed that the differentiation of mouse bone marrow mononuclear cells (BMM) into osteoclasts was reduced by this combination group. In addition, co-administration of LMT-28 and kaempferol inhibited the differentiation of CD4+ T cells into Th17 cells. Combination treatment with LMT-28 and kaempferol more significantly reduced IL-6-mediated gp130, STAT3 and ERK signaling in MH7A cells compared to individual treatments. Furthermore, combined treatment with LMT-28 and kaempferol inhibited cell proliferation, migration and invasion in RA-FLS and RA-chondrocytes. Conclusion: These results demonstrate that the combined administration of LMT-28 and kaempferol has a significant effect on suppression of RA symptoms by inhibiting Th17 differentiation and IL-6 signaling.

P1-088 Structural and biophysical characterization of an alternative IL-37 receptor complex

Andries J[1,2], Clancy D[1,2], Savvides S[1,2]
[1]VIB-UGent Center for Inflammation Research, [2]Department of Biochemistry and Microbiology, Ghent University

Interleukin-37 (IL-37), a member of the IL-1 cytokine family, has been described as an anti-inflammatory cytokine which acts to suppress innate immunity. Previous literature suggested that IL-37 engages IL-18Rα and SIGIRR to exert its anti-inflammatory functions. However, recently the role of IL-37 as an anti-inflammatory cytokine has been challenged. Instead, IL-37 was proposed to exhibit pro-inflammatory activity, specifically, by engaging IL-36R, a receptor activated by IL-36.
The emerging exciting notion of receptor sharing between IL-37 and IL-36, has sparked a renewed interest in the field and will require further study at the structural, biophysical and functional level. We have now characterized the interaction of IL-37 with IL-36R using both biolayer interferometry (BLI) and isothermal titration calorimetry (ITC). Interestingly, we observed a higher affinity of IL-37 towards IL-36R than the affinity displayed by IL-36γ towards IL-36R. However, in cellular assays IL-36γ shows higher pro-inflammatory cytokine production than IL-37. During my presentation, I will illustrate the structural similarities and discrepancies derived from ongoing structural undertakings by cryo-electron microscopy and X-ray crystallography complemented by structure predictions and modeling. In addition, I will present insights into how the respective interaction interfaces of the two cytokines with IL-36R might provide clues about their binding properties.
Collectively, these approaches are expected to yield important insights into the assembly and regulation of IL-36R, which will greatly contribute to a better understanding of IL-37/IL-36 biology in general. This may open opportunities to guide the development of novel therapeutics targeting the IL-1 family of cytokines and receptors.

P1-089 Novel antagonistic anti-TSLP Nanobodies targeting the low affinity IL-7Rα binding site

Markovic I[1,2], Thierens L[1,2], Verschueren K[1,2], Savvides S[1,2]
[1]Ghent University, [2]VIB-UGent Center for Inflammation Research

TSLP plays a crucial role in regulating Th2 immunity involved in allergic and inflammatory diseases such as asthma and atopic dermatitis. TSLP is produced by epithelial cells in various tissues and acts on DCs to promote Th2 differentiation and activation. In recent years, the development of anti-TSLP therapeutics has emerged as a promising strategy for treating Th2-driven diseases. While most of these inhibitors target high affinity TSLP-TSLPR interaction, the low affinity TSLP-IL7R interface remains poorly targeted.

To target this low affinity interface, we developed and identified a selection of camel-derived anti-TSLP single domain antibodies (VHHs) that bind to both TSLP and the TSLP:TSLPR complex. VHH candidates from four different CDR families were further narrowed down by CDR differences, and the final candidates were expressed and purified in E. coli. We show that these VHHs have a nanomolar affinity towards TSLP with binding profiles characterized by fast on-rates and variable off-rates. Moreover, cellular studies demonstrated antagonistic effects in TSLP-mediated STAT5 signalling. We structurally characterized these VHHs and their modes of binding to TSLP using X-ray crystallography at high resolution, validating and confirming the criteria of selection leading to targeting TSLP:IL7R interface.

Structural studies of TSLP-VHH complexes, supported by biochemical, biophysical, and cellular investigations, have yielded valuable insights into the mechanism of action of anti-TSLP VHHs. This has inspired efforts to improve the potency of these agents by protein engineering. Furthermore, we assessed the top candidates in Th2 immunity using human PBMCs and evaluated their potency in an atopic dermatitis context utilizing human keratinocytes and 3D skin models.

Collectively, our findings support the notion that strategies targeting a low affinity binding site in cytokine-receptor signaling complexes may provide opportunities for the development of potent cytokine and cytokine receptor antagonists.

P1-090 Detection of Cardiotrophin-like Cytokine Factor 1 (CLCF1) by flow cytometry and validation of a new conditional knock-out mouse model

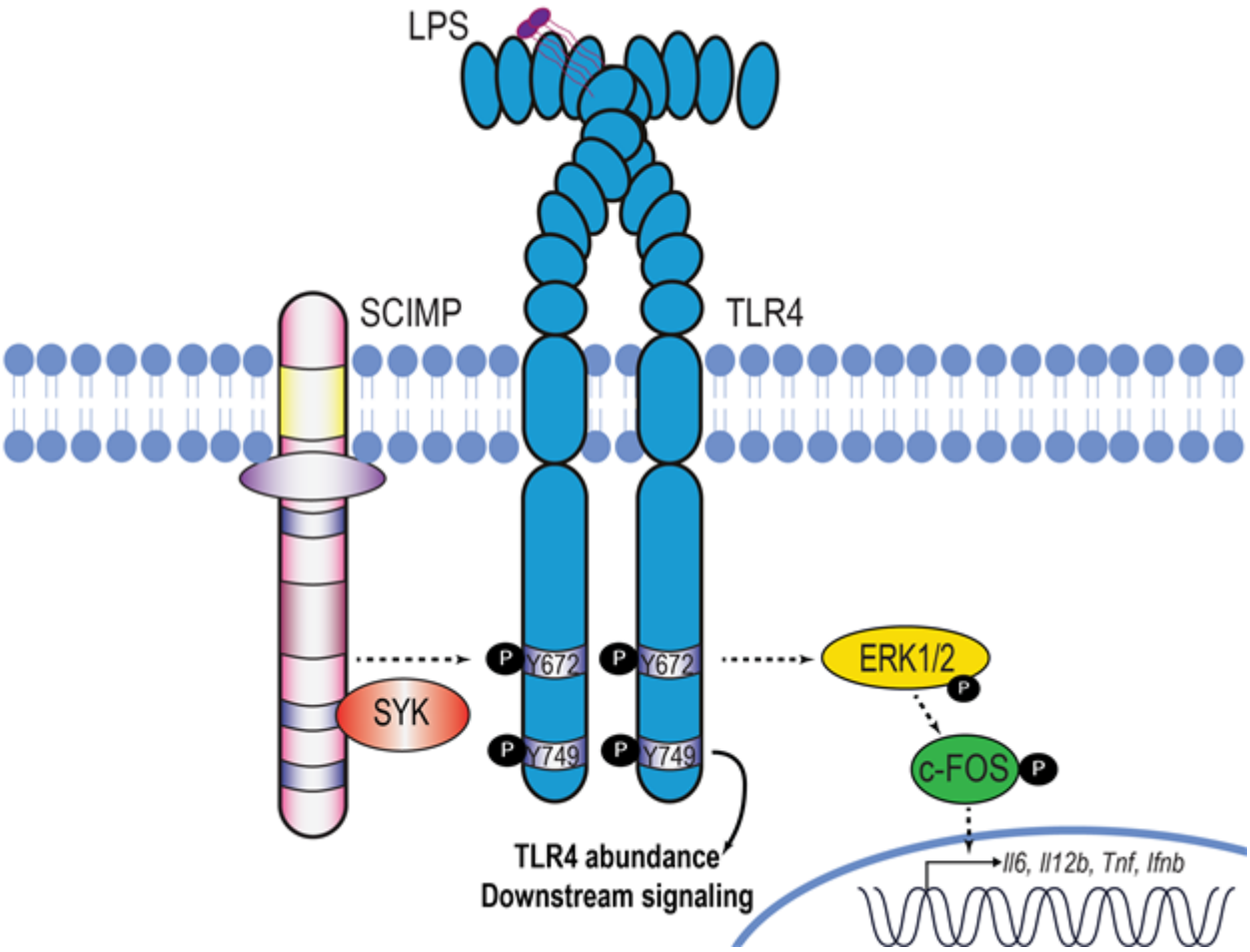
Laplante V[1], Rousseau M[1], Fajardo E[2], Pasquin S[3], Lesage S[3], Gauchat J[1]
[1]Université De Montréal, [2]Maisonneuve-Rosement Hospital Research Center, [3]Université de Montréal and Maisonneuve-Rosement Hospital Research Center

Cardiotrophin-like Cytokine Factor 1 (CLCF1) belongs to the IL6 family of cytokines and possesses pro-neurotrophic and immuno-modulating functions. Coding mRNA for CLCF1 has been detected in primary and secondary lymphoid organs (i.e. lymph nodes, spleen and bone marrow), as well as in the lungs and feminine reproductive organs. Modulation of CLCF1's mRNA levels has been associated with the Th17 polarization in CD4+ T cells. However, little information is available regarding CLCF1 protein levels in these tissues or the nature of the immune cells responsible for its production. This can be explained by a lack of in situ detection options for CLCF1. We have therefore developed a methodology for the detection of human and murine CLCF1 by flow cytometry in permeabilized cells. This technique has been validated using derivatives of the Ba/F3 cell line in which cDNAs coding for human and murine CLCF1 were introduced by transduction with recombinant retroviruses. Using this method, we have detected CLCF1 expression in murine splenocytes, mainly CD4+, CD8+ and differentiated T lymphocytes. Finally, we have validated our approach using a new CLCF1 conditional knock-out mouse model. The development of a method to detect CLCF1 by flow cytometry will be beneficial for the study of CLCF1's functions in the regulation of the immune response.

P1-091 TLR4 tyrosine phosphorylation: The old dog still has new tricks.

Curson J[1], Liu L[1], Luo L[1], Muusse T[2], Lucas R[1], Gunther K[1], Vajjhala P[2], Abrol R[1], Jones A[1], Kapetanovic R[3], Stacey K[2], Stow J[1], Sweet M[1]
[1]Institute for Molecular Bioscience (IMB), IMB Centre for Cell Biology of Chronic Disease and Australian Infectious Diseases Research Centre, The University of Queensland, [2]School of Chemistry and Molecular Biosciences (SCMB) and Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, [3]Friedrich Miescher Institute for Biomedical Research

Macrophages recognise and respond to molecular markers of danger through Toll-like receptors (TLRs) that signal by recruiting Toll/interleukin 1 receptor-like (TIR) domain-containing adaptor proteins. Historically, strategies for inhibiting aberrant TLR activity have been focused on TLR engagement and downstream signaling from canonical TIR domain-containing proteins such as MyD88 and TRIF. Recently we discovered that Slp65/76 and CSK interacting membrane protein (SCIMP), a member of the transmembrane adaptor protein family, acts as a novel non-TIR TLR adaptor protein in macrophages, facilitating recruitment of the tyrosine kinases Lyn and Syk to promote TLR4 tyrosine phosphorylation and selective production of the pro-inflammatory cytokines IL-6 and IL-12p40. To further delineate this signaling pathway, we investigated the regulation and function of two tyrosine residues within the TIR domain of TLR4, Y672 and Y749, which were predicted to be surface exposed. We first showed that both residues contribute substantially towards total LPS-inducible TLR4 tyrosine phosphorylation. Next, we functionally assessed the roles of both phosphorylation events in primary bone marrow-derived mouse macrophages (BMM) through retroviral reconstitution of Tlr4-/- BMM with the phospho-dead mimetics TLR4 Y672F and TLR4 Y749F. These experiments revealed that phosphorylation of TLR4 at Y749F and Y672F drives the production of a subset of LPS-inducible cytokines via distinct mechanisms. Y749 phosphorylation is required for maintenance of total TLR4 protein levels and signaling, whereas Y672 phosphorylation exerts its proinflammatory effects by selective triggering of an ERK-1/2 and c-FOS signaling module. Furthermore, SCIMP facilitates TLR4 Y672 phosphorylation to permit downstream inflammatory responses, with the tyrosine kinase Syk, likely phosphorylating both Y672 and Y749. These findings shed new light on proximal events in a central innate immune signaling pathway, revealing mechanisms by which post-translational phosphorylation differentially engages specific inflammatory responses, highlighting new avenues that can be exploited to target TLR-driven inflammation.


LPS
SCIMP
TLR4
SYK
P
Y672
P
Y672
P
Y749
P
Y749
ERK1/2
P
c-FOS
P
TLR4 abundance
Downstream signaling
Il6, Il12b, Tnf, Ifnb

P1-092 E3 ligases that promote antiviral immunity via type I interferon are associated with pathology in systemic lupus erythematosus

Cheang L[1], Jones S[1]
[1]Monash University, Center for Inflammatory Diseases

Background
In autoimmunity, intracellular damage-associated molecular patterns (DAMPs) such as dsDNA and RNA can trigger type 1 interferon (IFN) release via intrinsic TLR7 and TLR9 binding. This pathway is a major contributor to disease development in over half of patients with systemic lupus erythematosus (SLE), and is associated with poor response to glucocorticoid therapy and greater disease severity among patients. In antiviral immunity, several E3 ligases, particularly SPRY domain-containing proteins, promote this pathway by enhancing cytoplasmic sensing of nucleic acid DAMPs and activating type I IFN release.

Method
Seeking novel drug targets, we used a global transcriptomics approach to identify E3 ligases implicated in this pathogenic IFN pathway.

Results
We identified 280 E3 ligases whose expression was positively correlated with type I IFN in PBMC from patients with SLE. Half of these were also correlated with both disease severity and glucocorticoid dose, indicating high clinical relevance. A subset of the E3 ligases identified as being associated with clinical parameters in SLE was also directly modulated by IFN and steroid treatment, as their transcription was altered in healthy PBMC following acute IFN stimulation and/or dexamethasone treatment. Further studies underway are revealing the mechanisms by which a selected group of these E3 ligases is acting to modulate IFN release and disease activity, and contribute to steroid resistance, in SLE.

Conclusion
E3 ligases are gaining increased interest in recent years as potential targets for small molecule inhibition. Our data represent novel and significant findings regarding the functions of these E3 ligases in the immune system, and introduce new druggable targets for SLE and other IFN-driven diseases.

P1-093 The CRISPR activation system, a tool for the identification of the CLCF1 immune cell receptor

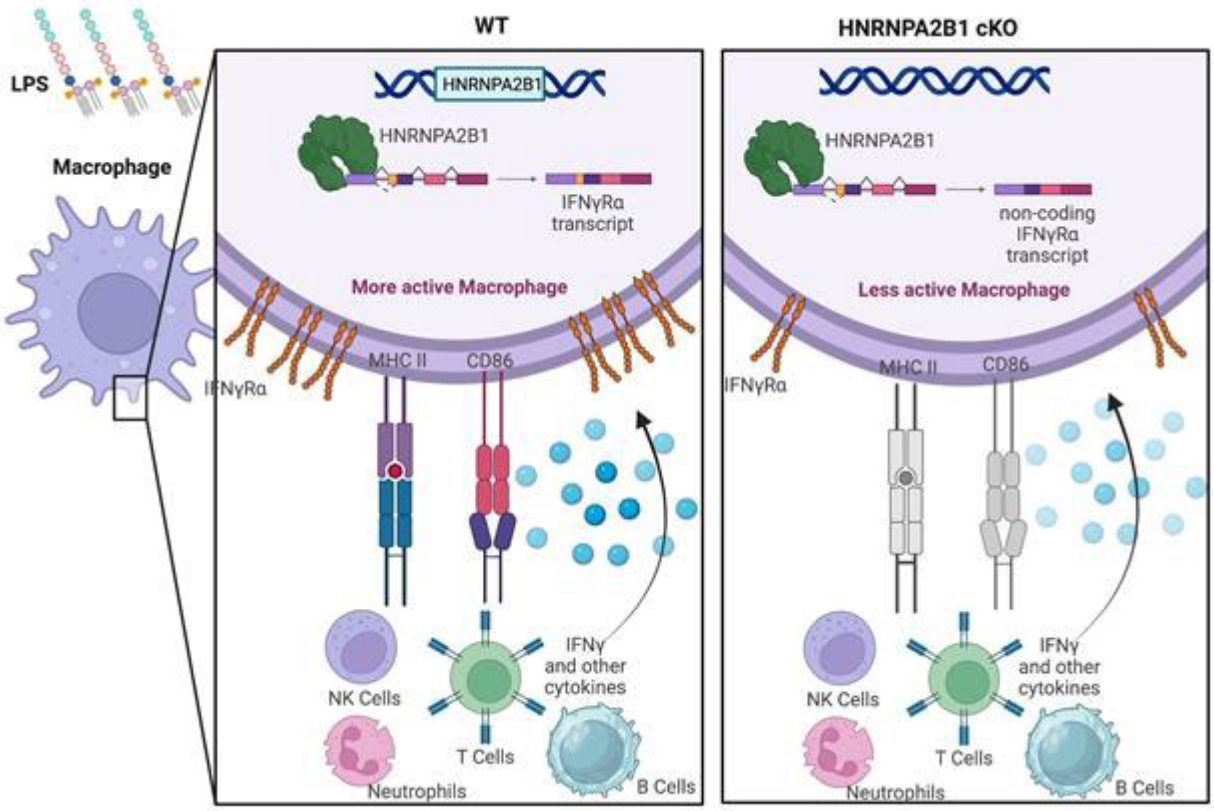
Rousseau M[1], Laplante V[1], Khelil A[1], Fajardo E[2], Lesage S[1], Gauchat J[1]
[1]Université De Montréal, [2]Centre de recherche de l'Hôpital Maisonneuve-Rosemont

The cytokine CLCF1 has been shown to activate B cells and macrophages and to induce the secretion of pro- and anti-inflammatory cytokines. In vivo, overexpression of CLCF1 leads to an expansion of B cells coupled with a humoral response, as well as an increase in myeloid cell levels. CNTFR, the CLCF1 canonical receptor, is not expressed by immune cells. The objective of the project is to investigate the expression of CLCF1 immune cell alternative receptor and to identify it. Using flow cytometry, with biotinylated CLCF1, we assessed CLCF1 binding on mouse splenocytes and human PBMCs. A strong signal was observed on fresh CD19+ cells, which was further amplified by CD40L stimulation. No binding could be detected using biotinylated CNTF, confirming that CNTFR was not involved. RNA sequencing will be performed to identify cytokine receptors differentially expressed by CLCF1-binding immune cells. In parallel, we are using the CRISPR activation (CRISPRa) approach to uncover CLCF1 immune receptor. A gRNA library covering all human membrane protein promoters will be transduced in Expi293 cells stably expressing the dCas9-VP64 derivative. Flow cytometry and deep sequencing will be used to identify the gRNAs enriched in cells in which CLCF1 receptor expression is induced. Our preliminary approach validation data show CLCF1 binding on Expi293 cells subjected to CRISPR activation with CNTFR gRNAs. Together, our study might provide further insights into the role of CLCF1 in immune regulation and lead to the identification of the CLCF1 immune receptor.

P1-094 The RNA binding protein, HNRNPA2B1, functions in macrophages to regulate IFN gamma signaling.

Mohammed Salih M[1], Carpenter S[1], Malekos E[1]
[1]Ucsc

HNRNPA2B1 is an RNA binding protein involved in transcript processing and maturation. We report on a novel role for HNRNPA2B1 in promoting interferon gamma signaling in macrophages. We generated an hnRNPA2B1 conditional knockout mouse (cKO) and crossed it to LysMCre to obtain a knockout in macrophages. HNRNPA2B1 KO mice were exposed to an endotoxic shock model where they displayed an overall impairment in pro-inflammatory cytokine production, especially IFN gamma. HNRNPA2B1 has been reported to act as a viability gene ,yet surprisingly, we observed elevated numbers of macrophages and neutrophils in the KO mice when challenged with LPS. We performed RNA-seq comparing WT and KO macrophages following LPS stimulation where the dominant dysregulated pathway was IFNgamma signaling including the receptors, JAK/Stats and many IRF transcription factors. Western blot analysis confirmed a decrease in STAT3 phosphorylation. Flow Cytometry revealed that KO macrophages expressed less IFNgRa on the cell surface when challenged with LPS in vitro and in vivo. We believe that this observed loss of the receptor on the cell surface of the KO macrophages is mediating the downstream effects observed. To understand what impact this altered IFNgamma phenotype might have during an infection we employed the macrophage targeted infection, Salmonella. HNRNPA2B1 KO mice were more susceptible to infection and failed to effectively clear the pathogen, similarly, they displayed a persistent disruption in pro-inflammatory cytokine production. Mechanistically we believe that loss of HNRNPA2B1 results in an increase in NGO transcripts of the IFNg receptor leading to less expression of the IFNg receptors and impairing all the downstream signaling events. Collectively, our data highlights an important role for HNRNPA2B1 in regulating the immune responses specifically within macrophages. Our results point to active involvement of HNRNPA2B1 in promoting interferon gamma response through modulating IFNgRa levels, which we hypothesize to occur through splicing modulation.


WT
HNRNPA2B1 cKO
LPS
Macrophage
HNRNPA2B1
HNRNPA2B1
IFNγRα transcript
More active Macrophage
IFNγRα
MHC II
CD86
NK Cells
T Cells
IFNγ and other cytokines
Neutrophils
B Cells
HNRNPA2B1
non-coding IFNγRα transcript
Less active Macrophage
IFNγRα
MHC II
CD86
NK Cells
T Cells
IFNγ and other cytokines
Neutrophils
B Cells

P1-095 Lymphocyte Regulomes in Health and Neurodegenerative Diseases

Shih H[1]
[1]National Institutes of Health

Lymphocytes play critical roles in maintaining homeostasis, immune surveillance, and host defense through cytokine production. Recent breakthroughs in research have shed light on the role of the immune system in neurodegenerative diseases such as Alzheimer's disease. These discoveries highlight the importance of cytokine regulation in lymphocytes for the prevention and treatment of these conditions. Therefore, understanding precise gene regulation for lymphocyte development and cytokine expression is essential to manage diseases without triggering autoimmunity. Our recent findings have revealed that the regulation of Th1 differentiation requires re-organization of the three-dimensional (3D) chromatin architecture at the interferon gamma (IFN-γ locus to prevent enhancers mis-targeting nearby cytokine such as interleukine-22 (IL-22). This 3D genomic structure facilitates initial lineage commitment, and disruption of this architecture leads to dysregulated IFN-γ and IL-22 production and increased susceptibility to Toxoplasma gondii infection. Interestingly, impaired loop formation does not impact cytokine production in NK cells, suggesting the dependency of higher-ordered chromatin architecture is lineage-specific. We next investigated how lymphocytes are regulated in Alzheimer's disease, a leading cause of human dementia. Using single-cell multiomic and computational approaches, we have identified a unique subset of CD8+ T cells expressing interferon-stimulated genes in the brain of a transgenic Alzheimer's disease mouse model with human risk genes amyloid precursor protein and presenilin-1. The regulomes of these T cells were shaped by interferon regulatory factor family, and this specific lineage feature was only regionally identified in the brains, but not in spleens or other tissues. Remarkably, blocking CD8+ T cell development significantly reduced amyloid plaque formation, a hallmark pathology in Alzheimer's disease, and rescued memory loss in these mutant mice. This suggests a potential role of T cells in disease progression and opens new avenues for novel therapeutic targets in the battle against this devastating neurodegenerative disorder.

P1-096 Autosomal recessive STAT2 mutation leads to a type I interferonopathy by defective negative regulation

Gruber C[1,2], Lee A[1,2], Buta S[1,2], Martin-Fernandez M[1,2], Bustamente J[3,4,5,6], Casanova J[3,4,5,7,8], Bogunovic D[1,2,9,10,11]
[1]Center for Inborn Errors of Immunity, Icahn School of Medicine at Mount Sinai, [2]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, [3]Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Santé et de la Recherche Médicale (INSERM) U1163, Necker Hospital for Sick Children, [4]University Paris Cité, Imagine Institute, [5]St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller University, [6]Study Center for Primary Immunodeficiencies, Necker Hospital for Sick Children, AP-HP, [7]Howard Hughes Medical Institute, [8]Department of Pediatrics, Necker Hospital for Sick Children, [9]Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, [10]Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, [11]Department of Microbiology, Icahn School of Medicine at Mount Sinai

Type I interferon (IFN) is an essential pro-inflammatory cytokine; failure to control its potency is life-threatening. Genetic defects along the IFN signaling pathway that serve to increase the production or signal transduction of IFN lead to a group of auto-inflammatory disorders known as type I interferonopathies.
Recently, homozygous gain of function (GOF) mutations in STAT2 have been discovered as an etiology of type I interferonopathy. Here, we identify a novel STAT2 GOF mutation (R223Q) in members of a consanguineous family afflicted with lethal auto-inflammatory disease. Similar to previous reports, the novel R223Q variant increases the late, but not early, interferon-stimulated-gene (ISG) induction in patient-derived cells and STAT2-reconstituted cell lines. Mechanistically, the proximal JAK-STAT signaling conferred by the R223Q variant was comparable to those of the wild type. However, mutant-expressing cells fail to negatively regulate late IFN-I signaling due to dysfunctional recruitment of USP18, a key negative regulator, to the interferon receptor. Taken together, this report further substantiates the unique mechanisms of a hyper-activating mutation with autosomal recessive inheritance, and better elucidates its biochemical underpinnings.

P1-097 Impact of IL-12 variants on immune parameters associated with cytokine toxicity.

Lanzar Z[1], Aldridge D[1], Perry J[1], Garcia K[2], Lupardus P[3], Hunter C[1]
[1]University Of Pennsylvania, [2]Stanford University, [3]Synthekine

Interleukin-12 (IL-12) is a type I, heterodimeric cytokine comprised of the subunits p35 and p40. IL-12 that acts upon natural killer (NK) cells and T cells to promote activation and cytolytic activity. There is interest in harnessing utilizing this cytokine as an anti-cancer therapeutic, but unfortunately IL-12 administered in murine studies and human clinical trials is linked to adverse events associated with NK cell activation and interferon gamma (IFNg) production. The data presented here utilizes engineered IL-12-Fc agonists in vivo to investigate and assess IL-12 toxicity via a range of immune parameters. Treatment with IL-12-Fc enhances IFNg production, drives global PD-L1 expression and activates conventional CD4 and CD8 T cells accompanied by severe weight loss. While these processes during infection are associated with a loss of suppressive regulatory T cells, the systemic inflammation driven by IL-12 toxicity did not have the same effect. Interestingly, IL-12 treatment did not lead to an overall decline in Treg numbers, but instead an expansion of the effector Treg (eTreg) subset. In contrast, the use of a partial agonist with decreased affinity for the IL-12rb1 receptor led to comparable activation of CD4/CD8 T cells yet preserved the homeostatic composition of the Treg compartment and did not lead to weight loss and toxicity. The production of IFNg downstream of IL-12 signaling is believed to be the primary driver of the toxicity associated with recombinant IL-12 treatment, however initial studies using KO mice suggests IL-12 treatment in the absence of IFNg led to comparable weight loss, but increased susceptibility and rapid demise. The studies presented herein highlight novel insight into the regulatory T cell compartment during IL-12 toxicity and present preliminary data regarding the possible anti-inflammatory role of IFNg in this IL-12 mediated toxicity.

P1-098 IL-31 produced by novel T cell subsets contributes to psoriasiform dermatitis

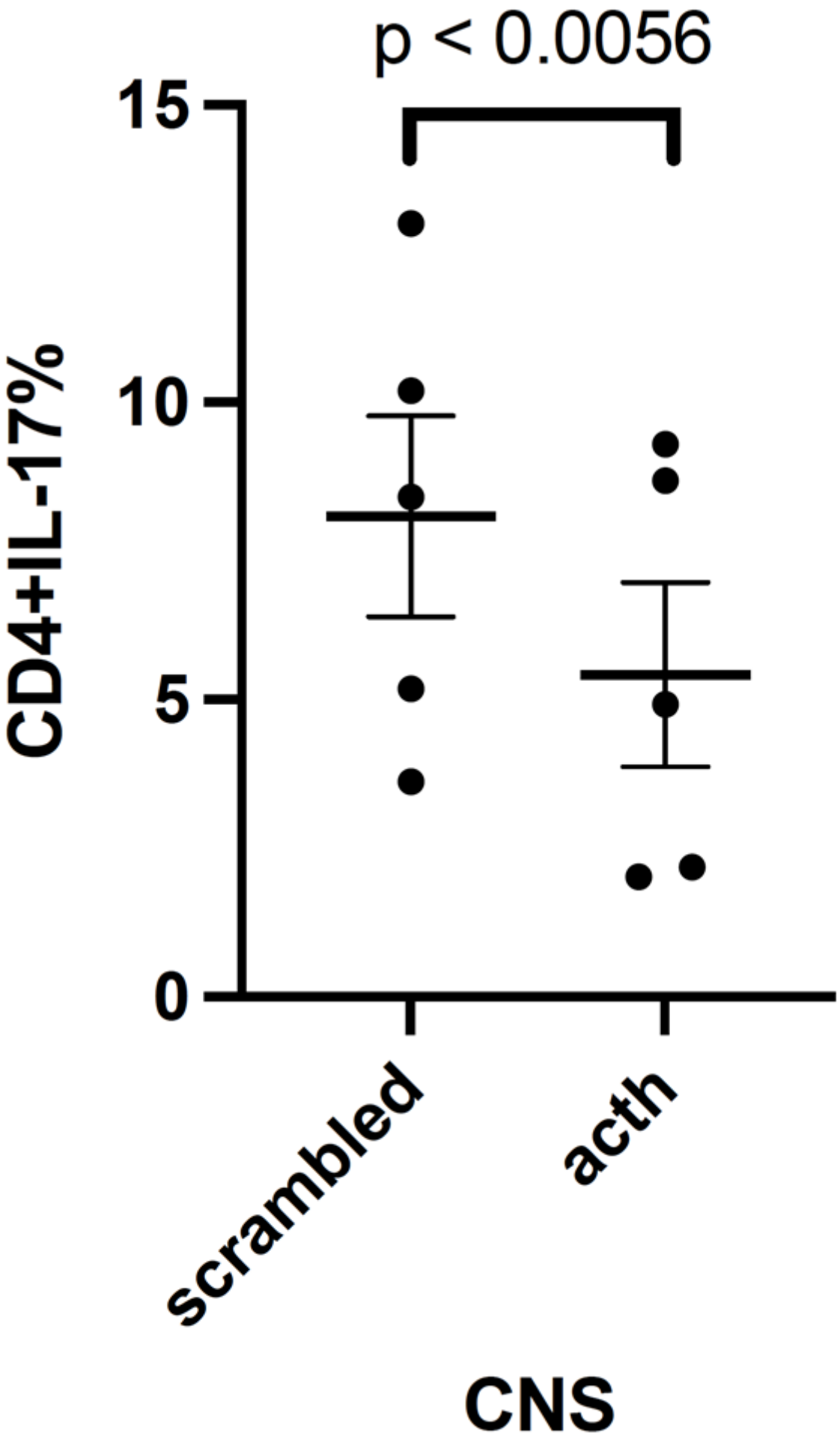
Fassett M[1,2], Lazarevsky S[1,2], Munoz-Sandoval P[1,2], Caston J[1,2], Mar D[2,3], Zhou C[2,3], Ansel K[2]
[1]Department of Dermatology; University of California, San Francisco, [2]Department of Microbiology and Immunology; University of California, San Francisco, [3]School of Medicine; University of California, San Francisco

IL-6 family cytokine IL-31 has been associated with a number of inflammatory skin conditions beyond atopy, including familial lichen amyloidosis, cutaneous T cell lymphoma, systemic sclerosis, and psoriasis. Despite GWAS data linking the Il31 locus with psoriasis, functional contributions of Il31 to Th17 inflammation and/or psoriasis pathophysiology have not been explored. Here, we describe expression of IL-31 in novel T cell subsets, including mouse and human CD4 T cells differentiated under Th17-like conditions. We also report a functional contribution of IL-31 to psoriasiform skin inflammation. Imiquimod-induced psoriasiform dermatitis was markedly diminished in Il31-deficient animals, with focal effects on skin-infiltrating myeloid cell subsets. This unexpected role for IL-31 in Th17-mediated inflammation has the potential to inform future management of psoriasis and related diseases.

P1-099 Oral ACTH inhibits interleukin-17 in the central nervous system after adoptive transfer of Th1/Th17 T cells in the mouse model of MS, experimental autoimmune encephalomyelitis

Dittel L[1], Dittel B[1], Brod S[1]
[1]Medical College Of Wisconsin

BACKGROUND: Experimental autoimmune encephalomyelitis (EAE) is an inflammatory autoimmune disease of the central nervous system (CNS) that resembles multiple sclerosis (MS) and provides a useful animal model for the evaluation of mechanisms of action for potential immunomodulatory therapies. We have previously shown that oral adrenocorticotropic hormone (ACTH) decreased either interleukin (IL)-17 or IFNg+ or both in the CNS during EAE.
OBJECTIVE: We wanted to examine whether oral ACTH showed a preferential effect on Th17 as opposed to Th1 phenotypes especially in the CNS.
DESIGN/METHODS: We therefore examined whether oral ACTH could inhibit EAE in the B6 mouse strain after adoptive transfer of equal quantities of Th17 CD4+IL-17+ and Th1 CD4+IFNg+ T cells generated after in vitro skewing. B6 mice were injected with a 1:1 ratio of Th1:Th17 T cells and were gavaged daily with control scrambled peptide (s-MSH) or 10 mcg ACTH
RESULTS: Ingested (oral) ACTH attenuated ongoing clinical EAE disease and decreased the frequencies of CD4+IL-17+ T cells in the spleen and in the CNS. There was no significant decrease in CD4+IFNg+ T cells in the CNS.
CONCLUSIONS: These findings suggest that there was preferential regulation of CD4+IL-17+ by oral ACTH compared to Th1 IFNg+ T cell in the CNS.


p < 0.0056
15
10
5
0
CD4+IL-17%
scrambled
acth
CNS

P1-100 The Stimulation trough Nucleic Acid-Sensing TLRs Induces Differential Production of Midkine and Pleiotrophin by Innate APCs

Said E[1]
[1]Sultan Qaboos University

Midkine (MK) and pleiotrophin (PTN) belong to the same family of cytokines. They have similar sequences and functions. Both have important roles in cellular proliferation, tumors and diseases. They regulate and are expressed by some immune cells. We have recently demonstrated MK production by some human innate antigen presenting cells (iAPCs) i.e. monocytes-derived dendritic cells (MDDCs), macrophages and pDCs stimulated through Toll-like receptor (TLR)-4. While PTN production was only documented in tissue macrophages. TLRs 3, 7, 8 and 9 are nucleic acid sensing (NAS) TLRs that detect nucleic acids from cell damage and infection and induce iAPC responses. We investigated whether NAS TLRs can induce MK and PTN production by human iAPCs, namely monocytes, macrophages, MDDCs, mDCs and pDCs. Our results demonstrated for the first time that PTN is produced by all iAPCs upon TLR triggering ($p<0.01$). Under all conditions IAPCs produced more PTN than MK ($p<0.01$). NAS TLRs and iAPCs had differential abilities to induce the production of MK, which was induced in monocytes and pDCs upon triggering through all NAS TLRs ($p<0.05$) and in MDDCs by TLRs 7/8 ($p<0.05$). TLR4 induced a stronger MK production than NAS TLRs ($p\leq0.05$) and macrophages significantly produced MK only upon triggering through TLR4 ($p < 0.05$). Monocytes produced higher levels of PTN after differentiation to macrophages and MDDCs ($p<0.05$). Altogether, the production of MK and PTN differs among iAPCs, with a higher production of PTN compare to MK and a selective induction of MK production depending on the NAS TLR. This highlights the differential capacity of NAS TLRs and innate APCs to induce the production of cytokines, and the potential important role of NAS TLRs and iAPCs in angiogenesis, tumors, infections and autoimmunity through the differential production of MK and PTN upon TLR triggering.

P1-101 Cytokine mimetics from parasites : a novel family of helminth TGF-β mimics bind immune cell receptors and drive differentiation of regulatory T cells

Cunningham K[1], Singh S[1], Mukundan A[2], van Dinther M[3], ten Dijke P[3], Hinck A[2]
[1]University Of Glasgow, [2]University of Pittsburgh, [3]Leiden University Medical Center

Background: Parasites have evolved sophisticated methods for manipulating the host immune response to benefit their long-term survival and circumvent therapeutic interventions. The pleiotropic cytokine TGF-β plays a pivotal role in regulating immunity and inflammation, promoting immune suppressive regulatory T cells (Tregs), which facilitate parasite establishment through down-modulation of protective immunity to intestinal helminths such as Heligmosomoides polygyrus. Results : We discovered that H. polygyrus encodes and releases a novel family of 10 TGF-β mimics (TGMs) which ligate mouse and human TGF-β receptors, induce canonical SMAD signalling, and drive differentiation of Tregs that can suppress inflammation in mouse models. TGMs bear no sequence similarity to TGF-β, instead comprising multiple domains distantly related to the complement control protein (CCP) family. In contrast to TGF-β in which the processed and C-terminal homodimer binds directly only to TGF-β receptor II (TβRII), TGMs are active as full-length monomeric proteins, organised in a modular 5-domain fashion, with domains 1/2 binding TβRI, and domain 3 binding TβRII. Domain 3 illustrates a remarkable convergent evolution in which the parasite protein precisely replicates the fingerprint of TGF-β interactions with TβRII, presenting a similar binding interface in 3-dimensional space held together by a completely different framework. Most recently, we have found that the C-terminal domains 4/5 bind additional cell-specific co-receptors on diverse cells. In the case of TGM-1, the co-receptor bound by domains 4/5 has been identified as CD44, a cell surface marker elevated on effector T cells and macrophages, and deletion of either domains 4/5, or of CD44, greatly reduces the potency of TGM-1. Furthermore, the equivalent domains of TGM-6 and TGM-7 target different determinants, expressed on fibroblasts and hepatocytes, respectively. Conclusion: Parasites have evolved a novel set of functional cytokine mimics which powerfully interact with mammalian immune cells, and which may target TGF-β signalling to specific cell subsets.

P1-102 IL-27-induced dendritic cells resist HIV infection via SPTBN1, autophagy, and YB-1 independent manners.

Chen Q[1], Sowrirajan B[1], Yang J[1], Laverdure S[1], Mele A[1], Sui H[1], Imamichi T[1]
[1]Frederick National Laboratory

Background: We previously demonstrated that IL-27 differentiates monocytes into Human immunodeficiency virus (HIV)-resistant macrophages or dendritic cells (DCs) using different differentiation reagents (cytokines or human serum); IL-27 also polarizes T cells into an HIV-resistant cell. Depending on cell types, HIV resistance is caused by the downregulation of the Spectrin beta, non-erythrocytic 1 (SPTBN1), induction of autophagy, or suppression of acetylation of Y-box B (YB-1). In the current study, we investigated the mechanism of HIV resistance in IL-27-induced DCs (27DCs).
Methods: CD14(+) monocytes were isolated from peripheral blood mononuclear cells (PBMCs) from healthy donors and differentiated to DCs in the presence of GM-CSF and IL-4 with or without 100 ng/mL IL-27 for seven days. Cells were infected with HIV-1, and the anti-HIV effect was monitored using a p24 antigen ELISA assay. Real-time RT-PCR and Western blotting were used to detect the expression of the genes and proteins of interest.
Results: Compared to control DCs (iDCs), in 27DCs, HIV replication was inhibited by 95%. However, the expression of SPTBN1 and acetylation of YB1, and the induction of autophagy were not changed between iDCs and 27DCs. A Venn diagram analysis between the differentially expressed genes in 27DCs compared to iDCs and the reported 2,214 HIV regulatory host genes identified nine genes as potential interests: ankyrin-repeat domain 22, guanylate binding protein-1, -2, -4, -5, Stabilin 1, Serpin family G member 1, Interferon alpha inducible protein 6, and Interferon-induced protein with tetratricopeptide repeats 3. Knockdown of each gene using siRNAs in 27DC induced a robust innate immune response; thus, we failed to identify the factor associated with HIV resistance. The overexpression of each protein did not affect HIV infection.
Conclusion: We could not define the mechanism of anti-HIV in 27DC; however, 27DCs resist HIV infection via different mechanisms with other IL-27-induced HIV-resisting cells.

P1-103 TBK1 and IKKε prevent cell death in response to TNF and IFNγ

Trivic T[1]
[1]First Faculty Of Medicine, Charles University

Inflammation is an essential immune system reaction to infection and tissue damage. TNF is a major proinflammatory cytokine released during such responses, and plays a crucial role in mediating inflammatory reactions. TNF was reported to synergize with IFNγ, another proinflammatory cytokine, to induce and promote cell death. The synergism between TNF and IFNγ in cell death has been implicated in several pathological conditions, including autoinflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and psoriasis. However, the precise mechanism behind this process remains enigmatic. Here we aimed to understand how these two cytokines cooperate to induce cell death. Our findings reveal that the closely related kinases TBK1 and IKKε are crucial in inhibiting cell death in response to TNF and IFNγ. TBK1/IKKε deletion or inhibition enhances TNF/IFNγ synergistic cell death in various cell lines resistant to the combined cytokine stimulus. Our findings suggest complex molecular mechanisms regulating the synergism between TNF and IFNγ in cell death.

P1-104 Maternal immune activation causes fetal demise in allogeneic- but not syngeneic-mated dams

Arreguin M[1], Kennedy E[1], Jurado K[1]
[1]Microbiology Department, Perelman School of Medicine, University of Pennsylvania

Pregnancy requires maternal immune tolerance toward a semi-allogeneic fetus. Simultaneously, the fetus must achieve immune developmental milestones during gestation. Maternal inflammation during pregnancy can have long-lasting effects on the offspring. The maternal immune activation (MIA) model has uncovered the consequences of the maternal-secreted cytokines on offspring neurodevelopment. In the MIA model, a pregnant dam is injected with Poly (I:C), a synthetic double-stranded RNA viral mimic that causes an acute immune response in the mother within hours of injection. Studies have shown that IL-6 and IL-17A, cytokines that are increased in the maternal serum upon Poly (I:C) injection, mediate the MIA-induced behavior changes in offspring. This model is frequently used in the context of murine syngeneic (C57BL/6 x C57BL/6) pregnancies. Intriguingly, we have discovered MIA has the capacity to break tolerance in allogeneic (C57BL/6 x BALB/c) mating. At mid-gestation, we injected syngeneic- and allogeneic-mated dams with Poly (I:C) or PBS. We dissected the fetuses 24 and 48 hours later. Offspring from the syngeneic mating were phenotypically normal, with some fetuses showing minor signs of pathology. Strikingly, offspring from the allogeneic mating showed severe pathology, with all fetuses being resorbed into the maternal uterus. Given the observed pathology, we hypothesized that there would be elevated IL-6 in the serum of the allogeneic-mated group. Surprisingly, preliminary results showed higher levels of IL-6 in the syngeneic-mated group. We are working to determine the cytokine profiles in the amniotic fluid of both offspring groups and will use an MHC class II mismatch model to ask whether that alone is enough to cause fetal demise. Importantly, investigating the cause of the pathology observed in the allogeneic mating will increase our knowledge of fetal tolerance mechanisms. Understanding the consequences of maternal inflammation in allogeneic pregnancies will help inform pregnancy complications, and ultimately, improve offspring health outcomes.

P1-105 Gene Expression of Type2 and Non-type2 Inflammation in Chronic Rhinosinusitis with Nasal Polyps

Kim M[1]
[1]Department Of Otorhinolaryngology, Boramae Medical Center, Seoul National University

Background: Chronic rhinosinusitis (CRS) is classified into type 2 (T2) and non-T2 inflammation. T2 CRS presents as a severe form, CRS with nasal polyps (CRSwNP), which often occurs with asthma as a comorbidity worldwide. Some cases of non-T2 CRS show nasal polyposis and refractoriness, mainly in Asian countries. However, its mechanism remains elusive. Objective: To investigate a biomarker for refractoriness of non-T2 CRSwNP via RNA sequencing. Methods: RNA sequencing using nasal polyps (NP) and ethmoidal mucosa (EM) from CRS subjects and uncinate tissues from controls were performed, and differentially regulated genes (DEGs) were analyzed (cutoffs: expression change >2-fold, $p<0.01$). Immunofluorescence staining, ELISA, and in vitro experiments were performed for validation.
Results: We identified DEGs among T2-NP, non-T2-NP, T2-EM, non-T2-EM, and controls (NP versus controls: 1,252 genes, EM vs. controls: 505 genes, T2-NP vs. controls: 221 genes, non-T2-NP vs. controls: 734 genes, T2-EM vs. controls: 60 genes). KEGG pathway analysis showed that neutrophil extracellular trap (NET) formation, SLE, and the phagosome were enriched in non-T2-NP versus controls and non-T2-EM versus controls. Immunofluorescence staining confirmed that NETs were elevated in non-T2-NP. Cytokine analysis demonstrated that NETs were significantly related to refractoriness in non-T2-NPs. Isolated neutrophils also showed significantly higher elastase activity and NET expression when treated with non-T2-NP-derived lysates from refractory patients. Conclusion: This study demonstrated DEGs between T2 and non-T2 inflammation. These results suggest that NETs may contribute to refractoriness in non-T2-NPs and have promise as a therapeutic strategy for patients with refractory non-T2-NP.

P1-106 Galectin-4 Promotes Gastric Cancer Growth via Interaction with Truncated O-Glycans and Activation of the P38 Signaling Pathway.

Tian Y[1,2], Desamero M[2,3], Chambers J[4], Uchida K[5], Nio-Kobayashi J[6], Nakayama J[5], Ogawa S[6], Iwakura Y[7], Nakayama H[4], Wataru F[1], Kyuwa S[8,2], Kakuta S[3,9]
[1]Department of Animal Resource Sciences, Graduate School of Agricultural and Life Science, The University Of Tokyo, [2]Laboratory of Biomedical Science, Graduate School of Agricultural and Life Science,The University Of Tokyo, [3]Department of Basic Veterinary Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, [4]Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences,The University Of Tokyo, [5]Department of Histocytology, Division of Anatomy, Graduate School of Medicine, Hokkaido University, [6]Laboratory of Molecular Pathology, Shinshu University School of Medicine, [7]Center for Human Disease Models, Institute for Life Science and Medical Bioscience, Tokyo University of Science, [8]Center for Food Safety, Graduate School of Agricultural and Life Sciences, The University Of Tokyo, [9]Collaborative Research Institute for Innovative Microbiology, The University of Tokyo

Galectin-4, a member of the galectin family and a tandem repeat-type protein of the β-galactoside-binding proteins, plays various biological roles in diseases. The biosynthesis of O-glycans is regulated by α1,4-N-acetylglucosaminyltransferase (α4GnT). Gastric mucin, MUC6, contains unique O-glycans with terminal α1,4-linked N-acetylglucosamine residues (αGlcNAc), which act as natural antibiotics against Helicobacter pylori (H. pylori), the leading cause of gastric adenocarcinoma. α4GnT is responsible for the formation of αGlcNAc, and mice deficient in the A4gnt gene, even without H. pylori infection, exhibit a prolonged progression to differentiated-type gastric adenocarcinoma in a hyperplasia-dysplasia-carcinoma sequence. However, the precise molecular mechanism underlying the development of gastric cancer in A4gnt knockout (KO) mice remains unclear. Based on immunohistochemistry (IHC) results, we chose galectin-4 for further research among the galectin family molecules to investigate its role in gastric cancer.
Galectin-4 possesses two carbohydrate recognition domains, and we considered whether it could bind to or interact with another molecule to modulate the development of gastric cancer. Recently, we found that lack of Galectin-4 suppressed the development of gastric cancer, but not dysplasia of gastric mucosa, in A4gnt KO mice. According to previous studies, we selected the truncated glycan structure Galβ3GalNAc, also called T antigen, catalyzed by C1GALT1, as a potential candidate. Lectin staining revealed a significant presence of the Galβ3GalNAc glycan structure during the growth period of gastric cancer. Therefore, we conducted gene knockdown experiments targeting the LGALS4 gene encoding Galectin-4 and C1GALT1 gene in human gastric cancer cell lines, and found these gene knockdown strongly affected cell proliferation. Through RNA-seq analysis, we identified an interaction between C1GALT1 and Galectin-4. Western blotting analysis confirmed that both C1GALT1 and Galectin-4 promote the growth of gastric cancer cells by mediating the P38 MAPK signaling pathway. These results suggested that Galectin-4 is a promising target for gastric cancer therapy.

P1-107 IL6 knockdown abrogates a genotoxic bystander effect following chemotherapy exposure in a model of the human bone marrow.

Asurappulige H[1], Thomas A[1], Morse R[1]
[1]University Of The West Of England, Bristol

We have developed in vitro models of the human bone marrow which resemble in vivo chemotherapy outcomes in humans[1] and predict in vivo animal genotoxicity[2]. Chemotherapy treatment upregulated cytokine expression in our microenvironment, where IL-6 was the most highly secreted. We have shown that high IL-6 ('storm doses') can induce DNA damage in vitro, which increases when IL-6 is used in combination with other cytokines[3]. As our complex multicellular model also demonstrated a chemotherapy-induced genotoxic bystander effect, we explored if IL-6 was central to this phenomenon.
The human bone marrow cell line HS-5 was transfected with siRNA targeted to IL-6, and knockdown was confirmed using ELISA. These cells were chemotherapy-pretreated, and then co-cultured with TK6 cells (modelling the bystander compartment) for 24 hours. TK6 were retrieved from the model and genotoxicity measured using the micronucleus assay.
Chlorambucil significantly increased HS-5 IL-6 secretion ($p<0.01$), which was significantly knocked down ($p<0.001$) at 72 hours. A similar outcome was seen for mitoxantrone, but this was not significant. ELISA confirmed a 50-70% IL-6 knockdown in medium from untreated and drug-treated cells for HS-5 alone, and by ~40% in co-cultured drug treated cells, inferring some IL-6 was taken up by the bystander cells. Micronuclei in bystander cells were shown to decrease by 17% and 50% in untreated and drug treated bystander cells respectively, with chlorambucil significantly reduced ($p<0.05$) to levels similar to the untreated control.
These data suggest that in a microenvironment modelling the complex mix of cytokine storm, IL-6 may play an important role in the health of bystander cells, and could be important in transplantation where these (donated) cells would have been unexposed to chemotherapy. Individuals with intrinsically high IL-6 secretion, could be most at risk, so intervention therapy may need to be considered.

[1]https://doi.org/10.1016/j.taap.2012.07.013
[2]https://doi.org/10.1093/mutage/geac009
[3]https://doi.org/10.1093/mutage/gead018

P1-108 Targeting IL-2RB-STAT5 Signaling to Preserve T Cell Stemness: A Promising Approach to Overcome Exhaustion in Adoptive Cell Therapy for Cancer

Shourian M[1], Ruisseaux C[1], Vallee A[1], El- Hachem N[1], Romero H[1], Bourdin B[1], Kwarteng E[2], Haddad E[1], Lavallée V[1], Beltra J[3], Decaluwe H[1]
[1]CHU Sainte-Justine Research Centre/University of Montreal , [2]University of Texas Southwestern Medical Center, [3]University of Pennsylvania

Persistent antigen stimulation can induce T cell exhaustion, a dysfunctional state characterized by decreased T cell proliferation and effector function, leading to resistance and relapse in adoptive T cell therapy (ACT) of cancer. The success of ACT has been associated with the transfer of less differentiated stem-like T cells, highlighting the need for strategies to promote stemness during T cell expansion. In a mouse model of chronic viral infection using lymphocytic choriomeningitis virus clone 13 (LCMV Cl.13), we previously demonstrated a correlation between CD8+ T cell exhaustion and the expression of IL-2Rβ, a shared receptor for IL-2 and IL-15. Building upon this, our current study investigates the impact of blocking IL-2Rβ signaling on prevention of terminal exhaustion and the preservation of stem-like progenitor exhausted cells following LCMV Cl.13 infection. Furthermore, we explored the inhibition of Signal Transducer and Activator of Transcription 5 (STAT5) and its effects on the exhaustion characteristics in CD8+ T cells expanded in vitro with IL-2 or IL-15. Inhibition of STAT5 resulted in decreased expression of inhibitory receptors while maintaining T cell stemness characteristics, such as increased expression of CD62L, Slamf6, TCF1, and effector cytokines. RNA sequencing analysis revealed that inhibiting STAT5 during in vitro expansion increased the expression of stemness hallmark genes, which correlated with improved tumor control and survival in a mouse melanoma model. Moreover, our findings extend to human CAR-T cell expansion, where inhibition of IL- 2/IL-15 signaling via STAT5 augmented the generation of stem cell memory and central memory T cells while decreasing the expression of inhibitory receptors. This study highlights the significant role of both IL-2 and IL-15 stimulation in impairing T cell stemness and suggests that modulating this pathway via STAT5 could enhance the efficacy of adoptive cell therapy.

P1-109 Combined Lactobacillus casei Shirota and gamma-aminobutyric acid supplementation alleviate IMQ-induced murine psoriasis-like skin inflammation

Ho T[1], Lin B[1]
[1]Department of Biochemical Science and Technology, College of Life Science, National Taiwan University

Background: Psoriasis is a chronic autoimmune skin disorder that affects approximately 1-3% of the global population. The prevalence of psoriasis has been increasing in Taiwan in recent years. The common skin symptoms of psoriasis are erythema and scales, which can lead to sleep disorders, depression, and anxiety. Gamma-aminobutyric acid (GABA) is known as an inhibitory neurotransmitter that has anti-inflammatory effects. Our previous study has shown that Lactobacillus casei Shirota (LcS) has immunomodulating effects. Therefore, this study aims to investigate whether supplementing with both GABA and LcS might have a more effective improvement on skin symptoms and inflammation in an imiquimod (IMQ)-induced psoriasis-like murine model.
Methods: The experimental procedures were to divide C57BL/6 mice into a control group (Ctrl), an IMQ-induced group (IMQ), a combined LcS and GABA supplemented group (LcS+GABA/IMQ), and a dexamethasone (Dex)-treated positive control group (IMQ/Dex). The LcS+GABA/IMQ group fed a diet containing GABA and daily tube-feeding with LcS for 4 weeks before IMQ induction. Then, mice were treated with IMQ or vaseline (Ctrl) for 5 consecutive days, and all groups were sacrificed on the 6th day.
Results: Our data shown that the LcS+GABA/IMQ group showed significantly decreased erythema, skin thickness, and total psoriasis area severity index (PASI) score compared to the IMQ group. The skin histological images also confirmed that the epidermal thickness of the LcS+GABA/IMQ group was significantly lower, and the pro-inflammatory cytokines IL-17A/F and IL-22 contents in the skin homogenates were also decreased.
Conclusion: These results demonstrated that supplementation with LcS and GABA alleviated psoriatic-like skin symptoms and inflammation, suggesting that dietary supplements may prevent or alleviate the severity of psoriasis.

P2-001 An Emerging Paradigm of Heterogeneous Midkine Expression in Thyroid Cancer

Anastasiadou D[1], Di Cristofano A, Karagiannis G
[1]Albert Einstein College of Medicine

Midkine (MDK) is a pleiotropic heparin-binding growth factor, contributing to normal tissue homeostasis and disease development. MDK expression is increased during carcinogenesis, acting as mediator for the acquisition of cancer hallmarks. Tissue-wide gene expression analysis reveal that MDK is upregulated in most human carcinomas. In the thyroid, MDK has been associated with increased metastatic potential in the context of papillary thyroid cancer, a relatively non-aggressive thyroid tumor. We thus theorized that more aggressive types of thyroid cancers may be linked to increased MDK expression/function from an earlier onset. To gain such insights, we developed a digital pathology infrastructure to investigate MDK expression in a characterized mouse model of Anaplastic Thyroid Carcinoma (ATC). Mice with conditional ablation of tumor suppressors, (Pten, p53), in thyrocytes [Pten, p53]thyr-/- develop thyroid carcinomas with mixed follicular and anaplastic components, and histological hallmarks of high aggressiveness, including giant cells, bone metaplasia, and muscular/tracheal invasion. MDK was more highly ex-pressed in anaplastic, compared to follicular components of the same animal tumors. When the above hallmarks were microanatomically demarcated within the anaplastic component, it was found that MDK expression was higher near and around giant cell islets, and within anaplastic lesions invading beyond the cartilaginous rings of tracheal mucosal epithelium. Based on evidence that MDK does not solely function in the extracellular space, but can also be endocytosed within tumor cells, whereby it exerts tumor-promoting functions, we quantified the intra/extranuclear MDK fraction using intensity thresholding immunofluorescence. Interestingly, MDK+ follicular lesions, were characterized by nuclear MDK expression, whereas MDK was mostly secreted in the extracellular space of anaplastic lesions, implying different modes of MDK trafficking, secretion, and function. The take home message is to work towards establishing and developing gain- and loss-of-function experiments, to elucidate mechanistic underpinnings on the role of MDK in the development of aggressive ATC lesions.

P2-002 Deciphering Aryl Hydrocarbon Receptor's Impact on Cryptopatch Development, ILC3 Subsets, and Epithelial Responses

Gnafakis S, Kofoed Branzk M[1], Diefenbach A[1]
[1]Institute of Microbiology, Infectious Diseases and Immunology, Charité-Universitätsmedizin Berlin

Cryptopatches, which comprise Rorc(γt)-expressing Innate Lymphocyte cells 3 (ILC3s) and Dendritic cells (DCs), are localized in the lamina propria near intestinal crypts and emerge around two weeks after birth. A subset of these structures accumulates B cells and undergoes transformation into isolated lymphoid follicles (ILFs). Investigating Aryl Hydrocarbon Receptor (AhR) deficiency, we find that AhR-deficient mice exhibit a notable scarcity of Cryptopatches/ILFs. Furthermore, the deletion of AhR in Rorc(γt)-expressing cells leads to a reduction in both the size and quantity of these formations. Intriguingly, our analysis reveals that the absence of AhR impacts the CCR6+ and NKp46+ ILC3 populations in terms of their numerical distribution and proportions. However, experiments involving the transfer of different ILC3 subsets, guided by the CCR6 and NKp46 surface markers, to immunodeficient Rag2-/- ; Il2rg-/- mice, illuminate that only the CCR6+ RORγt+ ILCs have the potential to give rise to Cryptopatches and ILFs. The AhR-deficiency in Rorc(γt)-expressing cells coincides with a downregulation of Il22, which is sensed by the IL-22R-expressing epithelial cells. To delve into the implications of IL-22 within the intestinal epithelium, we generated enteroid cultures and subjected them to recombinant IL-22 treatment. Our findings validate the downregulation of genes tied to lipid absorption and carbohydrate metabolism in these intestinal cells. As we progress, our focal points encompass unraveling AhR's role in Cryptopatch formation, comprehending its potential impact on the diverse subsets of ILC3s. and elucidating its impact on Intestinal Epithelial Cells (IECs), which are also known to respond to AhR ligands.

P2-003 Rebalancing STAT signaling with non-natural cytokine receptor combinations to modulate immune cell functionality

Rodriguez G[1,2], Saco J[7], Lee M[2,3], Abhiraman G[1,2,4], Saxton R[1,5], Su L[1,5], Picton L[1,5], Yen M[1,5], Ren J[1,5], Blish C[3,4,6], Ribas A[7,8,9,10], Garcia K[1,5]
[1]Department of Molecular and Cellular Physiology, Stanford University School of Medicine, [2]Program in Immunology, Stanford University School of Medicine, [3]Department of Medicine, Stanford University School of Medicine, [4]Stanford Medical Scientist Training Program, Stanford University School of Medicine, [5]Howard Hughes Medical Institute, Stanford University School of Medicine, [6]Chan Zuckerberg Biohub, [7]Division of Hematology-Oncology, Department of Medicine, University of California Los Angeles, [8]Jonsson Comprehensive Cancer Center, UCLA, [9]Parker Institute for Cancer Immunotherapy, [10]Broad Stem Cell Research Center, UCLA

Recombinant cytokines have provided therapeutic benefits in a variety of disease contexts, but they only recapitulate the signaling properties of the natural parent cytokines. The plasticity of cytokine receptor JAK/STAT pairings enables the induction of new or modified cytokine receptor signals with non-natural STAT profiles, which could potentially exhibit enhanced therapeutic properties. Using a synthetic “orthogonal” IL-2Rβ and γc receptor system (Sockolosky et al., Science 2018), we previously found that altered combinations of pSTAT5/pSTAT3/pSTAT1 exhibit emergent properties on engineered T cells over the pSTAT5-dominant signal of IL-2 (Kalbasi et al., Nature 2022). These engineered receptors enhanced stemness and mitigated T cell exhaustion in the setting of adoptive cell therapy. A limitation of this finding is that, while several natural cytokines exhibit strong pSTAT5 signaling on T cells (i.e. IL-2, IL-7), or pSTAT3 signaling (i.e. IL-21, IL-10), no natural cytokine exhibits simultaneous induction of pSTAT5, pSTAT3, and pSTAT1. Thus, we sought to endow IL-2-mediated pSTAT5-signaling on natural T cells with augmented pSTAT3 and pSTAT1 by engineering the IL-2 cytokine, eliminating the need to engineer cells. We created synthetic IL-2 analogs that rebalance pSTAT signaling profiles on natural cells through induced proximity of cytokine receptor combinations not normally formed in nature. We characterized the effects of combining the signals of γc family receptors in human T and NK cells. In CD4+ and CD8+ T cells, we observe differential modulation of pSTAT1, pSTAT3, and pSTAT5 signaling relative to the parent cytokines, as well as proliferation and cytokine production in response to stimulation. We also observe the mitigation of T cell exhaustion in long-term, repeated antigen challenge assays. In NK cells, we observe enhanced cytotoxicity and activation. These findings provide the foundation for a new catalog of engineered cytokine therapeutics based on compelling new receptor combinations.

P2-004 The extracellular IFI16 protein triggers TLR4-mediated inflammation via the PYRIN domain

Iannucci A[1], Lacarbonara D[1], Caneparo V[1], Raviola S[1], Martinello G[1,2], Bajetto G[1,2], Miggiano R[3], Gariglio M[4], De Andrea M[1,2]
[1]CAAD - Center for Translational Research on Autoimmune and Allergic Disease, Intrinsic Immunity Unit, University of Piemonte Orientale, [2]Department of Public Health and Pediatric Sciences, Viral Pathogenesis Unit, University of Turin, [3]Department of Pharmaceutical Sciences, University of Piemonte Orientale, [4]Department of Translational Medicine, Virology Unit, University of Piemonte Orientale

Background. The interferon-γ-inducible protein 16 (IFI16) is a nuclear phosphoprotein belonging to the PYHIN family of proteins that acts as a DNA/RNA sensor and antiviral restriction factor. In addition, IFI16 can be released in the extracellular space upon a variety of stimuli and be found in the sera of patients with autoimmune diseases. We have previously shown that IFI16, per se or upon binding to LPS, acts like a damage-associated molecular pattern (DAMP), propagating danger signals and amplifying IL-6 and IL-8 secretion.
Methods and Results. Here, using antibodies directed against either the N- or the C-terminal domains of IFI16 along with a panel of IFI16 recombinant domains that span the PYRIN, HINA, or HINB domains and truncated proteins that lack either the PYRIN or HINB domains (IFI16ΔPYRIN or IFI16ΔHINB, respectively), we demonstrated that the PYRIN domain of IFI16 is sufficient to induce inflammation when added to human macrophages. In addition, IFI16-PYRIN/TLR4 interaction has been confirmed using immunoprecipitation and surface plasmon resonance analysis. We also showed that point mutations in the PYRIN domain that involve highly conserved amino acids across the different PYHIN family members inhibited the IFI16-induced inflammatory activity. In silico analysis revealed that these amino acids are not conserved in other PYRIN-carrying proteins not belonging to the PYHIN family, which were indeed unable to induce TLR4-dependent inflammation.
Conclusion. Collectively, our findings reveal unprecedented inflammatory activity of the IFI16-PYRIN domain, which is conserved among the different PYHIN family members, as a DAMP able to trigger TLR4-mediated inflammation in macrophages. These results could lead to the generation of new drug candidates to be exploited for dampening the inflammatory response in autoinflammatory diseases.

P2-005 IL6-MEDIATED PARACRINE SENESCENCE DRIVES THE INTERCELLULAR COMMUNICATION WITHIN THE P2-005 CYTOMEGALOVIRUS MICROENVIRONMENT IN RENAL EPITHELIAL CELLS

Raviola S[1], Iannucci A[1], Caneparo V[1], Lacarbonara D[1], Castiglioni F[1], Lo Cigno I[1], Favero F[1], Corà D[1], Cantaluppi V[1], Landolfo S[2], Gariglio M[1], De Andrea M[2]
[1]University of Eastern Piedmont, [2]University of Turin

Background: Human cytomegalovirus (HCMV) is an opportunistic pathogen causing severe diseases in immunosuppressed individuals. To establish the optimal milieu for its replication, HCMV induces a profound changes in cellular homeostasis that resemble senescence. Senescent cells produce and secrete a complex combination of factors, collectively referred to as the senescence-associated secretory phenotype (SASP), that mediate most of their immune-modulatory effects, including induction of paracrine senescence in neighboring cells.
Methods and results: Here, we have experimentally demonstrated that renal proximal tubular epithelial cells (RPTECs), a natural setting of HCMV infection and disease, fully support HCMV replication and undergo a senescence program upon infection, a process referred as virus-induced senescence (VIS). We demonstrate that HCMV-induced SASP triggers a harmful secretory phenotype with the ensuing induction of paracrine senescence in uninfected surrounding cells, mainly driven by IL-6. Consistently, our transcriptome analysis showed a specific enrichment of the IL-6/JAK-STAT3 signaling pathway only in HCMV-infected RPTECs. When the IL-6R inhibitor Tocilizumab (TCZ) was added in the course of infection, a dramatic decrease in the total number of cells expressing nuclear NF-κB and the DNA-damage marker γ-H2AX was observed, mainly in the bystander uninfected cells. Moreover, the addition of TCZ to the UVB-inactivated conditioned medium from infected RPTECs significantly affected its capability to trigger senescence when applied to fresh target cells.
Conclusion: Altogether, our findings demonstrate that HCMV infection of RPTECs, which are naturally susceptible to infection and associated with virus-related disease in vivo, elicits a senescence-like program characterized by stable growth arrest and secretion of senescence-associated inflammatory cytokines. Intriguingly, this IL-6-driven paracrine senescence represents a mechanism of disease amplification in kidney tubular epithelial cells upon HCMV infection.

P2-006 Development of citraconate derivatives as novel ACOD1 inhibitors

Chen C[1], Zhao M[2], Waqas F[1], Kany A[3], Büssow K[2], Pessler F[1]
[1]Biomarkers for Infectious Diseases, TWINCORE Centre , [2]Department of Structure and Function of Proteins, Helmholtz Centre for Infection Research, [3]Drug Development and Optimisation, Helmholtz Institute for Pharmaceutical Research Saarland

In response to proinflammatory stimulation, macrophages undergo profound metabolic rewriring to support the biosynthetic and bioenergetic requirements of the cell. This metabolic shift can affect immune cell effector functions. cis-Aconitate decarboxylase (ACOD1, also known as IRG1) converts the tricarboxylic acid (TCA) cycle intermediate cis-aconitate to itaconate during macrophage activation. Itaconate is being intensely investigated as a link between metabolism and immunity and has immunomodulatory properties. Abnormal synthesis of itaconate by ACOD1 has also been reported in severe sepsis and tumors, suggesting that ACOD1 is a promising drug target for immunology as well as oncology. We previously identified citraconate, an isomer of itaconic acid, as the first ACOD1 inhibitor. We subsequently identified three citraconate analogs as substantially more active competitive ACOD1 inhibitors. All three analogs had lower 50% inhibitory concentrations (IC50) than citraconic acid, but comparable low toxicity (50% cytotoxic concentration, IC50 around 60 mM), resulting in much higher selectivity indexes (SI, CC50/IC50, approx. 60000). In vitro ADME profiling of citraconate and Analog 1 revealed high metabolic stability in mouse liver microsomes and S9 fractions, high plasma stability and low plasma protein binding. We then investigated the effect of ACOD1 inhibition on metabolism. As expected, ACOD1 inhibitors abolished the increase of succinate and mesaconate after LPS/IFNγ stimulation. Furthermore, ACOD1 inhibitors increased levels of citrate, cis-aconitate, isocitrate, and lactate. When added to LPS/IFNγ-stimulated dTHP1 cells, all there ACOD1 inhibitors reduce IL-6 after 12 hours of stimulation while only analog 2 also reduces IL-1β. Both citraconate and analog 1 reduced reactive oxygen species (ROS) levels while analog 2 increased ROS. These inhibitors will prove valuable for probing the physiological function of ACOD1-mediated itaconate synthesis and as starting points for the development of ACOD1-targeting small molecule drugs.

P2-007 Erythroid progenitors: unexpected orchestrators of fetal immunity

Clements R[1,2], Song D[2], Campbell A[2], An H[2,3], Addison M[2,3], Chou S[2,3], Einsenlohr L[2,3], Jurado K[2]
[1]Swarthmore College, [2]University of Pennsylvania, [3]Children's Hospital of Philadelphia

Red blood cells (RBCs) are not typically considered active immune mediators. This dogma stems from the fact that RBC precursors discard organelles as they mature, thus losing the ability to alter gene expression in response to stimuli. Intriguingly, nucleated RBC progenitors and precursors (nRBCs) circulate in human fetuses and neonates. Due to evolutionary pressure for successful reproduction, circulation of nRBCs during pregnancy is likely important. However, the role of nRBCs in utero remains unknown. To define the role of human nRBCs, we queried single cell RNA-seq data and found that transcriptomics support nRBCs as putative immune mediators.
Unexpectedly, we found that nRBCs express antigen processing & presentation machinery. nRBCs constitutively express MHC II & co-stimulatory molecules, hallmarks of specialized antigen-presenting cells. We further demonstrated that nRBCs internalize and cleave antigens. Currently, we aim to determine if nRBCs present antigens to T cells and to characterize the elicited response.
In addition, we found that nRBCs express pattern recognition receptors capable of detecting pathogens and initiating an antimicrobial response. We demonstrated that nRBCs respond to viral infection through upregulation of antiviral genes, interferons/interferon-stimulated genes, MHC I, and pro-inflammatory cytokines/chemokines. These data suggest a putative functionality for nRBCs in mediating antiviral immunity in utero. We are currently investigating antiviral functions of nRBCs. Together, our findings shed light on an unexpected orchestrator of fetal immunity. Knowledge of the fetal immune system has potential to help us understand health and disease during pregnancy, as a neonate, and likely every stage of life after.

P2-008 IL-22 modulates microbiota-induced serotonin production toregulate early life gut motility

Rabahi S[1], Salloum Y[1], Gros G[1], Garcia baudino C[1], Mikdache A[1], Diabangouaya P[1], Feijoo C[2], Levraud J[3], Guendel F[4], Eberl G[4], Hernandez P
[1]Institut Curie, [2]Fish Immunology Laboratory, Faculty of Life Sciences, Universidad Andres Bello, [3]Institut des neurosciences Paris-Saclay, [4]Institut Pasteur

Cytokines are small secreted proteins that promote tissue defense and homeostasis. Among key cytokines in barrier organs like the gut, interleukin-22 (IL-22) primarily targets epithelial cells, protecting this organ from pathogens and promoting tissue repair. Although it is well established that IL-22 dysregulation can lead to inflammatory bowel disease (IBD) and cancer, whether this cytokine plays a role in the developing gut remains unclear. We have used the zebrafish model to determine the function of il22 in gut development maturation. We generated IL-22-deficient zebrafish and found a novel role of this cytokine in modulating gut peristalsis during early life. Indeed, il22 KO fish showed dysregulated expression of several neuronal markers in the gut, of serotonin expression as well as impaired peristalsis and food transit.

In addition, using an il22-reporter line that we generated, we identified enteroendocrine cells as the main source of il22 in the larval gut. Furthermore, il22 was expressed by a enteroendocrine subtype that also expressed trpa1 (transient receptor potential cation channel, subfamily A, member 1). Trpa1 is best known as a sensor for pain, cold and itch and it has been recently shown to sense tryptophan-derived metabolites produced by bacteria. Accordingly, we found that treatment of fish with chemical activators of this channel increased il22 expression in enteroendocrine cells.

We are currently deciphering the mechanisms by which microbial-induced enteroendocrine cell production of IL-22 modulates serotonin release to regulate gut motility during early life. Altogether, this project will provide a better understanding of IL-22 function in gut development and maturation which could hold significant relevance for potential therapeutic applications.

P2-009 Effect of NT-I7, a long-acting interleukin-7, on CD4 T cells during chronic LCMV

Niavi C[1], Lee J[1], Valanparambil R[1], McGuire Jr D[1], Wolfarth A[2], Ferrando-Martinez S[2], Lee B[2], Ahmed R[1]
[1]Emory Vaccine Center and Department of Microbiology and Immunology, Emory University School of Medicine , [2]NeoImmuneTech, Inc.

Failure of the immune system to respond effectively during chronic disease is largely attributed to T cell exhaustion. With current immunotherapies, some of the effector CD8 T cell functions are potentially restored. Using a chronic LCMV model, our lab has previously shown that the exhausted CD8 T cell pool is heterogeneous, with PD1+TCF1+ stem-like CD8 T cells being the subset responsive to immunotherapy. Ongoing work has demonstrated that administration of a long-acting IL-7, NT-I7 (rhIL-7-hyFc, efineptakin alfa), is able to selectively expand stem-like CD8 T cells. Although the CD8 T cell response in chronic viral infections is well characterized, much less is known about the role of CD4 T cells. Because CD4 T cells play an important role in regulating CD8 T cell responses, we sought to characterize the CD4 T cell subsets and their response during chronic viral infection, as well as any effect NT-I7 has on these populations.
Our data show that administration of NT-I7 during chronic LCMV increases the number of antigen-specific CD4 T cells in lymphoid and non-lymphoid tissues. We also observed an increase of antigen-specific PD1+TCF1+ CD4 T cells. NT-I7 treatment increased TH1 antigen-specific CD4 T cells as described by Tbet+, Ly6c+, CX3CR1+, Tim3+ and CD150+ expression. Additionally, transcriptomic analysis identified a subset within the NT-I7-expanded CD4 T cells with enriched TH1 signature. In summary, our data suggest NT-I7 can potentially lead to an effective way to increase CD4 T cell help to CD8 T cells. Following the recent studies describing a progenitor CD4 population, we also identify a PD1+TCF1+ Slamf6+ CXCR5- population during chronic LCMV. Future studies are ongoing to describe the effect of NT-I7 on this subset, the mechanisms of CD4 T cell differentiation, their effect on B cell and antibody responses and to test combination therapy of NT-I7 with PD-1 blockade.

P2-010 NT-I7, a long-acting interleukin-7, promotes expansion and mobilization of virus-specific PD-1+TCF-1+ stem-like CD8 T cells during chronic viral infection

Lee J[1], Niavi C[1], Ahn E[1], Valanparambil R[1], Obeng R[2], Wolfarth A[3], Borgonia D[3], Ferrando-Martinez S[3], Kissick H[1], Choi D[3], Lee B[3], Ahmed R[1]
[1]Emory Vaccine Center and Department of Microbiology and Immunology, Emory University School of Medicine, [2]Department of Pathology, Case Western University, [3]NeoImmuneTech, Inc.

PD-1+TCF-1+ stem-like CD8 T cells, also referred to as Precursor or progenitor of exhausted CD8 T cells (Tpex), are a distinct subset identified as a crucial resource for sustained immune responses in chronic viral infections and cancer. Stem-like CD8 T cells are capable of slow self-renewal and serve as precursors providing proliferative bursts of effector CD8 T cells upon PD-1 blockade therapy, making them an important therapeutic target for effective antiviral and anticancer immunotherapy. IL-7 is a homeostatic cytokine critical for the maintenance of naïve and memory T cells through induction of proliferative and pro-survival signals. During chronic viral infection, the IL-7 receptor (CD127; IL-7Rα) is preferentially expressed by TCF-1+ stem-like virus-specific CD8 T cells as opposed to the TCF-1- terminally differentiated subset. In this study, using NT-I7, a long-acting hybrid Fc-fused recombinant human IL-7 (rhIL-7-hyFc, efineptakin alfa), we demonstrate that IL-7 treatment can selectively expand the crucial stem-like CD8 T cell population. Single-cell gene profile analysis revealed a unique cluster of proliferating TCF-1+ CD127+ cells induced by NT-I7 treatment. This cluster exhibited enrichment of a stem-like signature, including transcription factors preferentially expressed in stem-like cells and costimulatory molecules. They also displayed high levels of genes associated with the cell cycle and lymphocyte migration but low levels of gene encoding effector molecules and multiple inhibitory receptors. While stem-like cells preferentially reside in T cell zones of lymphoid organs and are rare in circulation, we observed the stem-like CD8 T cells were increased in circulating blood and peripheral tissues following NT-I7 treatment. Our data suggest the potential of NT-I7 to enhance antiviral and anticancer immunotherapies by amplifying the crucial stem-like CD8 T cell pool and mobilizing them to the peripheral target sites.

P2-011 Availability of IL-2 as a factor that regulates a transient expression of IL-10 by Th1 cells

Jankovic D[1], Kugler D[1], Oland S[1], Villarino A[2,3], O'shea J[2], Sher A[1]
[1]Laboratory of Parasitic Diseases, NIAID, NIH, [2]Molecular Immunology and Inflammation Branch, NIAMS, NIH, [3]Department of Microbiology and Immunology, University of Miami

Host resistance against intracellular pathogens frequently depends on the development of a Th1 lymphocyte response which if left uncontrolled can be detrimental. We have previously shown that in the case of Toxoplasma gondii infection autocrine IL-10 derived from conventional parasite-specific Th1 cells regulates their response preventing immunopathology and mortality. Interestingly, the production of IL-10, in contrast to IFN-gamma, is transient in Th1 lymphocytes. Although Th1 cells acquire the ability to produce IL-10 as a consequence of the normal immune response to T. gondii the specific pathways involved in its regulation remain poorly understood. IL-27 has been shown to play a critical role in promoting IL-10 production by Th1 cells. Because IL-27 can signal via STAT1 and STAT3, we employed mice that selectively lack expression of one or the other transcriptional factors in CD4+ T cells and showed that STAT1- but not STAT3-dependent signaling pathway is required for the generation of IL-10+ Th1 cells during T. gondii infection. When gene expression in IL-10+ vs. IL-10neg Th1 effectors was analyzed, we observed that IL-10+ Th1 cells express higher levels of several transcriptional factors (T-bet, Blimp-1) that in common with IL-27 are known to be strong suppressors of IL-2 production. Indeed we subsequently found that when exposed to IL-2, IL-10+ Th1 cells rapidly lose the ability to produce IL-10. Our results are thus consistent with a model in which during systemic infection the mobilization of large numbers T lymphocytes reduces the availability of free IL-2 causing the “collapse” of Treg cells while simultaneously favoring the transfer of their regulatory function to conventional pathogen-specific CD4+ T lymphocytes through the triggering of transient IL-10 expression in Th1 effectors.

P2-012 Reovirus Z-RNAs Activate ZBP1 Dependent Cell Death

DeAntoneo C[1,2]
[1]Fox Chase Cancer Center , [2]Drexel University College of Medicine

Reovirus (ReoV) is an enteric dsRNA virus which can infect a wide range of mammals and induce intestinal and autoimmune diseases due to the cell death elicited during infection. The mechanisms by which ReoV induces such cell death are unclear. For decades ReoV was thought to cause mainly apoptosis in infected cells. Recent research has shown that ReoV can also activate necroptosis, a caspase-independent form of inflammatory programmed cell death reliant on the kinase RIPK3 and its substrate MLKL. Necroptosis is highly immunogenic because it results in the release of damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), and intracellular cytokines, whereas apoptosis is more immunologically ‘silent’. The mechanisms by which both necroptosis and apoptosis are activated during ReoV infection, and the immunological consequences of this activation, are largely unknown. I have found the innate immune sensor Z-DNA Binding Protein 1 (ZBP1) initiates both apoptosis and necroptosis during ReoV infections. I have also discovered ReoV generates Z-RNA, the left-handed conformation of double-helical (ds)RNA, in infected cells. Together, these new findings allow us to hypothesize that (1) ReoV T3D produces cytoplasmic Z-RNAs, which are activating ligands for ZBP1; and (2) ZBP1-triggered necroptosis drives virus pathogenesis by both promoting virus release and inducing detrimental inflammation in the brain and heart. In this project, I test these hypotheses by identifying the ReoV-generated Z-RNA ligands for ZBP1, determining the mechanism of ZBP1 activation, and investigate the role of ZBP1-mediaed cell death to host survival during ReoV infection.

P2-013 Type I and II IFN crosstalk during Mycobacterium tuberculosis infection

Fattinger S[1], Kotov D[1], Parisi B[1], Vance R[1,2]
[1]UC Berkeley, [2]HHMI

The host immune response against viral and bacterial pathogens relies on cellular communication mediated by cytokines. Interferons (IFNs) are a major class of cytokines which result in the expression of thousands of IFN stimulated genes (ISGs). While type I IFNs signal through IFNAR and promote anti-viral responses, type II IFN signals through IFNGR and has been implicated in responses against bacteria and other intracellular parasites. Despite their rather distinct role in the immune response against pathogens, a potential crosstalk between type I and II IFN signaling has recently been suggested. However, how this crosstalk is mediated remains poorly understood. Here, using unbiased CRISPR screens in mouse and human immune cells, we are addressing how type I IFN signaling suppresses type II IFN response. The CRISPR screen in mouse cells combined with RNAseq analysis of IFN stimulated cells revealed a list of potential candidates mediating this crosstalk. This hit list is currently being validated. Furthermore, we are investigating if this crosstalk might explain the difference in disease outcome for Mycobacterium tuberculosis (Mtb) infection associated with type I and II IFNs.

P2-014 Glutamine deficiency delays viral clearance of SARS-CoV-2 in Syrian hamster

Oh E[1], Park S[1], Lee S[1]
[1]College of Veterinary Medicine, Chungbuk National University

Since viruses completely depend on host cell metabolism for replication, they induce metabolic reprogramming in host cell to their advantage. Recent studies have suggested that metabolic changes in viral infection play a critical role in shaping immune response and are often associated with viral pathogenesis. Metabolomic and transcriptomic studies shed light on the importance of glutamine (Gln) metabolism viral replication and anti-viral immune responses. Gln deficiency can be occurred during oxidative stress-inducing chronic diseases or conditions including old age and comorbidities such as smoking, hypertension, diabetes, or tumor. In this study, we investigated an association between Gln deficiency and COVID-19, which has been identified in COVID-19's progression in to sever. To mimic Gln deficiency in hamster, we depleted the endogenous Gln levels using 6-diazo-5-oxo-L-norleucine (DON), glutaminase antagonist. DON-treated and COVID-19-infected hamster resulted in exacerbated clinical symptoms. Interestingly, theses findings were associated with increased inflammatory responses and altered viral tissue tropism. Viral clearance was delayed in various organ including the brain, heart, liver, and large intestine as well as the lung, primary target organ, so the viral loads were detected higher than COVID-19-infected control hamster on 7days post-infection (dpi). Transcriptome analysis of lung revealed that DON treatments delayed type I IFN response and innate immune response including activation of macrophages and neutrophils. We also observed that oral administration of Gln (2g/kg/day) to the DON-treated group reduced the viral loads in lung on 7dpi. These data indicate that endogenous Gln is crucial for mounting anti-viral immunity and Gln deficiency can lead to persistent and severe COVID-19. Therefore, this study suggests that glutamine metabolism may be a regulatory factor important in the acquired immunity to SARS-CoV-2 infection and glutamine could be a promising therapeutic agent in COVID-19 treatments.

P2-015 A long-acting form of recombinant human IL-7 shows biologic effect in both a pre-clinical model and a clinical study of Idiopathic CD4 Lymphopenia

Pérez-Díez A[1], Liu X[1], Bennett A[1], Anderson M[1], Wolfarth A[2], Ferrando-Martinez S[2], Lee B[2], Lisco A[1], Sereti I[1]
[1]NIAID, NIH, [2]NeoImmuneTech, Inc.

Background: Idiopathic CD4 lymphopenia (ICL) is characterized by low numbers of CD4 lymphocytes (<300/µL blood), sometimes accompanied by CD8 lymphopenia. Patients often suffer opportunistic infections and there is no established treatment for ICL. Using a PBMC transfer humanized mouse model, we previously observed that T cells from 50% of the tested ICL patients phenocopied ICL syndrome by not being able to reconstitute immunodeficient mice as Healthy Controls (HC) cells do. Using this ICL pre-clinical model, we tested NT-I7 (efineptakin alfa; NeoImmuneTech, Inc.), a long-acting form of human IL-7, as a potential immunotherapy for ICL.
Methods: Mice received PBMC from either HC or eight ICL patients. ICL mice were either left untreated or treated the same day subcutaneously with NT-I7. Mouse blood was sampled at days 3, 7, 14, 21 and 28, when spleen was also collected, to compare CD4 and CD8 T cell reconstitution, their IL-7Rα expression and TCR clonality. We also weighted the mice to detect potential graft versus host disease (GvHD). In a single clinical study, CD4 and CD8 lymphopenia was treated with NT-I7, and blood lymphocytes were quantified thereafter.
Results: In seven out of eight patients tested, NT-I7 treatment increased T cell reconstitution in treated compared to untreated ICL mice, reaching similar levels to HC mice. IL-7Rα downregulation on treated T cells lasted 2-3 weeks. Treated T cells were more polyclonal than untreated T cells in three out of four patients tested and GvHD was not increased in NT-I7 treated mice.
The NT-I7 treated patient experienced 2- and 3-fold rise in CD4 and CD8 cell numbers, respectively, two months after a single dose of NT-I7.
Conclusion: Altogether, these data suggest NT-I7 has a potential therapeutic role in patients with ICL.

P2-016 A baseline cytotoxic immune phenotype that stems from CD16+ NK cells is specifically associated with reduced responses to adjuvanted T-dependent Prevnar vaccine among older adults

Ucar D[1]
[1]Jackson Laboratory

Pneumococcal infections can cause serious illness and death among older adults. Two vaccines are available for protection capsular polysaccharide vaccine Pneumovax and adjuvanted T-dependent Prevnar. An in-depth understanding of how older adults respond to these vaccines and whether there are baseline predictors for responsiveness is unknown. To fill these gaps, we recruited and vaccinated 39 older adults (60+ years old) with Pneumovax (n=19) or adjuvanted Prevnar (n=20). Both vaccines induced strong antibody responses and a shared plasmablast transcriptional signature (IGHG2, IGHA1, IGHA2) induced ten days after vaccination. Yet there was significant heterogeneity in responsiveness at the individual level. Pre-vaccination flow cytometry and bulk RNA-seq data revealed a baseline activated phenotype associated with weaker Prevnar responses: increased expression of cytotoxic genes (NCAM1, GNLY), increased proinflammatory Th17, decreased Th1 cell abundance. Using scRNA-seq, we showed that this phenotype stems from increased abundance and cytotoxicity of CD16+ NK cells and is specifically associated with T-dependent Prevnar responses. Moreover, CD16+ NK cell abundance significantly correlated with Th1 (positively) and Th17 cells (negatively) and NK-T cell interactions were more significant in non-responders, together suggesting NK immuno-suppressive effects on T cells. Interestingly men were more likely to have this cytotoxic phenotype and mounted weaker responses to Prevnar. In contrast, baseline expression of other genes (notably CD47) was predictive of Pneumovax responses. This systems vaccinology study uncovered the significance of CD16+ NK cells for modulating responses to conjugated Prevnar vaccine and revealed distinct baseline predictors for Prevnar and Pneumovax to guide future recommendations.

P2-017 Distinct baseline immune characteristics associated with responses to conjugated and unconjugated pneumococcal polysaccharide vaccines in older adults

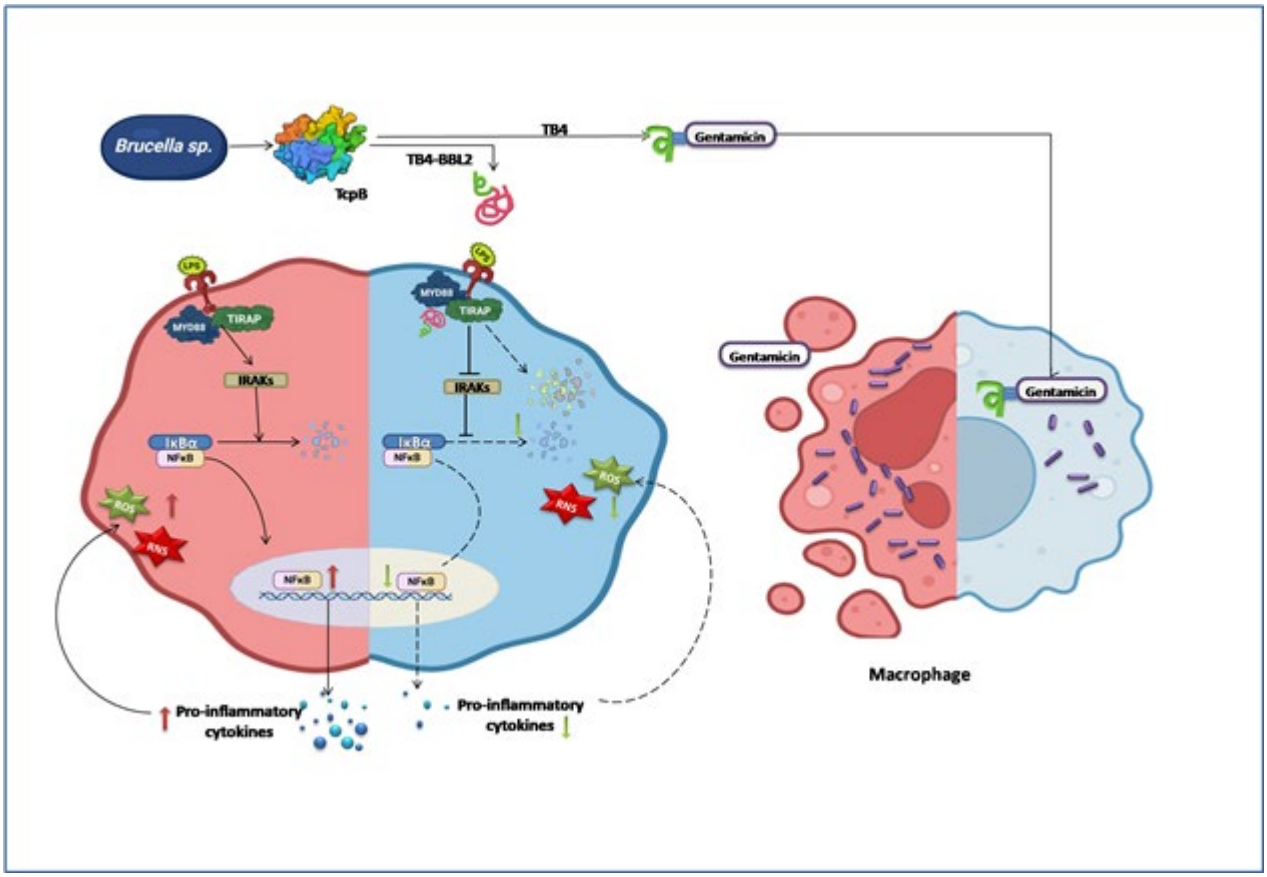
Ucar D[1]
[1]Jackson Laboratory

Streptococcus pneumoniae (Pneumococcus) infections lead to life-threatening diseases and are responsible for ~450,000 hospitalizations and 22,000 deaths annually in the US, particularly affecting older adults. A capsular polysaccharide vaccine PPSV23 (Pneumovax®) and conjugated/adjuvanted polysaccharide vaccines (e.g., PCV13, Prevnar®) are used to prevent these infections, yet underlying responses, and baseline predictors for these alternative vaccines remain unknown. To fill these knowledge gaps, we recruited and vaccinated 39 older adults (60+ years old) with PCV13 or PPSV23 and characterized serum antibody responses using opsonophagocytosis assay (OPA), peripheral blood leukocyte composition using flow cytometry, and transcriptional profiles using bulk and single-cell RNA-seq. Both vaccines induced strong antibody responses at day 28 and similar plasmablast transcriptional signatures at day 10, however, baseline transcriptomics data revealed two mutually exclusive gene sets that are significantly associated with responsiveness to PCV13 and PPSV23. Gene sets associated with PCV13 responsiveness was enriched in cytotoxicity-associated molecules and termed as the CYTOX signature. Single cell RNA-seq data showed that the CYTOX signature stems from CD16+ NK cells. Non-responders have more CD16+ NK cells and their cells express cytotoxic genes at higher levels compared to responders. Baseline flow cytometry data showed that having a lower Th1 and higher Th17 frequency is linked to reduced PCV13 responses. Frequency of CD16+ NK cells significantly correlated with Th1 and Th17 frequencies. Men were more likely to display this cytotoxic phenotype and mounted weaker responses to PCV13 compared to women. Baseline expression levels of a distinct gene set was predictive of PPSV23 responses. This first precision vaccinology study for pneumococcal vaccine responses of older adults uncovered seminal findings with significant translational and clinical implications. Our study provides the first framework for stratifying older adults for the selection of one of two different pneumococcal vaccines based on distinct and novel baseline predictors.

P2-018 A chimeric peptide derived from the Brucella virulent protein, TcpB, attenuates the production of pro-inflammatory cytokines in macrophages and mice.

Nandi B[1], Radhakrishnan G[1]
[1]National Institute of Animal Biotechnology

Toll-like receptors (TLRs) are essential mediators of innate immunity against microbial infections. TLR recognition by pathogen-associated components triggers a signalling cascade that results in the production of pro-inflammatory cytokines, free radicals and anti-microbial peptides, which helps to resolve the infection. Although TLRs elicit protective immune responses, their hyperactivation leads to excessive production of pro-inflammatory cytokines resulting in various inflammatory disorders, including sepsis. Therefore, therapeutics that interfere with TLR signalling can serve as ideal drugs to treat inflammatory diseases caused by aberrant activation of TLRs. The intracellular bacterial pathogen, Brucella encodes the virulence protein, TcpB, which negatively regulates the TLR2/4-mediated production of pro-inflammatory cytokines through the degradation of the adaptor protein, TIRAP. TcpB consists of an N-terminal phospholipid-binding motif, which imparts cell permeability to TcpB and a C-terminal TIR domain required to interfere with TLR signalling. Our study focused on synthesizing peptides from TcpB, followed by evaluating their cell permeability and anti-inflammatory properties. Subsequently, we generated the chimeric peptide, TB4-BBL2, which exhibited both cell-permeability and anti-inflammatory properties. We report that TB4-BBL2 was efficiently internalized by macrophages through cholesterol-mediated endocytosis and the internalized peptide could efficiently suppress LPS-induced NF-κB activation, production of pro-inflammatory cytokines and generation of free radicals. TB4-BBL2 peptide interacted with TLR adaptor proteins and promoted their degradation to attenuate the TLR4 signalling. Furthermore, we demonstrated that TB4-BBL2 could efficiently suppress the production of LPS-induced proinflammatory cytokines in the murine model of sepsis. We have also shown that the conjugation of Gentamicin with the TcpB-derived, cell-permeable peptide improved the cell permeability and bactericidal activity of Gentamicin against the intracellular bacterial pathogens in macrophages and mice. These findings suggest that the functional peptides from TcpB protein can serve as potential drugs for treating various infectious and inflammatory disorders.


Brucella sp.
TcpB
TB4
TB4-BBL2
Gentamicin
LPS
MYD88
TIRAP
IRAKs
IκBα
NFκB
ROS
RNS
Pro-inflammatory cytokines
Gentamicin
Gentamicin
Macrophage

P2-019 Chronicle of Inflammatory Changes in Liver and Adipose Tissue of High-Fat Diet Mice: Implications for Obesity-Induced Insulin Resistance

Bae H[1], Shin S[1], Yoo J[1], Kim S[2], Kwon E[1]
[1]Kyungpook National University, [2]Omixplus, LLC

Chronic inflammation is a common feature of metabolic disorders such as obesity and type 2 diabetes, where immune cells like macrophages infiltrate adipose tissue and secrete pro-inflammatory cytokines. However, the specific mechanisms underlying immune cell infiltration in response to a high-fat diet (HFD) are not well understood. In this study, we used gene set enrichment analysis to observe a time-dependent response to HFD in liver and epididymal tissues. Our data revealed a correlation between early abnormal innate immune responses in the liver and later inflammatory responses in adipose tissue, which ultimately leads to systemic inflammation. The study also highlighted dysregulated mitochondrial translation and abnormal mitochondrial energy metabolism, particularly NADH accumulation in the mitochondrial matrix, as potential key regulators of insulin resistance development. Overall, this study provides valuable insights into the molecular mechanisms underlying the development of HFD-induced chronic inflammation and metabolic disorders.

P2-020 Histone deacetylase 7 controls inflammatory cytokine production and systemic glucose metabolism

Wang Y[1,2], Das Gupta K[1,2], Miller G[3], Sullivan M[4], Ramnath D[1,2], Curson J[1,2], Abrol R[1,2], Gunther K[1,2], Wan Y[4], Burgener S[1,2], Schroder K[1,2], Clouston A[3,5], Irvine K[6], Karunakaran D[1,7], Sweet M[1,2,8]
[1]Institute for Molecular Bioscience (IMB), The University of Queensland, [2]IMB Centre for Inflammation and Disease Research, The University of Queensland, [3]Envoi Specialist Pathologists, [4]Glycation and Diabetes Complications, Mater Research Institute–The University of Queensland, Translational Research Institute, [5]Centre for Liver Disease Research, Faculty of Medicine, The University of Queensland, [6]Mater Research Institute, The University of Queensland, Translational Research Institute, [7]Monash Biomedicine Discovery Institute and Victorian Heart Institute, Monash University, [8]Australian Infectious Diseases Research Centre, The University of Queensland

Background: Histone deacetylase 7 (HDAC7) is a cytoplasmic lysine deacetylase in macrophages, controlling metabolic and inflammatory responses. In murine bone-marrow derived macrophages (BMMs), HDAC7 activates glycolysis and promotes the secretion of inflammatory mediators, such as IL-1β and CCL2, in response to lipopolysaccharide (LPS) stimulation. CCL2 mediates macrophage infiltration in multiple inflammatory diseases and exacerbates insulin resistance in diet-induced murine liver disease models. Additionally, HDAC7 mRNA levels are elevated in liver biopsies from patients with advanced chronic liver disease, thus suggesting a role for HDAC7 in low-grade inflammatory disease such as non-alcoholic steatohepatitis.
Methods: Myeloid-Hdac7 overexpressed (MacHDAC7) and myeloid-deleted (Hdac7 KO) mice were fed on a diet containing high fat, high cholesterol and high sucrose (HFHCHS) for 24-36 weeks, with glucose metabolism assessed at multiple time points. BMMs from MacHDAC7 and Hdac7 KO mice were used for investigating mechanisms by which HDAC7 amplifies LPS-inducible CCL2 production.
Results: Overexpression of Hdac7 in myeloid cells in vivo enhanced hepatic gene expression of Il1b and Ccl2. MacHDAC7 mice fed on a HFHCH diet demonstrated significantly higher fasting blood glucose and glucose intolerance compared to wildtype control mice, indicating dysregulated glucose metabolism. Conversely, Hdac7 KO mice manifested with lower fasting blood glucose levels and improved glucose tolerance. Moreover, myeloid-Hdac7 affected glycogen storage and glucose in the liver. In BMMs, HDAC7 enhanced LPS-inducible CCL2 production via a mechanism requiring its deacetylase activity and the glycolytic enzyme pyruvate kinase isoform 2 (PKM2). However, the specific mechanism was distinct from that by which HDAC7 promotes PKM2-dependent IL-1β production.
Conclusion: Myeloid-Hdac7 regulates liver glucose homeostasis and insulin response in a diet-induced obesity and chronic liver disease model. The pro-inflammatory phenotype of HDAC7 in macrophages is licensed by the deacetylation of PKM2. Whether myeloid-HDAC7 regulates hepatic glucose metabolism through CCL2-mediated immunometabolic control is currently under investigation.

P2-021 Glutamine-dependent solute carrier protein mediates pro-inflammatory cytokines to regulate glutamine production in tumor microenvironment to support gemcitabine chemoresistance in pancreatic cancer.

Kang H[2], Kim J[2], Lee D[2], Kim M[2], KIM H[1], Park J[1]
[1]Department of Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine,
[2]Department of Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine; Brain Korea 21 PLUS Project for Medical Science, Yonsei University

Pancreatic Ductal Adenocarcinoma (PDAC) is known as one of the fatal cancers that have a high mortality worldwide. Gemcitabine is one of well-known drugs to treat pancreatic cancer patients. However, since gemcitabine tends to cause drug resistance in pancreatic cancer, it is significant to discover novel therapeutic agents for gemcitabine resistance therapy. The present study represents the first study evaluating the relationship between gemcitabine resistant cancer cells and tumor microenvironment in PDAC. Here, we observed regulation of SLC protein, as a glutamine transporter, disrupts production of inflammatory cytokines from cancer cells as analyzed via both cell and human data. And, we found interleukin-8 (IL-8) was the most highly expressed pro-inflammatory cytokine among the other cytokines with related to the level of glutamine transporter. Also, a previous study reported that IL-8 binds to its receptor, CXC motif chemokine receptor 2 (CXCR2) in fibroblasts. Weakening both cancer-derived IL-8 and CXCR2 hindered glutamine production from surrounding fibroblasts in pancreatic tumor microenvironment. Therefore, suppression of fibroblasts-derived glutamine influx into gemcitabine resistant PDAC cells sensitized from gemcitabine as proved by down-regulation of NRF2 and GPX4 expression. Further, our findings showed that suppression of SLC6A14 effectively decreases tumor size and growth in gemcitabine-resistant pancreatic cancer in vivo. Altogether, our results indicate that both SLC transporter and IL-8 are involved in oncogenic effect in PDAC by inducing glutamine production from tumor microenvironment and could be novel potential candidates to improve weakness of gemcitabine monotherapy in drug resistance in PDAC.

P2-022 STK-026: A Novel IL-12 Partial Agonist for Cancer with Reduced NK-mediated Toxicity which Results in an Improved Therapeutic Window

Koliesnik I, Emmerich J, Tran K, Bauer M, Totagrande M, Jayaraman B, Buffone C, Balasubrahmanyam P, Chaturvedi D, Rokkam D, De Waal Malefyt R[1], Zuniga L, Greb H, Ratti N, Vivona S, Rosen D, Kastelein R, Lupardus P, Oft M
[1]Synthekine

Interleukin-12 (IL-12) is a pro-inflammatory cytokine composed of p35 and p40 subunits produced by antigen-presenting cells to stimulate Th1 cells, cytotoxic CD8 T cells and NK cells. IL-12 has potent anti-tumor properties in multiple preclinical models, however clinical applications of IL-12 have been hampered by severe dose-limiting toxicities. Preclinically, IL-12 toxicity is mediated by NK cell activation .

Here we report on a novel human IL-12 partial agonist (STK-026) with diminished binding to IL-12Rβ1. STK-026 is designed to more selectively engage antigen activated T-cells, which strongly upregulate IL-12Rβ1 upon activation, and to reduce stimulation of NK cells or resting T cells, which express modest levels of IL-12Rβ1.

To explore anti-tumor efficacy and toxicity in mouse syngeneic tumor models, we generated a half-life extended mouse surrogate of the IL-12 partial agonist (mSTK-026) and compared it to a similarly engineered half-life extended version of wild type mouse IL-12 (mIL-12wt Fc). At efficacious doses, systemic administration of mIL-12wt Fc induced significant weight loss and lethality characterized by early proinflammatory cytokine release and systemic NK cell activation. Conversely, mSTK-026 was well tolerated and avoided the robust and rapid NK cell activation and peripheral NK count decreases seen with mIL-12wt Fc. Both mSTK-026 and mIL-12 WT Fc showed similar robust single-agent anti-tumor efficacy in syngeneic tumor models with mSTK-026 showing a remarkably higher therapeutic index. Depletion of NK cells did not diminish anti-tumor efficacy of both IL-12s and efficacy of mSTK-026 was associated with intratumoral CD8 T cell activation and myeloid cell reprogramming. Moreover, combinations of mSTK-026 with systemic immunotherapies further enhanced anti-tumor activity without compromising tolerability.

Overall, mSTK-026 retained anti-tumor efficacy without induction of severe toxicities compared to mIL-12wt Fc. These data suggest IL-12 partial agonists may represent a novel immunotherapy approach to maintain efficacy while avoiding dose limiting toxicity associated with IL-12 therapy.

P2-023 IL-2Rbeta/IL-2Rgamma Surrogate Cytokine Agonists (SCAs) Induce Activation of T and NK Cells In Vitro and In Vivo.

De Waal Malefyt R[1], Buffone C, Balasubrahmanyam P, Gangopadhyay A, Ramadass M, Totagrande M, Koliesnik I, Tran K, Bauer M, Semana M, Ali M, Roupe J, Ratti N, Jayaraman B, Vivona S, Rokkam D, Kastelein R, Lupardus P, Oft M
[1]Synthekine Inc

Heterodimerization of the intermediate affinity Interleukin-2 Receptor (IL-2R) chains IL-2Rβ and IL-2Rγ initiates a signaling cascade in T and NK cells that results in proliferation and Interferon-gamma (IFN-γ) production. Normally, IL-2 dimerizes these receptor chains and induces phosphorylation and downstream activation of pathway associated STAT transcription factors. Here, we established a modular way to dimerize IL-2Rβ and IL-2Rγ chains in a cytokine-independent manner using surrogate cytokine agonists, composed of linked hIL-2Rβ- and hIL-2Rγ-specific heavy chain single domain camelid antibodies (VHHs), to induce physiological cellular responses.

Ten IL-2Rβ VHHs and six IL-2Rγ VHHs, all with low nanomolar affinity, were generated, linked as IL-2Rβ/IL-2Rγ VHH dimers in two orientations and tested in human T and NK cell functional assays. IL-2Rβ/IL-2Rγ VHH dimers were biologically active and induced pSTAT5 phosphorylation in the IL-2 dependent NK cell line NKL at various levels. IL-2Rβ/IL-2Rγ VHH dimers induced pSTAT5 phosphorylation in primary NK cells isolated from human peripheral blood and induced proliferation, and production of varied levels of IFN-γ depending on the specific IL-2Rβ/IL-2Rγ VHH combination. In addition, IL-2Rβ/IL-2Rγ VHH dimers induced pSTAT5 phosphorylation, proliferation and IFN-γ production in CD3/CD28 activated CD4 positive and CD8 positive T cells from PBMC. Certain IL-2Rβ/IL-2Rγ VHH dimers exhibited preferential T- or NK cell biased activities.

Two IL-2Rβ/IL-2Rγ VHH dimers and an IL-2Rβ/IL-2Rγ selective IL-2 control were tested in vivo using wildtype and heterozygous hIL-2Rβ/hIL-2Rγ knock-in (KI), double-transgenic mice. In KI mice, the administration resulted in systemic T cell and NK cell activation with dose dependent changes in lymphoid cell populations. Furthermore, in vivo responses correlated with signal strength and bioactivity observed in vitro. Overall, the results illustrate how surrogate cytokine agonists can differentially trigger receptor activation which can induce functional outputs in a cell type specific manner.

P2-024 IL-6-dependent reduction of REDD1 expression – a novel crosstalk between inflammation and metabolism

Köhler N[1], Schaper F[1], Dittrich A[1]
[1]Otto-von-Guericke University

Background: Interleukin 6 (IL-6) is a pleiotropic cytokine and a strong activator of mammalian target of rapamycin (mTOR). In contrast, mTOR activity is negatively regulated by regulated in development and DNA damage responses 1 (REDD1). REDD1 is encoded by the stress-responsive gene DNA-damage-inducible transcript 4 (DDIT4), whose transcription increases e.g. upon hypoxia, DNA damage, or when cells are exposed to metabolic stress such as fasting or glucose deprivation.
Methods: REDD1 expression was analysed in STAT3-deficient cells reconstituted with STAT3 mutants. Cellular localization, phosphorylation, DNA binding, and transcriptional activity of STAT3 was determined.
Results: Expression of REDD1 is reduced by IL-6. This reduction is independent of REDD1 protein degradation. Instead, IL-6 reduces REDD1 mRNA transcription, which depends on the expression and activation of signal transducer and activator of transcription 3 (STAT3). STAT3 is mostly seen as a potent activator of transcription. However, evidence exists, that STAT3 also represses gene expression. Little is known about the molecular mechanisms involved in STAT3-dependent gene repression. We identified the functional domains of STAT3, responsible for reduction of REDD1 transcription. The N-terminal domain and tyrosine 705 are crucial for IL-6-dependent reduction of REDD1 expression. STAT3 is recruited to the REDD1 promoter upon stimulation with IL-6, however, binding of STAT3 to canonical STAT binding sides found within the REDD1 promoter is not necessary for the reduction of REDD1 expression. Neither expression nor IFN-γ-induced activation of STAT1 contributes to the regulation of REDD1 mRNA and protein expression suggesting that the reduction of REDD1 expression is specific for STAT3 and IL-6.
Conclusion: In summary, we present a novel, non-canonical STAT3-dependent mechanism for reducing the expression of REDD1. This transcriptional repression increases the functions of STAT3 proteins beyond classical transcriptional activation of cytokine-regulated target genes to a more complex function in modulating gene expression in metabolism and cellular stress.

P2-025 CCL3-CCR5 axis in Kupffer cells plays a detrimental role in acetaminophen-induced acute liver injury

Ishida Y[1], Kuninaka Y[1], Nosaka M[1], Kimura A[1], Mukaida N[1], Kawaguchi M[1], Kondo T[1]
[1]Wakayama Medical University

Background: Acute liver injury caused by acetaminophen (APAP) is characterized by leukocyte infiltration with production of various cytokines and chemokines. Since CCR5 and its ligands regulate leukocyte chemotaxis and activation, we investigated the role of CCR5 in APAP-induced liver injury.
Methods: Wild-type (WT, C57BL/6), Ccr5-/- and Ccl3-/- mice were intraperitoneally administrated with APAP at the dose of 600 mg/kg. In some experiments, mice were intraperitoneally administrated with anti-CCL3 (250 µg/mouse, i.p.) or Maraviroc (10 mg/kg, i.p.) at 1 h before or 2 h after APAP challenge.
Results: Expression of Ccr5 and its ligand Ccl3 was significantly increased after injection of APAP into mice. CCR5 protein was detected in F4/80+ macrophages, CD3+ T cells, and Pan NK+ NK cells infiltrating the liver after APAP challenge. Ccr5-/- and Ccl3-/- mice had reduced liver injury after APAP administration compared to WT mice. Infiltration of neutrophils, macrophages, CD3+ lymphocytes, and NK cells was significantly suppressed in Ccr5-/- mice compared to WT mice. Ccr5-/- mice had decreased expression of Ifng and Nos2, molecules that have a detrimental role in APAP-induced liver injury. Administration of CCL3 neutralizing antibodies and maraviroc, a CCR5 antagonist, ameliorated APAP-induced liver injury. In intact livers, resident Kupffer cells were found to express CCR5. In line with this founding, pre-depletion of resident Kupffer cells by clodronate liposome treatment showed a significant reduction in APAP-induced liver injury compared to controls. Furthermore, bone marrow (BM) chimeric mice of Ccr5-/- recipients transfused with WT or Ccr5-/- BM cells had improved APAP-induced liver injury compared to WT recipients transfused with WT or Ccr5-/- BM cells. In vitro, the addition of CCL3 to isolated resident Kupffer cells increased the expression of Ifng and Nos2.
Conclusion: Our findings suggest that the CCL3-CCR5 axis of Kupffer cells may play a detrimental role in APAP-induced liver injury.

P2-026 IL-33 mediates steroid resistant eosinophilic inflammation dependent on the functions of allergen specific memory CD4 T cells

JUNG Y[1], Choi A[1]
[1]Korea University

Allergen-specific memory CD4 T cells induce relapse and aggravation of allergic asthma. In addition, it was reported that ST2hiCD44hi Th2 cells contributed to the development of lung inflammation that is steroid resistant upon exposure to IL-33. Thus, we hypothesized that allergen-specific memory CD4 T cells are converted into pathogenic memory CD4 T cells by IL-33 for inducing steroid resistant-allergic asthma. To test this hypothesis, we confirmed that allergen-specific memory CD4 T cells derived from the challenge with a mixture of OVA and house dust mite extract induced steroid sensitive inflammation in mice. Further, we examined whether IL-33 converts steroid responsiveness of allergic lung inflammation by mediating allergen-specific memory CD4 T cells. Unexpectedly, airway eosinophilia and expression of IL-33 receptor on allergen-specific CD4 T cells were declined by steroid when challenged with either IL-33 or allergen only. However, when rechallenged with a mixture of allergen and IL-33, eosinophilia and CD11ahi allergen-specific CD4 T cells, indicating newly emigrated CD4 T cells into airways, were not decreased regardless of steroid. Moreover, FTY720 treatment before rechallenging also did not impair steroid resistance of airway eosinophilia and allergen-specific CD4 T cells, suggesting that allergen-specific memory CD4 T cells are locally developed from the lung parenchymal region into steroid resistant-inducing pathogenic memory CD4 T cells. When macrophages were depleted by the injection of F4/80-specific antibody, the numbers of eosinophils and IL-4+ allergen-specific CD4 T cells in the lung decreased with steroid treatment upon rechallenge. Altogether, we propose that IL-33 alters the roles of macrophages and possibly other cells in lung microenvironments to develop steroid resistant asthma mediated by allergen-specific memory CD4 T cells.

P2-027 The influence of Lactobacillus fermentum 2I3 CCM 7158 on selected morphological parameters and cytokine profile in caecum of chickens

Herich R[1], Szabóová R[1], Karaffová V[1], Levkut M[1], Revajová V[1], Ševčíková Z[1], Gancarčíková S[1], Koščová J[1], Faixová Z[1]
[1]University of Veterinary Medicine and Pharmacy in Košice, Slovakia

One-day-old chickens were divided into 2 groups: 1. control group (n = 24; CG), 2. experimental group (n = 24; LB) with per oral application of Lactobacillus fermentum 2I3 CCM 7158. Animals were fed by standard starter feed mixture with free access to water. Lactobacillus fermentum 2I3 CCM 7158 was individually per orally administered in a dose of 0.2 ml with a concentration of 109 CFU/ml to animals for first 7 consecutive experimental days in the LB group. The experiment lasted 12 days with two samplings on day 7 and 12.
Caecal samples (1 cm2; n = 12) were routinely processed and morphometrically evaluated at 100x magnification. The relative gene expression of cytokines (IL-1β, IL-18, IL-15, TGF-β4, IL-10) was evaluated using the quantitative Real-Time PCR.
During the application of probiotic lactobacillus was observed significant ($P < 0.001$) influence on the villus height, villus cut surface, the middle of villus width as well as the number of Goblet cells in the caecum of chickens at the end of experiment in comparison to control group. Simultaneously was recorded a significant stimulatory effect of the used strain on the gene expression of selected cytokines compared to the control group ($P < 0.05$, $P < 0.01$, $P < 0.001$).
Because of such positive influence of caecal micro-environment we can conclude, that application of used strain could reduce risk of the development of campylobacteriosis in chickens and prevent its outbreaks in human population.
This study was supported by following projects – APVV-21-0129 of Slovak Research and Development Agency and VEGA 1/0554/21 of Slovak Scientific Agency.

P2-028 Interferon-alpha induces cellular autophagy and mitophagy by inhibiting Akt/mTOR pathway in Hypothalamic POMC neurons.

Lim Y[1], Chu P[1], Chen S[1]
[1]Institute Of Neuroscience, National Chengchi University

Accumulating data suggest that Interferon-alpha can act as neuronal modulator affecting to neural physiology. IFN-α is a pleiotropic cytokine with wide clinical applications to treat autoimmune diseases, viral diseases, and cancer by chronic treatment. However, the chronic treatment of IFN-α leads to various neurological diseases, including depression. POMC neurons is the neuronal population located in hypothalamic arcuate nucleus (ARC) regulating several essential physiological functions such as appetite, sleep, glucose metabolism, and energy homeostasis. In this study, we aimed to investigate the effect of IFN-α on neuronal characteristics and functions. We examined the cellular response of the mHypoA-POMC/GFP1 cell to the chronic treatment of IFN-α. This immortalized cell line has been previously proven that key metabolic responses and neuronal hypothalamic neuron function are maintained and can be used to mimic hypothalamic neuron functions. We found that chronic treatment of IFN-α initiates the Interferon signaling including STATs were upregulated. Significantly, autophagy was activated as observed by autophagosome formation and the up-regulation of autophagic and mitophagy markers, including LC3, Beclin-1, and PINK1. In contrast, chronic IFNα treatment downregulates the activity of Akt/mTOR/AMPK signaling pathways, consequently promoting the formation of the autophagosome on hypothalamic neurons. Additionally, the brain tissue of IFN-α chronic injection on mice showed that double immunofluorescence staining demonstrates co-localization of hypothalamic neuronal and autophagy protein with POMC and LC3. In summary, our study revealed that Interferon-alpha induced autophagy and mitophagy in hypothalamic POMC neurons during long-term treatment.

P2-029 IL-33 regulates tissue remodeling via MAPK/ NF-kB signal pathway in chronic rhinosinusitis

Lee H[1], Moon J[1]
[1]Department Of ORL-HNS, Korea University Medical Center, Seoul, South Korea

Purposes: IL-33 has been implicated in several inflammatory upper airway diseases including chronic rhinosinusitis. This study aims to investigate the effects of IL-33 on myofibroblast differentiation and extracellular matrix production and to investigate the underlying molecular mechanisms of IL-33 in sinonasal fibroblasts.
Methods: Sinonasal tissues were obtained from healthy, chronic rhinosinusitis without nasal polyposis (CRSsNP), and chronic rhinosinusitis with nasal polyposis (CRSwNP). Sinonasal fibroblasts were isolated and cultured. Expression levels of α-smooth muscle actin (SMA), fibronectin, IL-33 receptor (ST2), phosphorylated MAP kinases (p38, ERK, and JNK) and activation of NF-κB were determined by qPCR and Western blotting and/or immunofluorescent staining. Migration of fibroblast was measured by trans-well migration assay and wound scratch assay. Contractile activity was measured by a collagen gel contraction assay. siRNA for ST2 was transfected to down-regulate ST2 expression.
Results: IL-33 and ST2 expression levels were upregulated in the CRSwNP, compared to healthy control and CRSsNP. IL-33 mRNA expression positively correlated with the expression of α-SMA, fibronectin and type 1 collagen. IL-33 significantly increased mRNA and protein levels of α-SMA, fibronectin and collagen type 1. These mRNA and protein levels were dose-dependently increased significantly by IL-33 in fibroblasts. Migration and collagen gel contraction were also increased by IL-33 treament. Inhibition of ST2 by siRNA treatment significantly decreased protein expression of ST2, α-SMA, fibronectin induced by IL-33. Stimulatory molecular mechanism of IL-33 was involved in ERK, p38, JNK phosphorylation and NF-κB activation. Their specific inhibitors blocked myofibroblast differentiation and extracellular matrix production in fibroblasts. These findings were also observed in ex vivo inferior turbinate organ culture.
Conclusions: These findings suggest that IL-33-stimulated myofibroblast differentiation and extracellular matrix production through ST2/MAPK/NF-κB signaling pathway in sinonasal fibroblasts, which may contribute to the development of CRSwNP.

P2-030 Insights into the potential of Interleukin-37 as an anti-inflammatory therapeutic.

Bujotzek A[5], Tiefenthaler G[5], Lariviere L[5], Rudloff I[1,3], Cho S[1,3], Whisstock J[2], Nold M[1,3], Schumacher F[4,5], Ellisdon A[2], Nold C[1,3]
[1]Hudson Institute of Medical Research & Monash University, [2]Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, Monash University, [3]Department of Paediatrics, Monash University, [4]Roche Pharma Research and Early Development, Roche Innovation Center, [5]Roche Pharma Research and Early Development, Roche Innovation Center

BACKGROUND: An estimated 5-8% of the world's population is afflicted by severe inflammatory diseases that cause tremendous suffering and entail a major socioeconomic cost. In many areas of the globe, such diseases are among the leading causes of death, particularly in women younger than 65 years in the case of autoimmune aetiologies. Therefore, improved therapeutics are urgently needed.
Methods: Expression of IL-37 in E.Coli or mammalian purification of IL-37Fc fusion. Testing: in vitro, ex vivo and in vivo (murine model of disease).
RESULTS: Here we highlight the potential of interleukin (IL-)37 as an anti-inflammatory therapeutic Its powerful anti-inflammatory functions govern principal pathways of innate and adaptive immunity and inflammation, including signaling by pattern recognition receptors such as Toll-like receptors (TLRs), cytokines such as IL-1 and tumor necrosis factor (TNF), and type 1-, 2- and 3-polarised adaptive immune responses. Many of the mediators curbed by IL-37 are themselves important targets of inhibitory drugs in current clinical use (e.g. IL-17 blocked by secukinumab in psoriasis or TNF by infliximab in several autoimmune diseases).
CONCLUSIONS: Given such broad inhibitory effects on clinically relevant targets, there is great interest in utilising IL-37 itself and derivations thereof as broad-spectrum anti-inflammatory therapeutics. However, despite potent anti-inflammatory effects in several disease models, unmodified native IL-37 is poorly suited to therapeutic development. In collaboration with the Roche Innovation Center our team engineered a stable and potent anti-inflammatory IL-37-Fc fusion with enhanced therapeutic potential.

P2-031 Anti-fibrotic and anti-polyp effects of IL-37 in chronic rhinosinusitis

Shin H[1], Khalmuratova R[1], Kim Y[1]
[1]Seoul National University College of Medicine

Chronic rhinosinusitis is an inflammation of the sinus mucosa that lasts for more than 12 weeks and is a representative intractable disease that sometimes accompanies polyps. IL-37 is known to suppress both innate and acquired immunity. However, the role of IL-37 in chronic rhinosinusitis has not been fully elucidated.
IL-37 expression was evaluated by collecting tissues from 15 normal subjects, 15 patients with chronic sinusitis without polyps, and 15 patients with chronic sinusitis with polyps, respectively. The number of IL-37-expressing epithelial cells in normal and polyp tissues of polyp patients was significantly lower than that of normal subjects. A chronic rhinosinusitis murine model (n=20) was prepared using OVA/SEB, and intranasal IL-37 treatment group was compared with PBS treatment group or dexamethasone treatment group. Both the number of polyps and epithelial disruptions were significantly reduced in the IL-37 treated group compared to PBS. In addition, it was confirmed that the expression of vimentin and α-SMA in the IL-37 treatment group was significantly reduced compared to PBS.
Through this study, IL-37 inhibited the fibrosis and nasal polyps, suggesting the possibility of using it as a treatment for chronic rhinosinusitis.

P2-032 Next-generation sequencing analysis revealed lunasin ameliorated inflammation, oxidative stress and glucose uptake in C2C12 myotubes

Huang P[1], Chiang C[2], Hsieh C[1]
[1]Department of Biochemical Science & Technology,National Taiwan University, [2]Graduate Program of Nutrition Science, National Taiwan Normal University

Background and objectives: Obesity, accompanied by chronic low-grade inflammation, contributes to risk factors for metabolic dysfunction. Lunasin, a seed peptide, has been shown to possess various bioactive properties such as anti-carcinogenic, anti-inflammatory, and anti-oxidative effects. Previous data showed that lunasin decreased pro-inflammatory cytokines interleukin (IL)-6 and monocyte chemoattractant protein (MCP)-1 productions, and improved glucose uptake. Additionally, pro-inflammatory cytokines are related to the reactive oxygen species (ROS) generation. This study aimed to investigate how lunasin regulates oxidative stress and glucose utilization in C2C12 myotubes in the obese condition and its possible molecular mechanisms.
Methods: C2C12 cells were challenged by palmitic acid (PA) to mimic the obesity-induced insulin resistance, and ROS and cell vitality were analyzed. C2C12 cells in the presence or absence of insulin represents the fasting or feeding phase. Cellular RNA was extracted and analyzed by next generation sequencing (NGS). The biological mechanism was analyzed used the Kyoto Encyclopedia of Genes and Genomes (KEGG).
Results: Lunasin reduced ROS generation in C2C12 cells induced by PA and H2O2. In addition, lunasin increased the proportion of healthy cells in the PA and H2O2 challenges. In NGS, insulin induced cell cycle through up-regulation of mki67 and down-regulation of inmt genes expression in volcano plots. Interestingly, lunasin increased the piccolo gene expression, indicating an improvement of insulin secretion. The KEGG analysis showed that insulin stimulation boosted molecules involved in the cell cycle, while lunasin enhanced the insulin secretion and oxidative phosphorylation pathways, no matter insulin presence or absence. Moreover, lunasin decreased diabetic cardiomyopathy and ROS related-pathways by down-regulation of cxIII .
Conclusions: The study has revealed that lunasin suppressed inflammation, oxidative stress and enhanced cell vitality and insulin secretion, resulting to promote glucose utilization in C2C12 myotubes, might benefit to metabolic disorders in obese conditions.

P2-033 IRF3-mediated CXCL1 expression regulates neutrophil infiltration in bacterial pneumonia

Yongliang Z[1]
[1]National University Of Singapore

Background:
Bacterial pneumonia is a leading cause of illness and hospitalization. Neutrophils are indispensable for controlling bacterial infection, but exaggerated neutrophil response could cause acute lung injury. The interferon regulatory factor 3 (IRF3) is known as the master regulator of type I interferons. However, if IRF3 plays a role in regulation of neutrophils in pneumonia is unclear.
Methods:
IRF3 and IFNAR wildtype (WT) and knockout (KO) mice were infected with Burkholderia thaliandensis (Bt) or Klebsiella pneumoiae (Kp) to examine their functions in bacterial pneumonia. Immune infiltration and cytokines/chemokines expression in the lung were examined. Neutrophil NETs formation was determined in the lung of the mice. In addition, transcriptome analysis of the lung from WT and KO mice were carried out to identify important pathways/molecules in the pathogenesis of bacterial pneumonia.
Results:
IRF3 KO mice were resistant to lethal Bt and Kp pulmonary infection, which was associated with reduced bacterial burden and lung injury compared to WT mice. The resistance of IRF3 KO mice to lethal pulmonary infection was independent of type I IFNs, but dependent on neutrophils. Decreased neutrophil infiltration in the lung of KO mice was associated with lower levels of various inflammatory cytokines/chemokines and lower NETs formation compared to WT. Transcriptome analysis showed that pathways that are important for leukocyte migration and regulation of inflammation were downregulated in KO. Further investigation showed that IRF3-regulated CXCL1 was important for neutrophil recruitment upon pulmonary bacteria, thereby regulating lung injury and disease outcome.
Conclusion:
Deficiency of IRF3 resulted in resistance to lethal pulmonary bacterial infection, which is independent of type I IFNs, but dependent on its regulation of neutrophil recruitment through CXCL1. These findings suggest that IRF3-CXCL1 axis is important for neutrophil recruitment during bacterial pneumonia and could be targeted to develop therapeutic intervention for treatment of the disease.

P2-034 Plexin B1 controls the size of the Treg pool in vivo and limits allergic airway inflammation

Chapoval S[1], Gao H[1], Fanaroff R[1], Keegan A[1]
[1]University of Maryland School of Medicine

Plexin B1 is a high affinity receptor for semaphorin class 4 family members, Sema4A and Sema4D. We showed previously that Sema4A deficient mice displayed enhanced allergic airway inflammation accompanied by fewer Treg cells, while Sema4D deficient mice displayed reduced inflammation and increased Treg cell numbers. We investigated the effect of global Plexin B1 deficiency on the allergic airway responses to ovalbumin (OVA) or house dust mite (HDM) challenges using an intraperitoneal priming with OVA absorbed to Alum adjuvant followed by two OVA nebulizations or multiple intranasal HDM applications. In the classical OVA model, Plexin B1 deficiency led to increases in lung inflammation, mucus production, and Th2 cytokines. Spleen cells from Plexin B1-/- mice proliferated more robustly than WT cells in vitro to a variety of stimuli. CD4+ T cells from spleens of these mice expressed higher levels of the activation markers CD69 and Ki-67 as compared to WT mice. Spleen cells from naïve Plexin B1-/- mice secreted IL-4 and IL-6 whereas in vivo OVA-primed spleen cells produced IL-4/IL-5 when subjected to in vitro OVA restimulation. The upregulated allergic inflammatory response in Plexin B1-/- mice was associated with a lower number of Tregs in the lung tissues after OVA treatment. Despite higher Th2 cytokine levels in BALF of HDM-treated Plexin B1-/- mice, the gradings of tissue inflammation and mucus were not affected by the absence of Plexin B1. Our studies demonstrate a previously unrecognized in vivo link between Plexin B1 and Treg cells. These data add to the evaluation of Sema4A as a Plexin B1 ligand, as a regulator of Treg cell stability and activity in vitro and in vivo, and as a potential immunotherapeutic for allergic airway disease.

P2-035 Exogenous Lipoxin A4 attenuates IL4-induced Mucin Expression in Human Airway Epithelial Cells

Seo E[1], Lee S[2], Choi B[3], Park J[3], Kim B[4], Kim J[3]
[1]BKPlus21, School of medicine, Konkuk University, Seoul, Korea, [2]Department of Microbiology, School of medicine, Konkuk University, [3]Departments of Otorhinolaryngology-Head & Neck Surgery, School of medicine, Konkuk University, [4]Department of Otolaryngology-Head and Neck Surgery, Eunpyeong St. Mary's Hospital, College of Medicine, The Catholic University of Korea

Introduction: The proinflammatory cytokine interleukin-4 (IL-4) induces mucus hypersecretion by human airway epithelial cells and the MAP kinase signalling pathway may be important in terms of IL-4-induced MUC5AC gene expression. Lipoxin A¬4 (LXA4) is an arachidonic acid-derived mediator that promotes inflammation by binding to the anti-inflammatory receptors (ALXs) or the formyl-peptide receptor like-1 (FPRL1) protein expressed by airway epithelial cells. Here, we explore the effects of LXA4 on IL-4-induced mucin gene expression in, and secretion from, human airway epithelial cells.
Methods: We co-treated cells with IL-4 (20 ng/mL) and LXA4 (1 nM) and measured the expression levels of mRNAs encoding MUC5AC and 5B via real-time polymerase chain reaction; protein expression levels were determined by Western blotting and immunocytofluorescence. The ability of IL-4 and LXA4 to suppress protein expression was determined by Western blotting.
Results: IL-4 increased MUC5AC and 5B gene and protein expression. LXA4 suppressed IL-4-induced MUC5AC and 5B gene and protein expression by interacting with the IL4 receptor and mitogen-activated protein kinase (MAPK) pathway, including both phospho-p38 MAPK and phospho-extracellular signal-regulated kinase (phospho-ERK). IL-4 and LXA4 increased and decreased, respectively, the number of cells that stained with anti-MUC5AC and 5B antibodies.
Conclusions: LXA4 may regulate mucus hypersecretion induced by IL4 in human airway epithelial cells.

P2-036 Interaction between Innate Immune Cells and DRG Neurons Leads to the Epigenetic Memory upon Atopic Dermatitis condition

Lee J[1], Kim S[1], Park J[2], Choi J[2], Kim L[3]
[1]Brain Korea 21 FOUR Project for Medical Science, Yonsei University College of Medicine, [2]Department of Biomedical Sciences, Korea University College of Medicine, [3]Yonsei University College of Medicine

Background: Atopic dermatitis(AD) is one of the most common inflammatory dermatologic disease characterized by severe pruritus. The core pathophysiology underlying AD is Th2-skewed inflammation within the skin. Previous research have been tried to delineate the relationship between skin inflammation and severe pruritus, which have led to the concept that the skin inflammatory repertoire might influence the dorsal root ganglion (DRG) neurons to be 'sensitized' for pruritus. In this regard, we tried interpreting those phenomena by 'epigenetic memory' of neurons.

Methods: We tried anlayzing the public AD skin and DRG scRNA-seq dataset to infer cell-to-cell interactions between the skin cells and DRG neurons. Subsequently, the downstream pathways and hallmark transcription factor associated with the candidate receptors on DRG neurons were analyzed. We also profiled the genome-wide chromatin accessibility map of the DRG neurons by ATAC-seq with the MC903-induced AD mouse model's DRG neurons. We have combined the scRNA-seq analysis with ATAC-seq analysis to identify the core ligand-receptor interactions between skin cells and DRG neurons and the master transcription factor which might be able to engage in the sensitization of DRG neurons to pruritus.

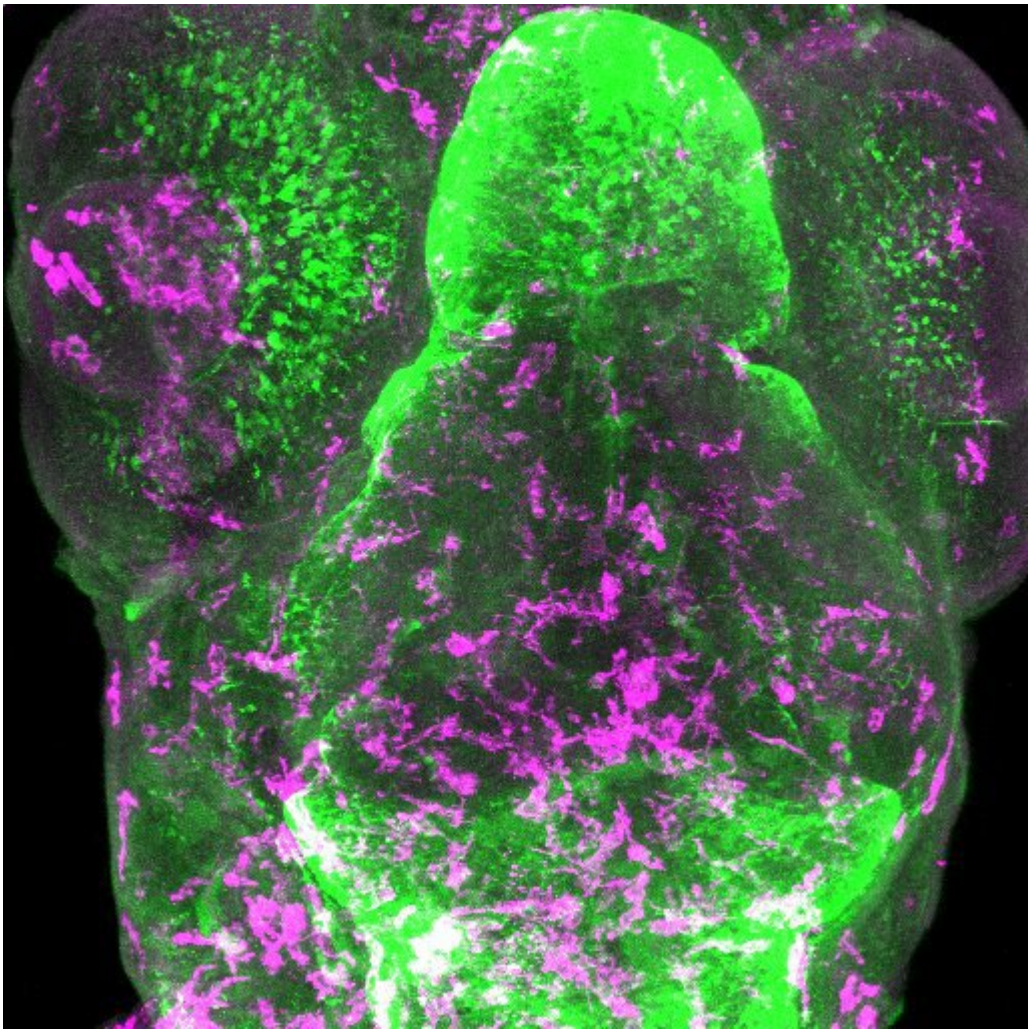
Results: We found that, among the enriched immune cells in AD, innate immune cells rather than adaptive immune cells primarily interact with DRG neurons. Also, by combining the scRNA-seq and ATAC-seq analysis, we could identify the master regulator which might take part in inducing the epigenetic change in DRG neurons to memorize the pruritus.

Conclusion: By combining the transcriptomic & epigenomic assays, we were able to get insight on the change of gene regulatory landscape in DRG neurons in atopic dermatitis-like environments. This study also ighlights that the concept of epigenomic memory is able to be applied with various tissue and cell types.

P2-037 Illuminating antiviral inflammation and neuroinflammation

Wiggett H[1,2], Froc C[6], Champelovier D[4], Levraud J[2], Bousso P[1,3], Meijer A[5], Faught E[5]
[1]Dynamics of immune responses unit, equipe labellisée ligue contre le cancer, Institut Pasteur, [2]Team Neuro-infection and immunity, Institut des Neurosciences Paris-Saclay, Université Paris-Saclay, CNRS, Institut Pasteur, [3]INSERM U1223, [4]TEFOR Paris-Saclay, UAR 2010, CNRS, Université Paris-Saclay, Centre CEA, Bat 151, [5]Institut of Biology, Leiden University, [6]PSI-Co, Institute des Neurosciences Paris-Saclay, CNRS UMR9197, University Paris-Saclay

Type 1 interferons (IFN-Is) are the major cytokines in the antiviral immune response. They are essential in the clearance of viral pathogens and activate an array of downstream effector proteins via canonical jak/stat and non-canonical signalling pathways. Whilst IFN-Is have broad antiviral effects, their clinical usage is limited by side effects, most significantly by the occurrence of neuropsychiatric symptoms such as major depressive disorder. The detrimental effects of IFN-Is on the developing brain are further illustrated in heritable diseases characterised by IFN-I overproduction, termed interferonopathies, which currently have limited treatment options. Gaining insight into the molecular mechanisms of the IFN-I response in the central nervous system (CNS) is therefore vital in the development of tools for the modulation of inflammation and to determine the efficacy of IFN-I targetting therapies. Using zebrafish larvae, we are looking at the impact of viral inflammation and the IFN-I response on behaviour, gene expression and cellular morphology to identify how it affects the developing brain. Using whole larvae single cell sequencing, we have been able to identify new neuron-specific interferon stimulated genes, whose known functions suggest a role in IFN-I mediated neuropathology and psychiatric diseases. We are also developing optogenetic tools that will allow us to generate the sterile release of IFN-I in a spatially and temporally controlled manner. By utilising the optical accessibility of the zebrafish alongside fluorescent reporters for downstream IFN-I signalling cascades, this system will give us the opportunity to visualise the real time dynamics of the cytokine response in the whole organism.



P2-038 Diesel exhaust particle impairs nasal epithelial barrier through activation of aryl hydrocarbon receptor in rhinitis model

Ryu G[1], Shin H[2], Mo J[3]
[1]Samsung Medical Center, [2]Seoul National University College of Medicine, [3]Dankook University College of Medicine

Background: Diesel exhaust particles (DEPs) are major traffic-related air pollutants. They are closely related to the development and aggravation of respiratory diseases. In rhinitis, DEPs can downregulate tight junction proteins, which can lead to barrier disruption and disease exacerbation. We sought to explore whether DEPs aggravate inflammation through the aryl hydrocarbon receptor (AhR) activation in rhinitis and to demonstrate that blocking AhR can reduce inflammation and alleviate rhinitis aggravated by DEPs.
Methods: We examined inflammatory markers and tight junction molecules in DEP-exposed ovalbumin and polyinosinic-polycytidylic acid (poly I:C)-induced rhinitis mouse model and primary human nasal epithelial cells (HNECs) using immunostaining, quantitative real-time polymerase chain reaction, and immunoblotting. The effect of AhR blocking was also analyzed in the mouse model using CH223191, an AhR antagonist, and AhR knockout mice.
Results: Exposure to DEPs caused overexpression of interferon-γ and interleukin (IL)-17A, and infiltration of neutrophils and IL-17A+ cells in the poly(I:C)-induced rhinitis model. Tight junction molecules including E-cadherin, ZO-1, Claudin-1, and Claudin-3 were significantly decreased after DEP treatment. In an air-liquid interface culture of HNECs, DEPs increased cellular permeability and reduced tight junction proteins in a dose- and time-dependent manner. AhR and CYP1AP were activated by DEP exposure in the sinonasal mucosa of the mouse model and HNECs. Blocking AhR with an antagonist reduces DEP-induced inflammations and restored barrier dysfunction. The DEP/poly(I:C)-induced rhinitis model using AhR knockout mice showed decreased inflammation and ameliorated barrier disruption.
Conclusion: DEPs can activate AhR and aggravate inflammation in rhinitis through epithelial barrier disruption. Blocking AhR in airway epithelial cells might be a new therapeutic option to restore barrier damage caused by air pollutants in rhinitis.

P2-039 Cytokine affinity tuning using the AlphaSeq platform to generate targeted immuno-oncology therapeutics

Goodnight D[1], Adamo J[1], Homad L[1], Minch K[1], Engelhart E[1], Swanson R[1], Lopez R[1]
[1]A-Alpha Bio

Although cytokine therapies have demonstrated curative effects in some cancer patients, clinical use remains limited due to alarming toxicity profiles accompanying systemic administration. Next generation cytokine approaches include conditional signaling focused to sites of interest, such as the tumor microenvironment or specific immune cell populations. Here, we share a novel approach for generating detuned cytokine therapeutic candidates using the AlphaSeq platform, which involves the re-engineering of yeast agglutination and mating to quantitatively measure protein-protein interactions at a library-on-library scale. Using interferon alpha 2 (IFNA2) as an illustrative example, we show how AlphaSeq measures cytokine-receptor interactions and generates engineered cytokines with a broad range of affinities. A saturated mutational library was created for IFNA2 and subsequently screened against a second library consisting of human IFNAR2, species orthologs and off-target receptors, which allowed for parallel identification of hundreds of detuned variants against both human and mouse receptors in a single assay. Cytokine variants with lower affinity than parental IFNA2 were recombinantly expressed as Fc fusion proteins to orthogonally measure affinity with biolayer interferometry and characterize potency with an in vitro human PBMC phosflow assay, both of which showed strong correlation with AlphaSeq affinity measurements. Finally, detuned IFNA2 candidates were fused to anti-CD8 antibodies to demonstrate cell population-specific signaling. Candidate molecules showed >1000-fold greater potency in the targeted cell population than non-targeted populations. Our results show the AlphaSeq platform can accurately quantitate thousands of cytokine variant affinities simultaneously against multiple relevant receptors, enabling the selection of candidate immunocytokine antibody fusion proteins with exquisite cell population specificity. AlphaSeq's rapid, comprehensive affinity determination is being used to develop a portfolio of clinically relevant therapeutic immunocytokines.

P2-040 Type 2 immunity promotes neonatal pulmonary stem/progenitor activity through STAT6 signaling axis

Liao C[1], Chiu C[1], Chiang B[1]
[1]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University

Background
Studies have revealed that type 2 immunity is transiently elevated in neonatal lungs, so allergen exposure or oxygen supplementation during the neonatal period further exacerbated the type 2-mediated inflammation in the lungs. However, the effect of naturally elevated type 2 immunity on neonatal lung epithelium development remains unclear. In this study, we investigated the role of immunity on neonatal lung epithelium development through the pulmonary organoid model generated by the neonatal lung-derived stem/progenitor cells, the neonatal pulmonary SSEA-1+ cells.

Methods
To investigate the effects of type 1 and type 2 immunity on neonatal lung epithelium development, the neonatal pulmonary SSEA-1+ cells were embedded in 50% Matrigel for 3D culture with the supplements of IFN-γ or IL-13. The epithelium development was evaluated after 14 days of culture, including organoid generation and epithelial differentiation.

Results
The organoid generation was increased by IL-13 but suppressed by IFN-γ, suggesting the opposite roles of type 1 and type 2 immunity on neonatal lung epithelium development. The effect of IL-13 was associated with increased cell proliferation. To clarify the differences between transient elevated and dysregulated type 2-mediated inflammation on neonatal lung development, the neonatal lung stem/progenitor cells were stimulated with short-term or sustained addition of IL-13. Short-term IL-13 exposure could also enhance organoid generation with multiple cell type differentiation, such as basal, club, ciliated, and goblet cells. However, sustained IL-13 stimulation had led to abnormal epithelium development by increased goblet but decreased ciliated cell differentiation. In addition, the IL-13-induced cell proliferation and differentiation were not observed in STAT6-deficient cells, suggesting the role of STAT6 in mediating the stem/progenitor properties.

Conclusion
Our results suggested that the transiently elevated type 2 immunity in postnatal lungs might exert a beneficial effect to facilitate lung growth. However, dysregulated type 2-mediated inflammation disrupts epithelial homeostasis by allergic asthma-like feature development.

P2-041 Elucidating the Role of Steady-State Th1 Cells in CNS Physiology

Yoshida T[1], Nguyen M[1], Murray K[2], Grutzendler J[2], Palm N[1], Hafler D[2], Wang A[3]
[1]Yale School of Medicine, Department of Immunobiology, [2]Yale School of Medicine, Department of Neurology, [3]Yale School of Medicine, Department of Rheumatology

While T cells mediate immune surveillance, they also orchestrate diverse biologic functions, such as metabolic homeostasis, through communication with parenchymal cells within tissues. Here, we use flow cytometry and single-cell RNA sequencing to profile T cells in healthy mouse brain at steady-state, revealing functional, modulatory, and metabolic differences compared to the periphery. Among such differences was an unexpected Th1/IFN-γ and Th17/IL-17A signature in brain T cells ex vivo confirmed with direct measurement of intracytoplasmic IFN-γ and IL-17A expression. This steady-state extravascular T cell population in the brain was primed by the microbiome during weaning and originate from the periphery, namely the adipose and gastrointestinal tissues as demonstrated using a photoconvertible mouse model. This fat-brain axis can be further perturbed by food-depriving mice, resulting in the mobilization of T cells from the fat to the brain. These steady-state brain T cells play a role in regulating behavior, as IFN-γ-deficient mice and T-bet-deficient mice lacking the CD4 Th1 compartment exhibit behavioral differences. Identifying a robust CNS T cell signature provides the framework for understanding the diverse homeostatic functions of T cells in this highly specialized tissue and for modeling perturbed T cell: glia axes in neuroinflammation.

P2-042 CYTOKINES IN ASTHMA: TYPE 2 AND BEYOND

Asrat S[1], Scott G[1], srivatsan s[1], Lim W[1], Nagashima K[1], Ben L[1], Le Floc'h A[1], Murphy A[1], Yancopoulos G[1], Sleeman M[1], Orengo J[1]
[1]Regeneron Pharmaceuticals

Background: Asthma is a heterogeneous chronic airway disease with variable clinical phenotypes that are currently stratified into Type 2-high (T2) and T2-low endotypes. Cytokines play a key role as drivers of airway inflammation and remodeling associated with asthma. Targeting cytokines in asthmatic patients with licensed biologics (anti-IL-5/Rα, anti-IL-4Rα, anti-TSLP) and emerging therapies (anti-IL-33) has shown clinical benefit.

Methods: Primary human immune cells were stimulated in vitro with IL-4, IL-13, IL-5 or IL-33 and differential gene expression was assessed by RNAseq. Airway inflammation and fibrosis in the context of either anti-IL-4R, anti-IL-33 or anti-IL-5 was assessed in a mouse model of lung inflammation induced by intranasal administration of house dust mite extract.

Results: IL-4 and IL-13 drive distinct and overlapping pathways in airway inflammation suggesting that dual cytokine blockade is necessary to alleviate disease. IL-4 and IL-13 drive lung inflammation and remodeling by inducing immune cell infiltration, increasing cytokine and chemokine expression, impacting barrier integrity and mucus production, which in turn leads to impaired lung function; whereas IL-5 drives eosinophil maturation and survival. IL-33 is not just an alarmin that initiates an inflammatory response, but is also an unrelenting signal that once induced, retains the lung in a state of lasting inflammation and remodeled tissue, primed for exacerbations. Using head-to-head comparisons with various monoclonal antibodies, we determined that anti-IL-5 globally reduced eosinophil counts but did not impact inflammatory or functional measures of lung pathology; whereas, blockade of either IL-4/13 or IL-33 leads to resolution of inflammation and remodeling.

Conclusions: Eosinophils are not the sole contributor to asthma pathophysiology or lung function decline which emphasizes the need to block additional mediators to modify lung inflammation and impact lung function. Cytokines associated with airway inflammation such as IL-4, IL-13 and IL-33, drive distinct and overlapping pathways that lead to airway inflammation and remodeling.

P2-043 The effect of interleukin-1 on the development and function of neonatal SSEA1+ lung stem cells

Weng C[1], Chiang B[2]
[1]Graduate Institute Of Immunology, College Of Medicine, National Taiwan University, [2]Graduate Institute Of Clinical, College Of Medicine, National Taiwan University

Background
IL-1, which includes both IL-1α and IL-1β, has been implicated in neonatal lung dysplasia. However, the mechanism by which IL-1 disrupts lung development and regeneration in neonates remains unclear. SSEA1+ cells were a population of stem cells in the neonatal lung that had the ability to differentiate into both airway and alveolar epithelial cells. This study aims to determine whether IL-1 affects neonatal lung dysplasia and injury by regulating the function of neonatal SSEA1+ lung stem cells.

Methods
To investigate the role of IL-1 in regulating SSEA1+ lung stem cells, a 3D organoid culture system was employed. SSEA1+ lung stem cells were isolated from the lungs of neonatal mice embedded in matrigel, and cultured with or without IL-1α and IL-1β to follow the development of organoid formation.

Results
We found that stimulation with IL-1α and IL-1β altered the morphology of SSEA1+ lung stem cell-derived organoids. Furthermore, the organoids treated with IL-1 contained a higher proportion of SSEA1+ cells than those without IL-1. The expression levels of markers associated with airway epithelium cells were significantly decreased at both the RNA and protein levels following IL-1 stimulation, indicating that IL-1 inhibited the differentiation of airway epithelium cells from SSEA1+ stem cells. Although there was no difference in the expression levels of the type I alveolar cell (AT1)-associated gene HOPX1 between organoids with and without IL-1, the expression of another type I alveolar-associated gene, AQP5, was significantly decreased in response to IL-1 stimulation, suggesting that IL-1 altered the normal differentiation of AT1 cells.

Conclusion
Collectively, our findings suggest that IL-1 plays a critical role in impairing the differentiation of both airway and alveolar epithelial cells from SSEA1+ stem cells during the neonatal stage, potentially contributing to the development of abnormal lung morphology and function.

P2-044 Sorbus commixta promotes antimicrobial responses through Autophagy activation via the AMP-activated protein kinase pathway in Allergic asthma.

Lee H[1], Park J[1], Shin H[1]
[1]College of Veterinary Medicine, Chungnam National University

The induction of host cell autophagy by various autophagy inducers contributes to the antimicrobial host defense against inflammation conditions. In this study, we present a role for the newly identified natural compound from Sorbus commixta (Sc-JH) in the antimicrobial responses against allergic asthma models and macrophages. Sc-JH robustly activated autophagy, which was essentially required for all steps of autophagy responses in human and murine macrophages. Using an allergic asthma mouse model, we showed that Sc-JH-induced autophagy contributed to the regulation of inflammatory responses. We further showed that Sc-JH triggered AMP-activated protein kinase (AMPK) activation, which was required for antimicrobial responses against inflammation. Collectively, these data show that Sc-JH is a promising candidate for new anti-allergic asthma therapeutics by activating autophagy via AMPK-dependent signaling and suppressing excessive inflammation during allergy infections.

P2-045 Type 2 diabetics have an elevated level of NLRP3 inflammasome activation

Lee H[1], Park J[1], Shin H[1]
[1]Chungnam National University

Little is known about the ex vivo profile of inflammasome activation in type 2 diabetic patients. NLRP3 inflammasome in the pathophysiology of type 2 diabetes. In this study, we looked into the patterns of NLRP3 inflammasome activation in monocyte-derived macrophages (MDMs) from patients with type 2 diabetes who had never taken any medication before. In MDMs cultivated with autologous sera, type 2 diabetes patients had significantly higher levels of NLRP3, an apoptosis-associated speck-like protein containing a CARD, and proinflammatory cytokines than did healthy controls. When MDMs from type 2 diabetic patients were stimulated with various danger molecules, interleukin (IL)-1β maturation, IL-18 secretion, and caspase-1 cleavage were found to be upregulated. Finally, 2 months of treatment with the diabetes medication metformin significantly decreased the maturation of IL-1β in MDMs from type 2 diabetes patients through activating the AMP-activated protein kinase. Collectively, our findings indicate that metformin use as an anti-diabetic medication helps to modulate inflammasome activation in type 2 diabetes and that NLRP3 inflammasome activation is increased in myeloid cells from type 2 diabetic patients.

P2-046 Understanding mechanisms by which microbially derived metabolites regulate host gut inflammation.

Robinson E[1], Sonnenburg E[1], Sonnenburg J[1]
[1]Stanford University

Chronic inflammation is a rising health issue that affects millions of people worldwide. Emerging evidence shows that dysbiosis of the gut microbiota can lead to changes in the production of microbial-derived metabolites (MDMs), which contribute to inflammation and the onset of inflammatory diseases, such as cancer, non-alcoholic fatty liver disease, and inflammatory bowel syndrome. MDMs are absorbed into host circulation through the gut and regulate immune responses. A key immune cell type at the interface of host-microbe interactions is macrophages, which maintain tissue homeostasis, and are key contributors to chronic inflammation. To date, few MDMs with well-studied cellular and molecular mechanisms describing the interplay between microbes and our immune system are known. Here we aim to identify novel MDMs that positively and negatively regulate the host immune system through a direct effect on macrophages. Previously, we carried out a 17-week dietary intervention study that found distinct immunological trajectories based on diet and microbiota diversity. We utilized the untargeted metabolomics data from serum and stool samples from this study to identify MDMs that associate with the trajectory of immunological markers during the intervention. To validate these findings, we stimulated human macrophages to measure how pro-inflammatory cytokines expression and NFκB activation are altered upon MDM exposure. Next, we used primary mouse macrophages and determine the immune regulatory capacity of a number of MDMs conserved between mice and humans, including organic acids, amino acid derivatives, and carboxylic acid derivatives. We are now using CRISPR/Cas9 techniques to interrogate what part of the host inflammatory signaling pathway is disrupted by exposure to the metabolite in macrophages. This work highlights the power of taking a human-first approach to investigate bacterial metabolites that interact with human immune cells and be studied in animal models, a discovery pipeline focused on identifying novel therapeutic strategies for inflammatory diseases.

P2-047 Interferon-Alpha Subtype-Specific Effect Against Hepatitis B Virus in IFNAR-Humanized Mouse Model

Ashuo A[1], Li Y[1], Ye J[1], Hua T[1], Li Y[1], Chen J[1], Yuan Z[1]
[1]Key Laboratory of Medical Molecular Virology (MOE/NHC/CAMS), Shanghai Frontiers Science Center of Pathogenic Microorganisms and Infection, School of Basic Medical Sciences, Shanghai Medical College, Fudan University

Background: Hepatitis B virus (HBV) infection remains a major health burden worldwide with ~250 million individuals chronically infected, to whom a curative therapy is still not available. Interferon-α (IFN-α) includes numerous subtypes whose diverse functions remain largely unknown. IFN-α2 has been used for treating chronic HBV infection, but its efficacy is relatively low. We have identified human IFN-α14 as the most effective subtype against HBV in cell models and immunodeficient human-liver chimeric mice. It remains unclear how IFN-α14 activates the signaling and works in immunocompetent settings.
Methods: A gene targeting strategy was employed to generate the human IFN-I receptor (hIFNAR)-transgenic mice, by which the anti-HBV effect and immunomodulatory potential of IFN-α2 and -α14 was evaluated.
Results: In comparison to IFN-α2, IFN-α14 induced higher activation of STAT1/2 and IFN-stimulated genes, and synergistically elicited IFN-α and -γ signaling. We successfully constructed the IFNAR-humanized mouse model and detected the responsiveness to IFN-α2 and -α14 in vivo and ex vivo. A more potent anti-HBV effect of IFN-α14 was observed, while the adverse effects induced by IFN-α2 and -α14 were very similar at a well tolerable level. Compared to IFN-α2, IFN-α14 treatment induced higher percentages of IFN γ-producing CD8+ T cells as well as antigen-specific CD8+ T cells.
Conclusion: These results support that IFN-α14 elicits potent antiviral effect against HBV and could be more effective in immune modulation, which deepen our understanding of the divergent activities of IFN-α subtypes, with implications for improved IFN therapy and HBV cure strategies.

P2-048 Tensor factorization maps dysregulation of immune signaling in breast cancer patients

Orcutt-Jahns B[1], Lee P[2], Rodin A[2], Meyer A[1]
[1]University of California, Los Angeles, [2]City of Hope

Introduction
Metastatic cancer correlates with dysregulation of the immune system's response to cytokine stimulation, and greater alteration is correlated with worse clinical outcomes. However, while examples of dysregulation have been identified involving specific subsets of cells, the nature of this dysregulation has not been interrogated systematically.

Materials and Methods
To systematically profile the dysregulated responses in breast cancer, we stimulated human PBMCs from 18 healthy and 18 HR+ breast cancer patients with a panel of 8 cytokines and growth factors. Cells were then stained for cell type and response markers and responses in 23 cell populations were measured. We organized this data into a four-dimensional tensor and used tensor factorization to delineate patterns in the data (Fig. 1a).

Results/Conclusions
We examined components generated during tensor factorization to uncover their meaning across cell types, treatments, and markers (Fig. 1b-c). We found that patient-associated factors were informative of disease status (Fig. 1d). We next used predictive models to identify cancer status, and thus identify which components were most predictive of BC status (Fig. 1e). We found that CD8+ T cells showed lesser pSTAT3 responses to IL-10, in a pattern associated strongly with cancer status (Fig. 1f). Altered IL-10 responses were not explained by changes in the cytokine's cognate receptor. Upregulation of the abundance of checkpoint protein PD-L1 in B cells was also correlated with cancer status.

Discussion
We found that immune signaling in breast cancer patients was characterized by consistent patterns of altered receptor expression and altered capacity to respond to cytokine signals and that a reduction in capacity to respond to IL-10 and increased abundance of PD-L1 in B and cytotoxic T cells were among the patterns most associated with disease status. Resolving the patterns of dysregulation uncovered in our analysis may help to reestablish anti-tumor immunity.

P2-049 Construction of RNAi against Th2-related cytokines for the treatment of animal model of atopic dermatitis

Lai M[1], Chiang B[1]
[1]Graduate Institute Of Oral Biology, College Of Medicine, National Taiwan University

Background
The aim of this study is to investigate therapeutic strategies for atopic dermatitis (AD), a chronic disease caused by repeated allergic reactions, affecting approximately 10-20% of children worldwide. The pathogenesis of AD involves the crucial role of type 2 helper T cells (Th2 cells) that secrete interleukin 4 (IL-4) and interleukin 13 (IL-13). These Th2-related cytokines inhibit epidermal differentiation and suppress epidermal lipid synthesis, leading to the disruption of the skin barrier, sensitization of mast cells through the secretion of immunoglobulin E (IgE), and ultimately the release of histamine mediators that cause AD.

Methods
Therefore, the study aimed to explore the feasibility of relatively low-cost RNA interference (RNAi) technology as a potential alternative therapeutic approach. The study focuses on constructing RNAi targeting the key cytokines IL-4 and IL-13 in the pathogenesis of AD using RNAi to degrade their mRNA.

Results
The experimental results demonstrated that the application of a lentiviral vector to deliver siRNA could effectively decrease the expression of IL-4 and IL-13 inflammatory cytokines and reduce inflammatory cell infiltration. Moreover, the application of a hydrogel mixed with lentiviral vectors carrying siRNA targeting IL-4 and IL-13 was used to treat AD in mice, resulting in significant differences in the experimental outcomes.

Conclusion
In summary, the study aimed to investigate further the efficient delivery of siRNA to mice for the treatment of atopic dermatitis. This study might shed new insight on the novel ideas and approaches for the treatment of AD.

P2-050 Characterization of the exosomes secreted by regulatory T cells induced by B cells

Cheng I[1], Chiang B[1]
[1]Graduate Institute Of Immunology, College Of Medicine, National Taiwan University

Background
Regulatory T cells are a group of T cells with immunosuppressive activity. By expressing the Foxp3 transcription factor, they perform immunoregulatory functions and suppress the proliferation or hyperactivation of pathogenic T cells to maintain homeostasis. Our previous studies found that when CD4+CD25- T cells and B cells were co-cultured, induced a subtype of regulatory T cells, named "B-cell-induced regulatory T cells," also called Treg-of-B. In in vitro proliferative experiments, we demonstrated that Treg-of-B cells inhibited the proliferation of CD4+ T cells. Given this, we focused on the mechanisms of their immunosuppressive capability. In addition, some studies have pointed out that the exosomes of regulatory T cells are involved in their immunosuppressive. Therefore, we wondered whether the exosomes derived from Treg-of-B cells could contribute to the suppression of T-cell proliferation or cytokines secretion of T helper cells.

Methods
Mice splenic B cells and CD4+CD25- T cells were isolated to generate Treg-of-B cells which were then stimulated with plate-bond anti-CD3 and anti-CD28 antibodies or bone marrow-derived macrophages, and exosomes in the supernatant were further purified by ultracentrifugation. The Treg-of-B cells-derived exosomes were further characterized and also co-cultured with T helper cells to assay the suppressive effect on T helper cells.

Results
Treg-of-B cells expressed Treg-associated markers like PD-1, CTLA-4, ICOS, LAG3, and high levels of IL-10. In addition to specific molecules, such as CD9 and CD63, Treg-of-B cell-derived exosomes also expressed regulatory T cell-related inhibitory molecules, such as CD25, PD-1, and CD39. Moreover, Treg-of-B cell-derived exosomes slightly reduced the proliferation of Th1 cells and the IFN-γ level of Th1.

Conclusion
In this study, we further characterized the function of Treg-of-B cells and also isolated exosomes derived from these Treg-of-B cells. These results here further showed the potential of Treg-of-B cells-derived exosomes as the modulatory approaches for the downregulation of inflammatory condition.

P2-051 Enteric nerve system and immune cell interaction regulates intestinal inflammation

Chung K[1], Ko S[2]
[1]Seoul National University College Of Medicine, [2]Department of Biomedical Sciences, Seoul National University College of Medicine

Background/Aim
Recent studies have revealed that enteric nervous system (ENS) interacts with the immune system and regulates the inflammatory state in IBD. Still, its mechanism has yet to be fully known. In the present study, we applied neuromodulation tools using chemogenetics and ultrasound treatment in murine models of IBD. We practiced quantitative analysis of neurons and immune cells with cleared tissue 3D imaging from IBD patient biopsy samples.
Methods
First, we used AAV virus- clozapine-N-oxide (CNO) interaction to activate ENS in mice. The retrograde AAV virus (hM3Dq, 6.2 x 1010 vg) was injected into the 3-week-old mouse. After 2 weeks (5-week-old), CNO in drinking water was administered for 7 days. To induce acute colitis, 3% DSS in drinking water was given for 7 days with CNO (1mg/ml) for the experiment group. Second, we applied ultrasound to activate ENS in mice. To induce acute colitis, 3% DSS in drinking water was administered for 7 days with or without ultrasound. The bodyweight changes and histological scoring was evaluated. The 3D image analysis was performed using colonic mucosal biopsies from patients with ulcerative colitis, which was with neuronal and neutrophil markers.

Results
Neuronal modulation by virus and CNO resulted in attenuation of acute colitis assessed by disease activity index and histologic assessment. However, there was no significant difference in body weight change and colon length between the two groups. Neuronal modulation by ultrasound attenuated DSS-induced colitis in IL-10 KO mince. 3D analysis using clearing method detected total neuronal amount and neutrophil counts, showing the difference between control and patients with UC.

Conclusions
Neuronal modulation attenuated DSS-induce colitis in mice. 3D imaging analysis using colonic mucosal samples simultaneously exhibited ENS structure and immune cells, suggesting that ENS-immune interaction plays an essential role in patients with IBD.

P2-052 Folate deficiency enhancing high-fat high-fructose diet-induced kidney fibrosis in mice

Chan C[1], Lin B[1]
[1]Department of Biochemical Science and Technology, College of Life Science, National Taiwan University

Background: Modern diets with high-fat foods, fructose syrup, and less vegetables that result in an inadequate folate intake might be a risk factor for chronic disease. The prevalence of chronic kidney disease (CKD) increases concomitantly with obesity worldwide. A recent study reported that lower dietary intake of folate might be associated with an increased CKD risk. Therefore, we investigate the effects of folate deficiency (FD) on renal fibrosis.
Methods: Eight-week-old male C57BL/6 mice were fed with either a normal-fat (NF) diet or a high-fat containing high-fructose (HFF) diet with (NF-f1, HFF-f1) or without folate (NF-f0, HFF-f0) for 12 months. For in-vitro study, mouse glomerular mesangial cell lines (MES-13) were cultured with (f1) or without (f0) folate.
Results: HFF-f0 mice had the highest body weight, fat mass, and sera leptin levels. Thus, FD increased lipid accumulation and leptin production. HFF-diets enhanced IL-2, IFN-γ, and IL-17A/F secretions by splenocytes, and folate deficiency increased splenic IL-6 and TNF-α but decreased IL-10. Therefore, FD enhanced the pro-inflammatory activities of immune cells. Folate-deficient mice had higher urine protein than folate-sufficient mice, resulting in kidney dysfunction. Then, HFF-f0 mice had the highest renal leptin, IL-6, and TGF-β1 levels, further phosphorylated the pro-fibrotic signaling molecules, such as signal transducer and activator of transcription (STAT)3 and small mothers against decapentaplegic (Smad)2/3. Thus, FD exacerbated HFF diet-induced renal fibrosis in mice. Folate deficiency increased MCP-1, IL-6, and TGF-β1 productions of LPS-stimulated or LPS+leptin co-stimulated MES-13 cells. Higher collagen expression of leptin+TGF-β1 co-stimulated f0 MES-13 cells. Increased hypoxia-inducible factor (HIF)-1α expression and mammalian target of rapamycin (mTOR) activity were detected in $CoCl_2$-stimulated f0 MES-13 cells but not in mTOR-specific inhibitor rapamycin addition.
Conclusion: Long-term dietary folate deficiency exacerbated renal fibrosis may enhancing by hyperleptinemia, inflammation, and hypoxic stress. Inadequate folate status may be one of the risk factors for CKD.

P2-053 Higher hepatic pro-inflammatory cytokines in folate-deficient mice fed with high-fat high-fructose diet

Chan C[1], Chiu Y[1], Chen T[1], Lin B[1]
[1]Department of Biochemical Science and Technology, College of Life Science, National Taiwan University

Background: The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing year by year, which is associated with imbalanced dietary habits with low vegetables, high fat, and high fructose syrup intakes is worth studying. Vegetables are a good source of folate. Previous studies have demonstrated that the sera folate levels are lower in NAFLD patients than in healthy individuals, indicating that lower folate intake or metabolism is significantly associated with NAFLD. The purpose of this study is to investigate the effects of folate deficiency and high-fat high-fructose on liver damage and hepatic cytokine profiles.
Methods: Four groups of male C57BL/6 mice were fed either a normal-fat diet with folate (NF-f1), NF without folate (NF-f0), high-fat high-fructose diet with folate (HFF-f1), or HFF without folate (HFF-f0) for 12 months before sacrifice.
Results: Our data showed that folate deficiency significantly increased the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in HFF diet-fed mice. The HFF groups had significantly higher levels of steatosis, hepatocyte ballooning, and immune cell infiltration compared to the NF groups. The total NAFLD activity score and the expression of liver lipid synthesis-related genes, such as carbohydrate response element binding protein beta (ChREBPβ), acetyl-CoA carboxylase 1 (ACC1), and fatty acid synthase (FASN) were significantly higher in the HFF-f0 group. Hepatic pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-17A/F were significantly higher in the HFF-f0 group, as well as regulatory cytokine IL-10. The hepatic IL-17A/F level was positively correlated not only TNF-α, but also IL-6. For immunoregulation, hepatic IL-10 level was also positively correlated with IL-17A/F and TNF-α.
Conclusion: These results suggest that folate deficiency might promote steatosis, inflammation, and the development of NAFLD.

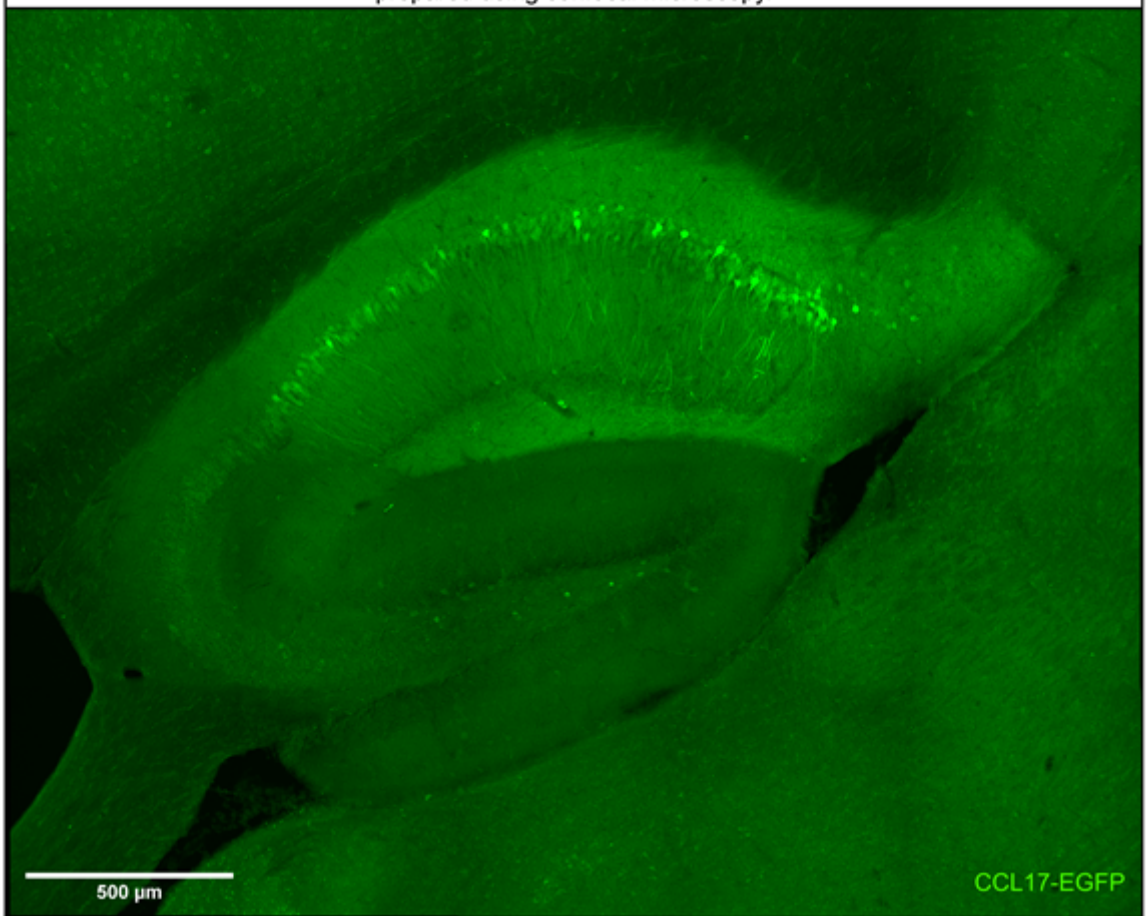
P2-054 Expression and functional role of the chemokine CCL17 in the developing murine brain

Eberhard J[1], Fülle L[1,2], Lösch J[1], Förster I[1]
[1]Immunology & Environment, LIMES Institute, University of Bonn, [2]Miltenyi Biotec

The chemokine CCL17 is best known for its role in facilitating interactions between T cells and dendritic cells. Previous research in our lab demonstrated a constitutive expression of CCL17 in hippocampal neurons of adult mice. Analysis of the tissue-resident macrophages of the brain, the microglia, showed that CCL17 is required to maintain the homeostatic morphology of these cells. Genetic deficiency for CCL17 resulted in an activated phenotype. Furthermore, CCL17 downmodulates the basal transmission at CA3-CA1 Schaffer collaterals in the hippocampus.
The expression of CCL17 during mouse brain development was analyzed utilizing CCL17-EGFP reporter mice. No CCL17+ neurons were detected in the embryonal brain parenchyma (E13.5 - E18), however, CCL17+ cells co-expressing Lyve1+ were present in the meningeal vessels of brains from mouse embryos. Following birth, CCL17+ neurons were evenly distributed in the cortex as well as the hippocampal area. Within three weeks, CCL17+ neurons vanished from the cortical area and were only detectable in the pyramidal band of the hippocampus.
CCL17 as well as CCL22 signal via the chemokine receptor CCR4. The functional role of CCL17, CCL22 and CCR4 in the murine brain remains incompletely understood. CCR4 could be involved in the CCL17-mediated maintenance of microglia morphology. Therefore, the influence of the CCL17/CCL22/CCR4-axis on microglia morphology was analyzed by immunohistology in mouse strains deficient in CCL17, CCL22, CCL17&CCL22 or CCR4. Interestingly, microglia from CCR4-deficient mice displayed distinct morphological changes compared to CCL17-deficient mice.
In conclusion, CCL17 is first expressed in the murine brain around the time of parturition and is responsible for sustaining microglia morphology and cell density. Additionally, CCL17 shows a tendency to promote neuroinflammatory processes, as in a mouse model of inducible neuroinflammation, deficiency of CCL17 resulted in a delayed progression of neuroinflammation.

CCL17-positive neurons in the CA1 area of the murine hippocampus

40µm brain section of perfused CCL17-EGFP reporter mice were stained for EGFP. Images were prepared using confocal microscopy.



P2-055 Inotodiol alleviate mucosal inflammation in eosinophilic CRS mouse model

Kim Y[1]
[1]Chungnam national university

In this study, we tried to investigate the therapeutic effect of inotodiol in mouse model of eosinophilic CRS, which are representative diseases in which mast cells and eosinophils play an important role in the pathogenesis. We also tried to find out which mechanisms of inotodiol might be involved in its therapeutic effect.
Forty mice were divided into 4 groups : control mice, NP mice , NP+Inotodiol(Ino) treatment, and NP+dexamethasone(Dexa). This experimental according to previously established protocol, murine model of chronic rhinosinusitis. Cytokines in nasal lavage fluid (NLF) and immunoglobulin in blood serum were measured. Nasal mucosa tissue were measured RT-PCR and histological analyses.
Th-associated cytokines, such as IL-4, IL-5 and MCT in NLF were significantly higher in the NP group than the control group, and the increased production of these cytokines in the NP group was significantly suppressed the NP+Ino group or the NP+Dexa group. mRNA expressions of Th-associated cytokines (IL-4, IL-5, IL-10, IL-13, IL-17A, IFN-r), pro-inflammatory cytokines (IL-6 and IL-1β), epithelium-derived innate cytokines (IL-25, and IL-33), and chemokines (CCL1, CCL2, and CXCL2) were significantly suppressed in the NP+Ino and the NP+Dexa groups compared to the NP group.
NP+Ino and NP+Dexa treatment groups decreased thickness and number of polyp like lesion and eosinophils than the NP group. The thickness of respiratory epithelium in the NP group was significantly thicker than that of the other group. The number of goblet cells were significantly decreased in the NP+Ino and NP+Dexa groups compared to the NP group. Mast cell degranulation found in the mucosal tissue tended to decrease in the nasal mucosa of Ino and Dexa-treated mice compared to CRS mice.
Our findings clearly suggest that inotodiol compromises the immunological function of eosinophils, mast cells, and inflammatory Th cytokines, consequently leading to improved mucosal inflammations of CRS.

P2-056 PD-L1-directed PlGF/VEGF blockade synergizes with chemotherapy by targeting CD141+ cancer-associated fibroblasts in pancreatic cancer

Jung K[1]
[1]Seoul National University

Pancreatic ductal adenocarcinoma (PDAC) has a poor 5-year overall survival rate. Patients with PDAC display limited benefits after undergoing chemotherapy or immunotherapy modalities. Herein, we reveal that chemotherapy upregulates placental growth factor (PlGF), which directly activates cancer-associated fibroblasts (CAFs) to induce fibrosis-associated collagen deposition in PDAC. Patients with poor prognosis have high PIGF/VEGF expression and an increased number of PIGF/VEGF receptor-expressing CAFs, associated with enhanced collagen deposition. We also develop a multi-paratopic VEGF decoy receptor (Ate-Grab) by fusing the single-chain Fv of atezolizumab (anti-PD-L1) to VEGF-Grab to target PD-L1-expressing CAFs. Ate-Grab exerts anti-tumor and anti-fibrotic effects in PDAC models via the PD-L1-directed PlGF/VEGF blockade. Furthermore, Ate-Grab synergizes with gemcitabine by relieving desmoplasia. Single-cell RNA sequencing identifies that a CD141+ CAF population is reduced upon Ate-Grab and gemcitabine combination treatment. Overall, our results elucidate the mechanism underlying chemotherapy-induced fibrosis in PDAC and highlight a combinatorial therapeutic strategy for desmoplastic cancers.

P2-057 Type I IFN receptor stabilization at hepatic metastatic site

Ferreira Espinoza L[1], Monestiroli A[1], Zannino L[1], Caruso F[1], Buttiglione V[1], Fagioli C[1], Clissi B[1], Ekalle-Soppo C[1], Zordan P[1], Pedica F[2], Grassi S[2], Doglioni C[2], Cipriani F[3], Aldrighetti L[3], Elmore U[4,5], Rosati R[4,5], Esposito A[5,6], Gnasso C[5,6], Lampasona V[7], Marzinotto I[7], Ferrara B[7], Piemonti L[5,7], Scotti G[8], Botrugno O[9], Tonon G[9], Tauriello D[10], Batlle E[11], Brendolan A[9], Guidotti L[1,5], Sitia G[1]
[1]Division of Immunology, Transplantation and Infectious Diseases. IRCSS San Raffaele Scientific Institute, [2]Pathology Unit, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, [3]Hepatobiliary Surgery Division, San Raffaele Hospital, [4]Department of Gastrointestinal Surgery, San Raffaele Hospital, [5]Vita-Salute San Raffaele University, [6]Experimental Imaging Center, IRCCS San Raffaele Scientific Institute, [7]Diabetes Research Institute, IRCCS San Raffaele Scientific Institute, [8]Center for Omics Sciences, IRCCS San Raffaele Hospital, [9]Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, [10]Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, [11]Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology

Background
Liver metastases from colorectal carcinoma (CRC) and pancreatic ductal adenocarcinoma (PDAC) are unfavorable prognostic factors and largely incurable diseases. The liver metastasis microenvironment (TME) is uniquely predisposed to induce a state of immune insensitivity that impedes the antitumor potential of the innate and adaptive immune response. In this context, the type I IFN system is evolving into a complex system that can lead to immune dysfunction through mechanisms based on the expression of immune checkpoint proteins and downregulation of IFNAR1 in the TME. Whether CRC and PDAC liver metastases trigger similar immune dysfunction is currently unknown.
Methods
To test whether hepatic metastatic TME deregulates IFNAR1 and promotes a state of immune dysfunction, we used cohorts of patients with synchronous primary and liver metastases CRC and mouse models of CRC and PDAC liver metastases.
Results
We found that several type I IFN subtypes are upregulated in CRC metastatic liver lesions, associated with increased IRGs, checkpoint inhibitors, genes associated with pathogenic inflammation, and degradation of IFNAR1. To clarify the cellular source of type I interferons, we examined mouse CRC cell lines and tumor organoids (MTO) and mouse PDAC cells and found that CRCs expressed the same type I IFN subtypes in vitro, whereas PDACs did not. We then treated mice with established intrahepatic CRC or PDAC tumors differentially expressing type I IFNs with IFNα and found that IFNα did not control CRCs but efficiently inhibited PDAC metastasis, consistent with differential expression of IFNAR1 by these tumors. Finally, we test whether stabilization of IFNAR1 by p38/PDK inhibitors in the hepatic microenvironment improves the therapeutic outcomes of IFNα therapy and immune checkpoint therapies.
Conclusions
Stabilization of IFNAR1 in CRC liver metastases is a promising new therapeutic approach to improve immunotherapies.

P2-058 Activation of futile thermogenic triacylglycerol cycling in adipocytes is controlled by IL-4

Michurina S[1], Stafeev I[1], Agareva M[1], Zubkova E[1], Menshikov M[1], Parfyonova Y[1]
[1]National medical research centre of cardiology named after academician E.I.Chazov

Background: Activation of thermogenesis in adipose tissue protects against excessive triacylglycerol (TAG) storage and development of obesity and cardiometabolic abnormalities. Anti-inflammatory cytokine IL-4 is known to activate thermogenesis in vivo by stimulation of beige adipocyte differentiation. Here we describe a novel mechanism of IL-4 action via stimulation of thermogenic TAG-cycling in mature adipocytes.
Methods: 3T3-L1 cells were differentiated into adipocytes under insulin, isobutylmethylxanthine, dexamethasone, and rosiglitazone treatment. Mature adipocytes were stimulated with recombinant IL-4 (50 ng/ml) for 24 h. Glucose oxidation in adipocytes was estimated on Seahorse XFe96. Evaluation of glucose uptake, lipogenesis and lipolysis was performed using [3H]-2-deoxyglucose and [14C]-glucose. Lipid droplets morphology and thermogenesis were assessed using confocal microscopy of cells stained with BODIPY493/503 and ERthermAC fluorescent probes. To assess the mechanism of IL-4 action we used inhibitors of lipases (orlistat, atglistatin).
Results: We demonstrated that IL-4 activated the uptake of [3H]-2-deoxyglucose in adipocytes. IL-4 did not affect lipogenesis, but increased lipid droplets fragmentation and lipolysis. In addition, IL-4 activates glucose oxidation (glycolysis and oxidative phosphorylation) and thermogenesis in adipocytes. Stimulation of oxidative metabolism and heat production depends on the activity of adipocyte triglyceride lipase (ATGL).
Conclusion: IL-4 simultaneously activates glucose uptake, lipolysis, and thermogenesis, which indicates metabolic reprogramming of mature adipocytes. Reciprocal regulation of lipolysis, thermogenesis and glucose catabolism indicates stimulation of thermogenic futile TAG cycling, leading to the dissipation of excessive energy of oxidative processes in the form of heat. This work was supported by RSF grant #22-75-10085.

P2-059 The Circadian gene Per2 regulates inflammation through targeting BMAL1 & GATA3 expression in vitro and in vivo experimental model

Kang T[1], Annamneedi V[1], Park J[1], Bae J[1]
[1]Sahmyook University

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disorder associated with defective skin barrier. Circadian rhythms (CR) regulates immune system by regulating immune cell number, cytokine concentration, surface marker abundance and immunological effector functions in rhythmically. The Clock gene Per2, one of circadian genes, has vital role in inflammation and it is up regulates inflammatory cytokines.
Methods: In this study we studied the Per2 role in RAW 264.7 by transfecting with Per2 siRNA for knockdown specific Per2 gene and atopic dermatitis animal model using establishing DNCB induced Per2-/- mice. RAW 264.7 cells were simulated with LPS for 12 hours, then measured circadian and cytokine gene expressions.
Results: We found that PER2 induce the inflammation and enhance the expression of pro-inflammatory cytokines such as TNF-□ and IL-6 by suppressing Bmal-1, which has anti-inflammatory activity, and PER2 is inducing nitric oxide (NO) level too. RAW 264.7 cells transfected with Per2 siRNA was not stimulated by LPS treatment and produced lower levels of inflammatory cytokines and NO by Bmal-1 up-regulation. We also examined activity of Per2 in DNCB induced atopic dermatitis animal model by using Per2+/+ and Per2-/- mice. We found Per2+/+ mice has higher number of Th2 cells, up-regulated Th2 cytokines and GATA3 expression, suppressed BMAL1 expression. Per2 -/- mice showed low levels of Th2 cytokines and IgE levels, less Th2 cells.
Conclusion: We hypothesized PER2 has prominent role to induce inflammation and atopic dermatitis

P2-060 Identifying microbial gene responsible for eye colonization and effects on in vivo IL-17 production

Rigas Y[1], Shane J[2], Treat B[6], Shanks R[2,4,5], St. Leger A[2,3]
[1]University Of Pittsburgh School of Medicine, [2]University of Pittsburgh Department of Ophthalmology , [3]University of Pittsburgh Department of Immunology, [4]University of Pittsburgh Department of Microbiology and Molecular Genetics, [5]The Campbell Laboratory for Infectious Eye Diseases, [6]St. Jude Children's Research Hospital

Previously, Corynebacterium mastitidis has been shown to stably colonize the ocular surface and induce a protective IL-17 response from γδ T cells. This protective immune response includes the recruitment of neutrophils, and secretion of anti-microbial factors that prevent infection (1). Despite this, the microbial factors governing C. mast colonization and immunogenicity are still unknown.
To investigate this, we generated a transposon mutant library to identify bacterial genes necessary for colonization and immune induction. Through screening, we identified a mutant that lacked the ability to colonize the ocular surface due to a mutation in a sortase gene, which we show controls how the bacterial surface is decorated with adhesins that are critical for efficient colonization of the ocular surface. Application of this mutant to the ocular surface did not result in the generation of in vivo γδ T cell immunity. To identify whether this mutant lacked immune stimulating factors or if colonization was a requisite to the generation of in vivo immunity, we investigated whether the mutant stimulated in vitro immune responses. This was done by exposing bone marrow derived dendritic cells with C. mast and assessing IL-1β release. Additionally, we monitored IL-17 production from γδ T cells after co-culture with C. mast exposed BMDCs. Results show that this mutant can sufficiently stimulate in vitro immunity.
In sum, we can conclude that the C. mast sortase gene is critical for ocular colonization but does not necessarily play a role in the recognition of C. mast by immune cells. Moreover, we can conclude that colonization is a requirement for the induction of in vivo ocular mucosal immunity, and multiple exposures to bacteria that immediately get washed away is not sufficient for in vivo immunity.

P2-061 Cytokine-based immunotherapy with long-acting human recombinant IL-7 in HPV-related diseases associated with idiopathic CD4 lymphopenia

Lisco A[1], Ye P[1], Anderson M[1], Brownell I[2], Pérez-Díez A[1], Liu X[1], Cho S[3], Ferrando-Martinez S[3], Lee B[3], Stermer K[3], Sereti I[1]
[1]National Institute of Allergy and Infectious Diseases, [2]National Institute of Arthritis and Musculoskeletal and Skin Diseases, [3]NeoImmuneTech, Inc.

Background: Skin and mucosal Human Papillomavirus (HPV)-related diseases represent the most common opportunistic infection in biallelic IL-7 genetic deficiency as well as in idiopathic CD4 lymphopenia (ICL), a clinical syndrome that is defined by CD4 counts <300 cells/□L in absence of any primary or acquired cause of immunodeficiency. To evaluate the safety and the immunological and clinical effects of IL-7 treatment in a patient with ICL and refractory skin and mucosal HPV disease, we designed a single patient dose-escalation protocol of NT-I7 (efineptakin alfa), a long-acting form of human recombinant IL-7.
Methods: The patient received 5 total doses of NT-I7 at an interval of at least 12 weeks from each prior dose. Clinical and radiological evaluations were employed to evaluate adverse events, changes in HPV-related lesions and the size of secondary lymphoid organs. The distribution of CD4 and CD8 T-cells in peripheral blood, skin and lymphoid tissue was evaluated by immunohistochemistry and flow-cytometry.
Results: Dose escalation to 720 μg/Kg was well-tolerated. The most common adverse events were low-grade fever, lymphadenopathy and decreased lymphocyte counts in the 2 weeks post-injection. NT-I7 treatment resulted in a consistent and durable increase of CD4 and CD8 counts over the course of the 3 years of study (Figure 1). Proliferation of all CD4 and CD8 T cell subsets was observed in peripheral blood with preferential expansion of CD8 T-cells expressing the skin homing cutaneous leukocyte antigen (CLA). HPV-related skin verrucous lesions regressed after the first 2 injections but subsequently recurred. Ablative surgical management resulted in stable regression of skin lesions and high-grade anal dysplasia.
Conclusion: Long-acting human recombinant IL-7 treatment resulted in consistent and prolonged T-cell reconstitution. IL-7 as neoadjuvant immunological therapy for skin and mucosal HPV-related diseases in immunocompromised hosts deserves further clinical evaluation.

P2-062 Playing with Fire: Exploring the Impact of Temperature on Human Immune Responses

Maloney E[1], Sugrue J[1], O'Farrelly C[3], Ni Cheallaigh C[3], Bourke N[3], Milieu Interieur Consortium[1], Duffy D[1]
[1]Translational Immunology Unit, Institut Pasteur, Université Paris Cité, [2]Frontiers of Innovation in Research and Education PhD Program, LPI Doctoral School, [3]Trinity College Dublin

Fever is a hallmark symptom of disease across the animal kingdom, and increased temperature is a cardinal sign of inflammation. Yet despite the evidence linking temperature fluctuation and immune responses, this relationship remains an understudied contributor to immune variation. We are combining large-scale human studies and precise ex vivo testing to understand how temperature-immune interactions vary within populations, and impact both disease manifestation and severity.

To address variation on a population scale, we are utilizing the Milieu Interieur project, a cohort of 1,000 healthy donors with extensive immunological datasets. Our preliminary analysis identified associations between sex, age, and body temperature in a non-febrile healthy range. We are now defining transcriptomic signatures of these associations' impact on immune pathways using standardized whole blood stimulations. Linear regression analysis further identified temperature as predictive of type II interferon responses in females and bacterial proinflammatory pathways in males. These findings potentially indicate new hypotheses for sex disparity in disease prevalence.

To interrogate temperature in a disease context, we examined how fever affects immune responses in COVID-19 patients (n = 49). Expanding on previous transcriptomic analysis showing perturbed type I interferon responses in hospitalized patients, we have incorporated RNA sequencing and discovered additional immunometabolic perturbations. These signatures persisted when comparing patients with fever versus those without, suggesting underlying immune modulation mechanisms. To further explore the impact of this hallmark symptom, we compared induced immune responses at healthy (36.8°C) and febrile (39°C) temperatures ex vivo (n = 18). Transcriptomic and proteomic analyses indicate interactions between temperature and LPS immune responses, with febrile temperature correlating with a decrease in both type I and type II interferon signalling.
With our experimental findings, we hope to provide new understanding of fundamental biology and apply these discoveries to relevant clinical questions in infection and autoimmunity.

P2-063 Il9r floxed mice: a novel tool to study IL-9 signaling in vivo

Baral I[1], Falduto G[1], Schwartz D[1]
[1]University of Pittsburgh

Background: Interleukin 9 (IL-9) is a major mediator of allergic disease that has also been tied to autoimmunity, antitumor immunity, and antihelminth responses. Yet, the cellular and molecular targets of IL-9 are poorly characterized in vivo due to an inability to specifically delete IL-9 receptor in different cell types.

Methods: The Il9r gene was analyzed and sgRNAs were generated around exons 1-8. sgRNA efficiency was tested in murine blastocysts. sgRNAs were introduced into expression vectors (Addgene plasmid 51132) and injected into murine embryos. Founders were tested using PCR and Sanger sequencing and crossed x 5 onto C57BL/6 to eliminate off-target mutations. IL-9 signaling was in bone marrow derived mast cells (BMMC) from Il9r flox mice, stimulated with various cytokines.

Results: Il9r-targeting sgRNAs displayed good efficiency around exons 2, 3, and 5. Based on these results, sgRNAs targeting exons 2-5 were injected to generate a premature termination at exon 2. PCR screening and Sanger sequencing confirmed insertion of sgRNAs at target sites. Four founder strains were generated, of which one was chosen for backcrossing and further experimentation with other strains cryopreserved. BMMC from Il9rflox/flox mice expressed IL-9 receptor and responded to IL-9 by phosphorylating STAT5, showing that IL-9 receptor signaling was not disrupted by loxP site insertion.

Conclusions and Future Directions: Insertion of LoxP sites does not disrupt IL-9 receptor signaling. Upcoming experiments using Tat-Cre and crossing to various Cre strains will be used to (1) confirm deletion of Il9r gene (exons 2-5) (2) confirm disruption of IL-9R signaling in Cre+ cells (3) Determine cellular targets of IL-9 in vivo.

P2-064 Biometabolites of Citrus unshiu Peel Enhance Intestinal Permeability and Alter Gut Commensal Bacteria

Lee S[1], Seo D[1], Park S[1], Kim H[1], Kim T[1], Joo I[2], Park J[2], Kang Y[3], Lim G[1], Kim D[2], Yang J[1]
[1]Pusan National University, [2]Daejeon University, [3]National Institute for Korean Medicine Development

Flavanones in Citrus unshiu peel (CUP) have been used as therapeutic agents to reduce intestinal inflammation; however, the anti-inflammatory effects of their biometabolites remain ambiguous. Here, we identified aglycone-type flavanones, such as hesperetin and naringenin, which were more abundant in the bioconversion of the CUP than in the ethanol extracts of the CUP. We found that the bioconversion of the CUP induced the canonical nuclear factor-κB pathway via degradation of IκB in Caco-2 cells. To check the immune suppressive capacity of the aglycones of the CUP in vivo, we orally administered the bioconversion of the CUP (500 mg/kg) to mice for two weeks prior to the 3% dextran sulfate sodium treatment. The CUP-pretreated group showed improved body weight loss, colon length shortage, and intestinal inflammation than the control mice. We also found a significant decrease in the population of lamina propria Th17 cells in the CUP-pretreated group following dextran sodium sulfate (DSS) treatment and an increase in mRNA levels of occludin in CUP-treated Caco-2 cells. Pyrosequencing analysis revealed a decreased abundance of Alistipes putredinis and an increased abundance of Muribaculum intestinale in the feces of the CUP-pretreated mice compared to those of the control mice. Overall, these findings suggest that the pre-administration of CUP biometabolites may inhibit the development of murine colitis by modulating intestinal permeability and the gut microbiome.

P2-065 Induction of Anisakis-specific IgE dependent on commensal bacteria in stomach during gastric Anisakis infection

Shimokawa C[1], Hisaeda H[1]
[1]National Institute of Infectious Diseases

There are four clinical manifestations of anisakiasis caused by Anisakidae: gastric anisakiasis, intestinal anisakiasis, extra-gastric anisakiasis, and anisakis allergy. Of these, anisakis allergy is most dangerous, because not only live anisakis but also dead anisakis can cause life-threatening anaphylactic shock including dyspnea within minutes to hours. Therefore, identification of allergens and elucidation of immune responses in anisakis allergy are urgent.

In this study, to evaluate IgE responses specific for anisakis, we established a mouse anisakis model by infecting mice with live anisakis using a gastric tube after intraperitoneal immunization with excretory and secretory (ES) or crude antigens (CA) prepared from anisakis collected from mackerels along the pacific coast. Unimmunized mice showed only a slight increase in specific IgE, whereas the immunized mice showed a marked increase in specific IgE after anisakis infection. This effect was stronger in mice immunized with ES than with CA. Furthermore, analysis of the gastric microbiota showed that more than 90% were Lactobacillus species in ES-immunized but not CA-immunized mice. We are currently conducting a comprehensive analysis of the detecting in the components of ES and CA using LC/MS, and are analyzing which components in ES increase Lactobacillus.

P2-066 Modeling of the human interleukin 12:receptor complex allows to engineer attenuated cytokine variants

Aschenbrenner I[1], Liebl K[1], Schiller L[2], Kerle A[1], Protzer U[2], Feige M[1]
[1]Center for Functional Protein Assemblies, Department of Bioscience, TUM School of Natural Sciences, Technical University of Munich, [2]Institute of Virology, TUM School of Medicine, Technical University of Munich/Helmholtz Centre Munich

Interleukin 12 (IL-12) as pro-inflammatory cytokine plays major roles in immune defense against intracellular pathogens. By activating T cells and increasing antigen presentation, it is also a very potent anti-tumor molecule. Strong immune activation and systemic toxicity, however, limit its potential therapeutic use so far.
Building on recent experimental structures of IL-12 related cytokine:receptor complexes, we here provide a high-resolution computational model of the human IL-12:receptor complex. We design attenuated IL-12 variants with lower receptor binding affinities based on molecular dynamics simulations, and subsequently validate their structural and functional characteristics experimentally. These variants show reduced activation of natural killer cells while maintaining T cell activation. This immunological signature is important to develop IL-12 for cancer treatment, where natural killer cells contribute to severe side effects.

Taken together, our study provides detailed insights into structure and dynamics of the human IL-12:receptor complex and leverages them for engineering attenuated variants to elicit fewer side-effects while maintaining relevant biological activity.

P2-067 Effects of Gamma-aminobutyric Acid on Cytokines Secretions of 3T3-L1 adipocytes

Chiu T[1], Lin B[1]
[1]Department of Biochemical Science and Technology, College of Life Science, National Taiwan University

Background: Obesity, a notorious issue in the realm of public health, has drawn worldwide attention for decades. Obesity stems from the hypertrophy of adipose tissue in individuals, which leads to chronic, low-grade inflammation both locally and systemically. Gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter in brain, has been shown to have anti-inflammatory potential in vivo and in vitro. Besides, studies have demonstrated that GABA alleviates insulin resistance in diabetic mice and attenuates leptin secretion in 3T3-L1 adipocytes, indicating a susceptible role of GABA in adipocyte metabolism and inflammation. The aim of current study is to investigate the effects of GABA on adipocyte inflammation and lipid accumulation.
Methods: For adipocyte inflammation, 3T3-L1 preadipocytes or mature adipocytes were treated with GABA for 24 hr, followed by LPS induction or not. For lipid accumulation, GABA was treated during different time period of adipocyte differentiation.
Results: The results showed that both MCP-1 and IL-6 secretion in preadipocytes and mature adipocytes were downregulated by 2-10 mM GABA, with an exception of 2 mM GABA treatment, which actually increased IL-6 production in mature adipocytes. On the other hand, 2 mM GABA treatment during differentiation decreased lipid accumulation in mature adipocytes; 10 mM GABA treatment showed the opposite effect.
Conclusion: These results suggest that GABA might attenuate inflammation and metabolism of adipocytes, and the effect depends on the dosage used.

P2-068 Tissue Resident IFNg-producing CD8 T Cells limits tissue damage to intestinal helminth infection by remodeling the stromal network

Westfall S[1], Gentile M[1], Atar-Karo D[1], Peng G[1], Gregorieff A[1], King I[1]
[1]Mcgill University

Combatting infection requires a balance between mounting an effective anti-pathogen immune response and limiting collateral tissue damage. During infection with large, multicellular intestinal helminths this balance is tipped such that tissue damage control is prioritized over parasite killing. Our previous studies demonstrated that infection of mice with the roundworm, Heligmosomoides polygyrus bakeri (Hpb), induces an early IFNg-dominant type 1 immune signature during the tissue-invasive stage of infection that limits tissue damage without reducing parasite burden: a defense strategy referred to as disease tolerance. However, the cellular source of IFNg and its downstream targets that mediate host tolerance remained unclear. Using a combination of cell transfer, bone marrow chimeric and gnotobiotic approaches, we demonstrate that tissue-resident CD8 T (Trm) cells are the critical source of IFNg during early Hpb infection. Importantly, CD8+ Trms are activated in an antigen-independent yet microbiota-dependent manner. Eliminating IFNg receptor signaling during Hpb infection resulted in enhanced myofibroblast remodeling of the submucosa and compromised recruitment of neutrophils to the site of infection. These structural and immune cell alterations were associated with accelerated helminth growth, enhanced intestinal damage and chronic defects in tissue repair. Overall, our studies describe an unexpected role for CD8+ Trm cells in mediating disease tolerance during intestinal helminth infection and reveal an immune-stromal cell network that could be targeted for protection against tissue injury.

P2-069 Anti-inflammatory effects of P. acidilactici isolated from canine feces against DSS-induced colitis

Lee T[1], Kim D[1], Jung D[1], Kim Y[1], Lee Y[1], Seo I[1], Kim W[1], Cho Y[1], Park J[1,2]
[1]Chonnam National University, [2]NODCURE Inc

Background : Canine chronic enteropathy (CE) is the representative type of inflammatory gastrointestinal disease, especially occurred on dog. CE is characterized by vomiting, abdominal pain, weight loss, and bloody stools like Inflammatory bowel disease. CE is generally treated with anti-inflammatory medications such as steroids, however, these medications have limitation. Therefore, we evaluated the protective effects of Pediococcus acidilactici NC-025 which is Lactic acid bacteria of canine with a positive role in the gut against DSS-induced colitis.
Methods : We isolated NC-025 from healthy canine feces. NC-025 was administered orally daily at 107, 109 CFU/mouse for the duration of the experiment. DSS was used for establishing IBD mouse model. After sacrificing the mice, the colon was used for histological evaluation and mRNA expression level evaluation. Mouse bone marrow derived macrophage and canine peripheral blood mononuclear cells were used for in vitro analysis.
Results : Administration of 107 CFU of NC-025 exhibited a protective effect against DSS-induced colitis, but not in 109 CFU of NC-025. Histological analysis also showed that 107 CFU of NC-025 administration contributed to maintaining epithelial integrity and intestinal mucosal protection against colonic lesions. In mRNA analysis, TNF-α gene expression was significantly decreased in 107 CFU group compared with PBS-treated group. MUC2 gene expression was significantly increased in those group. In vitro analysis, LPS-induced inflammatory cytokines such as IL-6 and TNF-α were significantly decreased by NC-025 treatment on mouse BMDMs. Similarly, LPS-induced inflammatory cytokine such as IL-6 and TNF-α were also significantly reduced by NC-025 treatment on canine PBMCs.
Conclusion : NC-025 administration alleviated mouse DSS induced colitis through reducing pro-inflammatory cytokine. These results suggest that NC-025 may have potential therapeutic agent for CE in dogs.

P2-070 Visceral mesenchymal stem cells from type 2 diabetes donorsactivate triglycerides synthesis in healthy adipocytes viametabolites exchange and cytokines secretion

Stafeev I[1], Agareva M[1,2], Michurina S[1,3], Shestakova E[4], Gavrilova A[4], Parfyonova Y[1,3], Shestakova M[4]
[1]National Medical Research Centre For Cardiology, [2]Institute of Fine Chemical Technologies named after M.V. Lomonosov, [3]Lomonosov Moscow State University, [4]Endocrinology Research Centre

BACKGROUND: In recent years, there has been an increase in the prevalence of obesity and type 2 diabetes mellitus (T2DM). Development of visceral instead of subcutaneous adipose tissue is pathogenic and increases the risk of metabolic abnormalities. We hypothesize that visceral adipocytes and stromal cells are able to deteriorate other fat depots metabolism via secretory mechanisms.
METHODS: We study the regulatory role of visceral adipose-derived stem cells (vADSC) from donors with obesity and T2DM or normal glucose tolerance (NGT) on healthy subcutaneous ADSC (sADSC) in the Transwell system. Lipid droplets formation during adipogenesis was assessed by confocal microscopy. Cell metabolism was evaluated by 14C-glucose incorporation analysis and western blotting. vADSC secretome was assessed by Milliplex assay.
RESULTS: We showed that both NGT and T2DM vADSC had mesenchymal phenotype, but expression of CD29 was enhanced, whereas expressions of CD90, CD140b and IGF1R were suppressed in both NGT and T2DM vADSC. Co-differentiation with T2DM vADSC increased lipid droplet size and stimulated accumulation of fatty acids in adipocytes from healthy sADSC. In mature adipocytes T2DM vADSC stimulated triglyceride formation, whereas NGT vADSC activated oxidative metabolism. Secretome of NGT vADSC was pro-inflammatory and pro-angiogenic in comparison with T2DM vADSC.
CONCLUSIONS: The present study has demonstrated the critical role of secretory interactions between visceral and subcutaneous fat depots both in the level of progenitor and mature cells. Mechanisms of these interactions are related to direct exchange of metabolites and cytokines secretion. This work was supported by Russian Science Foundation grant #22-15-00365.

P2-071 ONCOSTATIN M EXPRESSION by PERIPHERAL BLOOD MONOCYTES is ELEVATED IN INFLUENZA H1N1 INFECTION

Dvorkin-Gheva A[1,2], Botelho F[1,2], Richards C[1,2]
[1]McMaster Immunology Research Centre, [2]McMaster University

The gp130 cytokine Oncostatin M (OSM) functions broadly on cells of lung mucosa (epithelial, smooth muscle and fibroblasts) due to its receptor complex expression (OSMR/gp130) on such cells. OSM actions are limited on immune cells due to low expression of OSMR, however OSM ligand expression by monocyte/macrophages is induced by various agents including TLR-ligands. Influenza A (IFA) is a single-stranded (ss) RNA virus that causes a transient respiratory infection in healthy individuals, however some patients are affected severely. The role of OSM in severe lung inflammation due to virus infection is not clear. We queried the expression of OSM mRNA in peripheral blood monocytes of human patients infected with H1N1 influenza, by obtaining publically available dataset (GEO:GSE111368) published by Dunning et al (2018, Nature Immunol, doi:10.1038/s41590-018-0111-5), and used it to compare H1N1 Influenza patients to matching healthy controls (HC) using limma package. OSM mRNA expression in IFA infected patient PBM (severity level 1 of disease, n=39) was increased statistically significantly by 1.67 fold change [FC]) over HC (n=130) and further increased in correlation with severity by 2.47 FC in level 2 severity (n=28) and 2.56 FC in level 3 severity (n=27). Interestingly, these were higher fold changes than either IL-1 or TNF mRNA (which were not significant). We also examined the expression of OSM mRNA in human peripheral blood monocytes due the TLR-agonist that mimics single stranded RNA. Stimulation of PBMs by TLR-4 ligand LPS, TLR-3 ligand Poly I:C, and particularly TLR-7/8 ligand CL075 (ss RNA mimic) elevated OSM mRNA (5 Fold, 10 fold and 25 fold respectively (Mean of 3 different PBM donors). Since marked immunopathology due to IFA is generated in large part by overzealous inflammatory mechanisms, we propose that OSM is a marker of severity of H1N1 IFA infection and can drive severe immunopathology. (Supported by CIHR)

P2-072 THE CYTOSOLIC DNA SENSOR AIM2 PROMOTES HELICOBACTER-INDUCED GASTRIC PATHOLOGY VIA THE INFLAMMASOME

Dawson R[1,2], Deswaerte V[1,2], West A[1,2], Sun E[1,2], Wray-McCann G[1,2], Livis T[1,2], Kumar B[3], Ferrero R[1,2], Jenkins B[1,2]
[1]Hudson Institute Of Medical Research, [2]Monash University , [3]Monash Health

Helicobacter pylori (H. pylori) infection can trigger chronic gastric inflammation perpetuated by overactivation of the innate immune system, leading to a cascade of precancerous lesions culminating in gastric cancer (GC). However, key regulators of innate immunity that promote H. pylori-induced gastric pathology remain ill-defined. We recently showed that the innate immune cytosolic DNA sensor Absent In Melanoma 2 (AIM2) contributes to the pathogenesis of late-stage GC independent of inflammasomes and via cell migration. We therefore investigated whether AIM2 also contributed to earlier Helicobacter-induced gastric disease. We revealed that AIM2 mRNA and protein expression are elevated in H. pylori-positive versus H. pylori-negative human gastric biopsies. Similarly, chronic H. felis infection in wild-type mice augmented Aim2 gene expression levels compared to uninfected controls. Notably, gastric inflammation and hyperplasia were less severe in H. felis-infected Aim2-/- versus wild-type mice, evidenced by reductions in gastric immune cell infiltrates, mucosal thickness and pro-inflammatory cytokine and chemokine release. Additionally, H. felis-driven proliferation and apoptosis in both gastric epithelial and immune cells were largely attenuated in Aim2-/- stomachs. These observations correlated with decreased levels of the inflammasome effector protein, cleaved Caspase-1. Taken together, this work uncovers a pathogenic role for the AIM2 inflammasome in Helicobacter-induced gastritis, which contrasts an inflammasome-independent role for AIM2 in advanced gastric tumourigenesis.
We are now extending our investigation to other DNA sensors in gastric disease, with the goal of identifying other potential immune-based therapeutic targets. Expression of DNA sensors was screened in gastritis patients and a panel of GC cell lines, and subsequently tested functionally via CRISPR knockdown for their involvement in inflammatory processes with or without Helicobacter infection. Overall, this work furthers our understanding of the host immune response to a common pathogen and the complex and varying roles of DNA sensors at different stages of cancerous and pre-cancerous gastric disease.

P2-073 The role of factor X in cancer pyroptosis

Woo W[1], Cho M[1], Dho S[2], Kim L[2]
[1]BK21 PLUS Project for Medical Science, Yonsei University, [2]Yonsei University

Background: Liver cancer is the leading cause of cancer mortality worldwide. Despite the improved treatment of HCC, the 5-year survival rate is relatively lower than other cancers, therefore further research to identify a new target is needed.
Factor X is a human-specific protein with high homology to factor Y, both of which are known to activate apoptosis. Although factor X has been reported to share substrates with factor Y, recent studies have suggested that factorX may have a distinct role in cancer cell death.
Methods: To elucidate the mechanism of factor X in cancer, we generated a factor X knock-out cell line and performed RNA-seq analysis.
Results: Our study revealed that factor X regulates inflammatory cytokines, which are associated with pyroptosis. We demonstrated that factor X mediates pyroptosis by cleaving GSDME in response to sorafenib treatment.
Conclusion: Recently, switching from apoptosis to other types of programmed cell death such as pyroptosis has emerged as a new strategy for cancer treatment.
Our findings suggest that targeting factor X may represent a potential therapeutic approach for patients with liver cancer.

P2-074 Role of IL22Ra and its C-terminal region in cancer and inflammatory diseases

Puigdevall L[1], Stewardson C[1], Dhulst L[1], Dumoutier L[1]
[1]Uclouvain

IL-22 is a cytokine that acts mainly on epithelial cells such as keratinocytes, intestinal epithelial cells and pancreatic acinar cells. Depending on the context IL-22 can have beneficial or harmful effects. Indeed, when IL-22 production is controlled, it has beneficial effects such as in wound healing and defense against pathogens. Conversely, when IL-22 production is excessive, it can induce pathologies such as psoriasis and cancer. These dual effects suggest that partial targeting of IL-22 signaling could be beneficial.

The binding of IL-22 to its receptor induces the JAK-STAT pathway with a massive activation of STAT3. In addition to the canonical activation of STAT3 that is tyrosine-dependent, an “alternative” tyrosine-independent activation of STAT3 has been discovered. This mechanism relies on the coiled-coil domain of STAT3 and the C-terminal (C-ter) part of IL22Ra, which lacks tyrosines. Targeting this alternative mechanism could therefore help to partially reduce IL-22 deleterious effects.

In vivo experiments in mice with a deleted C-ter have shown that this alternative path is deleterious in psoriasis, mirroring what happens with Il22r-/- mice. However, in colitis and pancreatitis where IL-22 is beneficial, we saw no effect of the C-ter. This suggests that the role of the alternative mechanism is context dependent and may play an important role in pathologies associated with massive STAT3 activation. To test this hypothesis, we will investigate the C-ter's role in pancreatic cancer, another STAT3-associated disease. At the same time, we will map the interaction between the C-ter and STAT3 by co-crystallisation. This will allow us to develop a strategy to target this interaction using two complementary approaches: identification of cyclic peptides, and screening of a chemical library.

P2-075 Involvement of IL-24 in skin carcinogenesis

Choteau M[1], Puigdevall L[1], Stewardson C[1], Cheou P[1], Hendrickx E[1], Dumoutier L[1]
[1]de Duve institute

Interleukin-24 (IL-24) belongs to the IL-20 family, which acts on non-immune cells. Its two heterodimeric receptors are highly expressed on keratinocytes in which IL-24 promotes proliferation and induces the production of chemokines and antimicrobial peptides. Its role in the pathogenesis of several inflammatory skin diseases such as psoriasis or contact dermatitis has also been demonstrated. Beyond its role in the skin, IL-24 has been of particular interest in cancer research. Indeed, the intracellular overexpression of IL-24 in cancer cells, using adenovirus, inhibits their growth. This effect appears to be mediated by the induction of apoptosis in tumor cells but also by the inhibition of angiogenesis and the development of metastases. However, the tumor suppressor activity of IL-24 has never been demonstrated in the context of physiological expression.
In this study, we analyzed the development of skin papillomas in IL-24 deficient mice. We used the two-stage skin carcinogenesis model induced by dermal application of dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA). While DMBA induced mutations in epithelial cells, repeated application of TPA induced a chronic inflammatory response leading to cell proliferation and papillomas development. Contrary to what was expected, IL-24-deficient mice had fewer papillomas than WT mice and their tumors were also smaller. These results seem to be confirmed in mice deficient in IL-20Rβ, the common chain of the two IL-24 receptors, which are also protected against the development of papillomas. These results demonstrate that the role of IL-24 in tumor development remains unclear. The tumor context, the expression level of IL-24 and its mode of action (intracellular or via its extracellular receptor) may play a role in this apparent contradiction. We are currently investigating the mechanisms that lead IL-24 to have pro-tumor properties in the DMBA/TPA model.

P2-076 The expression map of the type III interferon receptor in the mouse

Piperakis A[1], GALANI I[1], Andreakos E[1]
[1]Laboratory of Immunobiology, Center for Clinical, Experimental Surgery and Translational Research, BRFAA, Athens, Greece

Organisms in order to defend themselves from invading pathogens, such as viruses, have developed mechanisms to eliminate them, such as the anti-viral cytokines interferons (IFNs). The latest addition to this family is type III IFNs or IFN-λ that are produced mainly by epithelial cells, as well as some immune cells, and, after binding to their receptor, they activate the expression of Interferon Stimulated Genes (ISGs), but not inflammatory factors, in opposition to type I IFNs. IFN-λ receptor, which is comprised of the unique IFNLR1, and the common IL-10R2 chain, is highly expressed on the surface of the respiratory, intestinal, and urogenital epithelium, but detailed information about its localization in specific cell types and other organs is lacking. This is largely due to the challenges posed for determining IFNLR1 presence. Here, we describe the development of a novel IFNLR1-td-Tomato reporter mouse, enabling the cell and tissue-specific monitoring of IFNLR1 expression in vivo. Using flow cytometry and immunohistochemistry, we describe IFNLR1 expression across cells and organs, including the respiratory and digestive tissues. Using adult mice and fetuses, we characterized the embryonic tissue localization of IFNLR1 and determined whether this is age-related. We further found that IFNLR1 presence in specific locations is related to the functional importance of the IFN-λ system. Altogether, our data provide a first map of the IFN-□ system with broader implications for the understanding of its role at barrier surfaces and beyond.

P2-077 Investigation of intestinal cell signaling pathways on local and systemic immunity to cancer

Yakou M[1,2], Ghilas S[1,2], Afshar-Sterle S[1,2], Huntington N[3], Ernst M[1,2], Mielke L[1,2]
[1]Olivia Newton-John Cancer Research Institute, [2]La Trobe University School of Cancer Medicine, [3]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University

Immunotherapy treatments targeting anti-PD1 and anti-CTLA-4 have revolutionized cancer treatment for some cancer types, however less than 10% of colorectal cancers (CRCs) respond to these immunotherapies. Investigating the molecular mechanisms underpinning the anti-tumour properties of immune cells, in particular intraepithelial lymphocytes (IELs) in the gut could uncover solutions to hurdles posed by colorectal cancer (CRC) and elucidate novel immunotherapy targets for improved treatment options.

Using scRNA sequencing, we have identified IELs with high expression of the cytokine receptor, IL-2Rα in the colon of naïve mice. While cytokines which signal through this receptor, such as IL-15 and IL-2, are well recognized as being critical for T-IEL development, their role in IEL function at steady state and in the context of CRC remains largely unexplored. Using pre-clinical mouse models where IL-15 and IL-2 signaling is altered, our preliminary data highlights a pro-tumorigenic role for these cytokines in MC-38 tumors located within the colon compared to subcutaneous tumors. We show that IELs have increased granzyme A and PD-1 expression in the colon tumors and yet exhibit greater tumor burden. Further investigation is ongoing to unravel the distinct mechanisms in colon vs subcutaneous tumors, by which IL-15 and IL-2 signaling controls anti-tumor immunity. This will allow us to better tailor CRC- specific immunotherapies that harness the full anti-tumor capabilities of IELs.

P2-078 Polyunsaturated lipid mediator networks determine COVID-19 severity and risk for critical illness

Papadaki M[1], Pavlos E[1], GALANI I[1], Triantafyllia V[1], Siouti E[1], Lambropoulou V[1], Salagianni M[1], Manioudaki M[1], PASXALIDIS N[1], Baillif V[2], Papelis D[3], Koukaki E[4], Rovina N[4], Koutsoukou A[4], Rapti V[5], Tamvakopoulos C[3], Dubourdeau M[2], Poulakou G[5], Andreakos E[1]
[1]Laboratory of Immunobiology, Centre for Clinical, Experimental Surgery and Translational Research, BRFAA, [2]Ambiotis SAS, [3]Division of Pharmacology-Pharmacotechnology, BRFAA, [4]ICU, 1st Department of Respiratory Medicine, National and Kapodistrian University of Athens, Medical School, 'Sotiria' General Hospital of Chest Diseases , [5]1st Department of Respiratory Medicine, National and Kapodistrian University of Athens, Medical School, 'Sotiria' General Hospital of Chest Diseases

Coronavirus disease-19 (COVID-19) caused by SARS-CoV-2 infection shows substantial phenotypic variability. At the root of COVID-19 lies the sudden development of hyper-inflammation attributed to dysregulated immunity and "cytokine storms" causing pneumonia and acute respiratory distress syndrome. However, bioactive lipid mediators (LM) derived from ω6 (AA) and ω3 (EPA and DHA) poly-unsaturated fatty acids via cyclooxygenase, lipoxygenase and cytochrome P450-dependent pathways are also critical regulators of inflammation. Still, their role in COVID-19 remains largely unknown.
Here, we employed a holistic approach involving the analysis of white blood cell transcriptomes, targeted lipidomics, cytokine and immune cell profiling in order to characterize bioactive LM networks during COVID-19, across the spectrum of disease severity, and identify molecules and pathways at hospital admission that are linked to disease pathophysiology and can also be used as biomarkers. First, we observed a major dysregulation of the lipoxygenase pathway in transcriptomics profiles of critically ill patients compared to less severe and milder forms of the disease. This was associated with dysregulated LM profiles in the blood of these patients and enhanced inflammation, and could be used to categorize patients according to their disease severity status. By applying feature selection techniques to characterize LMs that were most variable among patients, and Hazard-ratio models to identify LMs that can predict disease outcomes, we identified 20-HETE, a CYP450-dependent AA-first metabolite, as a novel biomarker for ICU admission. This was replicated in an additional group of patients and, importantly, in an additional vaccinated population.

In conclusion, our study characterized LM networks that are deregulated during COVID-19 development, and distinct LM profiles that are associated with disease severity, and uncovered 20-HETE as a novel highly promising biomarker with predictive ability for ICU admission. This sheds light into the pathophysiology of COVID-19 and helps improve diagnosis, prognosis and management of high-risk COVID-19 patients.

P2-079 SERTOLI CELLS MODULATE CYTOKINE ENVIRONMENT IN A MOUSE MODEL OF TESTICULAR INFLAMMATION

Porubska B[1], Hynkova M[1], Fikarová N[1], Somova V[1], Vasek D[1], Tlapakova T[1], Krulova M[1]
[1]Department of Cell Bilogy, Faculty of Science, Charles University

Cell therapies represent an important development in human medicine; stem cells are the prominent tool for such therapies. Sertoli cells (SCs) are the most important contributors of the spermatogonial stem cell niches, forming a blood-testis barrier, but also producing a variety of biomolecules to support germ cell development and creating an immunosuppressive environment necessary for germ cell survival. Additionally, SCs can be easily isolated from patient testicular biopsies performed routinely in fertility clinics. Currently, we have confirmed that SCs possess properties of mesenchymal stem cells, including migration of SCs into damaged testes, paracrine and contact-dependent action, and the donation of mitochondria to various recipient cells. Therefore, our objective was to introduce Sertoli cell (SC) therapy to protect testicular immune privilege, suppress inflammation, and support spermatogonial cells in males suffering from infections associated with the risk of impaired reproductive health. In an in vivo LPS-induced model of testicular inflammation, SC application suppressed inflammatory immune cell infiltration, supported the anti-inflammatory phenotype of resident immune cells, and protected developing germ cells. The presence of inflammatory and anti-inflammatory cytokines in serum and testicular homogenate was assessed by ELISA. The application of SC suppressed the production of inflammatory cytokines, while the levels of IL-10 and IL-4 were elevated. Therefore, the introduction of a new therapeutic approach using SC to address the cause of male infertility can help patients suffering from inflammatory testicular diseases.

P2-080 Mitochondria in intercellular communication between stem and immune cells

Somova V[1], Porubská B, Vašek D[1], Krulová M[1]
[1]Charles University, Faculty of Science, Department of Cell Biology

Introduction: Stem cells have the ability to modulate the immune response, including the phenotype of immune cells and the corresponding cytokine production. In addition to paracrine functions, mitochondrial transfer is an important mechanism through which stem cells reduce inflammation by balancing cellular stress. Furthermore, by supporting mitochondrial maintenance and biogenesis, stem cells could induce metabolic changes and regulate the phenotype of immune cells. However, the direct effect of mitochondrial transfer on cytokine production and the precise mechanism have not yet been discovered. By describing changes in the cytokine profile of immune cells after mitochondrial transfer, we aim to prove the regulatory mechanism behind the intercellular mitochondrial dynamics.
Methods: This study analyzed cytokine production and mitochondrial transfer between stem and spleen cells isolated from BALB/c mice in vitro. Samples from >3 independent experiments were measured using RT-qPCR, flow cytometry, and ELISA, and compared using suitable statistical analysis, e.g. One-way ANOVA. By performing bulk RNAseq, the differences in the transcriptional profile of acceptors and non-acceptors of mitochondria were described.
Results: Our study confirmed that immune cell activation induced intercellular mitochondrial dynamics and there was a direct effect of mitochondrial transfer on activated T and B cell cytokine production. We hypothesize that various mechanisms, including metabolic changes, mitophagy, and ROS production, play an essential role in these changes.
Conclusions: Stem cells transfer mitochondria to different populations of leukocytes, and the efficacy of transfer is enhanced by immune cell activation. Among other effects, mitochondrial transfer changes the expression and production of cytokines by activated immune cells. Therefore, we hypothesize that metabolic changes associated with mitochondrial acquisition are important mechanisms underlying the stem cell-induced phenotypic change of immune cells.

P2-081 Immune Cell Response during Cold Tolerance and Thermogenesis

Vašek D[1], Holíček P[2], Galatík F[3], Somova V[1], Fikarová N[1], Žurmanová J[3], Krulová M[1]
[1]Department of Cell Biology, Faculty of Science, Charles University, [2]SOTIO Biotech a.s., [3]Department of Physiology, Faculty of Science, Charles University

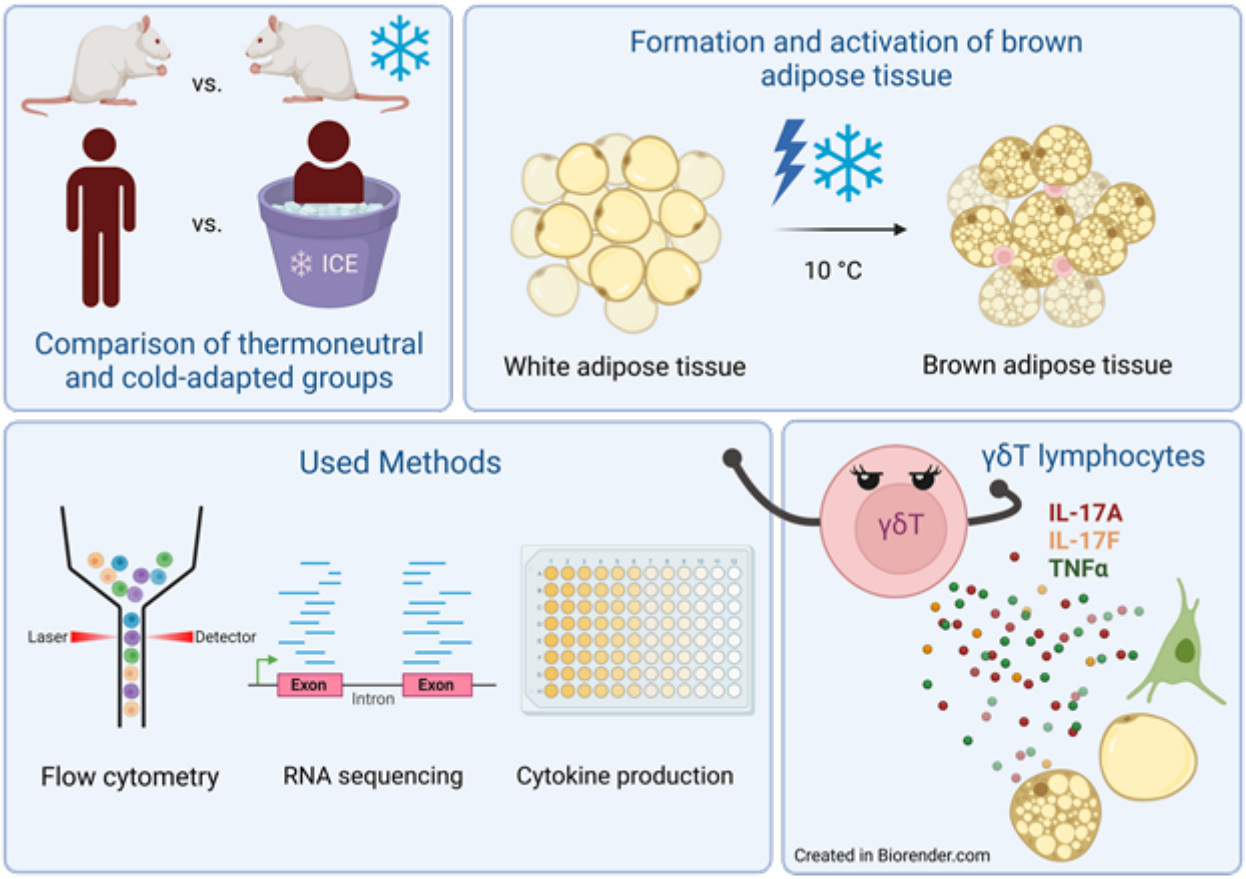
Background: Thermogenesis is essential for maintaining a constant temperature and for maintaining the physiological processes of organisms at a reduced temperature. The first phase of exposure to cold is associated with a stress reaction; subsequently, cold tolerance is induced. The acclimatization process is mainly related to the formation of brown adipose tissue and the white adipose tissue beiging, in which cytokines and immune cells play an essential role; however, immune cell dynamics at the systemic level have not yet been described. The study aims to describe the mechanisms of adaptation to cold associated with the immune system.

Methods: An animal rat model was used for the study of cold adaptation. The representation of immune populations was determined by flow cytometry. The presence and production of cytokines were determined by Luminex, ELISA, and cytometry. Fundamental findings were deepened by RNAseq and confirmed in human volunteers.

Results: Significant changes in the immune response to inflammatory stimuli indicated complex systemic modulation of immune reactions and were associated with altered metabolism of immune cells. We also described differences in cytokine production in various tissues and systemically in the serum of cold-acclimatized rats. Control of thermogenesis has been associated with a homeostatic population of γδT-cells in brown adipose tissue and the production of IL-17. Consequently, we observed an increase in the percentage of γδT-cells producing IL-17 in various tissues of cold-adapted rats. Changes in gene expression of γδT-cells were confirmed by RNAseq.

These results have also been confirmed in humans. Compared to controls, volunteers who regularly bathed in ice water showed an increased percentage of γδT-cells, including an altered phenotype of these cells and a difference in the levels of various cytokines.

Conclusion: Uncovering the immune-related processes associated with cold and subsequent metabolic changes can help develop therapeutic approaches to various diseases, such as obesity.


vs.
vs.
ICE
Comparison of thermoneutral and cold-adapted groups
Formation and activation of brown adipose tissue
10 °C
White adipose tissue
Brown adipose tissue
Used Methods
Laser
Detector
Exon
Intron
Exon
Flow cytometry
RNA sequencing
Cytokine production
γδT lymphocytes
γδT
IL-17A
IL-17F
TNFα
Created in Biorender.com

P2-082 IFN-α and Cytokine Production Tests: What Can They Tell Us?

Uno K[1], Kimura O[1,2], Fujita S[1], Yoshikawa T[1]
[1]Louis Pasteur Center For Medical Research, [2]Tokyo CA Clinic

Introduction and methods
The Louis Pasteur Center for Medical Research has been conducting HVJ-stimulated IFN production tests since the institute was founded in 1986. In these tests, IFN are collected after 24 hours and quantified using bioassay. Since 2011, we have been quantifying PHA stimulated cytokine production in serum collected after 48 hours (4-fold diluted heparinized whole blood with Eagle's MEM) using bio-plex panels (IL-2, IL-4, IL-5, IL-10, IL-12(p70), IL-13, GM-CSF, IFN-γ, and TNF-α).
Results and Discussion
Previous studies have shown that in diabetes, myelodysplastic syndrome, pulmonary tuberculosis, HCV hepatitis, HIV, MPO-ANCA nephritis, and various cancer patients, IFN-α production capacity is significantly lower than in healthy controls. This impairment leads to specific symptoms in each disease. For example, MPO-ANCA patients with impaired IFN production experience higher long-term susceptibility and vulnerability to infection. Furthermore, patients who developed hepatocellular carcinoma have lower mean IFN-α levels than cancer free HCV patients. In patients with lung, gastric/esophageal, colorectal, and bladder cancer, the mean IFN-α production was significantly higher in those who survived more than five years after their cancer diagnosis than in those who died within five years. Thus, measuring a patient's IFN-α production capacity may help predict infection resistance and prognosis in cancer patients.
In healthy subjects, IFN-α production showed a slight decrease with age. We also observed that marginal decline in IFN-α production can reverse even in individuals in their early sixties. Additionally, the ratio of IFN-γ production and IL-4(IL-5, IL-13) production allows us to create a profile of a patient's Th1 and Th2 balance. Cancer patients who became Th1-dominant after treatment experienced better prognoses. Thus, by combining IFN-α and cytokine production tests, a physician can better understand a patient's condition and adopt more effective treatment strategies, especially in patients with cancer.

P2-083 The role of Intracellular Interleukin-1α (IL-1α) in Breast Cancer Progression

Krishnamohan M[2], Schreiber G[1], Braiman A[2], Voronov E[2]
[1]Weizmann Institute Of Science, [2]Ben-Gurion University of Negev

BACKGROUND: Breast cancer is the most common malignancy in women and the second leading cause of female cancer-related death worldwide. IL-1α is a pleiotropic cytokine that affects inflammatory and immune responses and is a dual action 'alarm' pro-inflammatory cytokine, acting in both its intracellular and secreted forms.
The aim of this study was to assess the effects of intracellular IL-1α on the development of breast cancer in a mouse model of orthotopically injected triple negative 4T1 cells.
METHODS: We genetically manipulated 4T1 cells to suppress endogenous IL-1α production by using the CRISPR/Cas9 system. Endogenous IL-1α expression was assessed by FACS analysis. Three different IL-1α gene knock out (KO) clones were isolated and used in these experiments. We compared the effects of tumor-associated and microenvironment IL-1α on TNBC development and metastasis.
RESULTS: The injection of 4T1/IL-1α KO cells into BALB/c mice led to a significant decrease in local tumor development and lung metastasis, in comparison to control mice. The mechanisms involved in the effects of both tumor cell- and microenvironment IL-1α on the development of TNBC were studied. A decrease in the expression and secretion of most pro-inflammatory and pro- angiogenic molecules in local tumors was found in mice injected with 4T1/IL-1α KO clones. There was also a decrease in most chemokines that led to reduced recruitment of myeloid cells into tumors in these mice. We found fewer CD11b+ cells and MDSCs but an increase in antigen presenting MHCII+ cells in local tumors from mice injected with IL-1α KO cells. In tumors from mice injected with IL-1α KO cells, we found an increase in interferon- producing killer dendritic cells, which possess tumor killing properties.
CONCLUSION:
We found that tumor-associated IL-1α is essential for the development of local breast cancer and lung metastases in mice.

P2-084 Osteoclastogenic cytokines enriched in bone metastases induce the formation of osteoclast-breast tumor hybrids that can potentially facilitate bone metastatic disease progression

Lim K[1], CHOW K[1]
[1]City University Of Hong Kong

Tumor hybrid cells in cancer biology have garnered greater attention with the discovery of circulating hybrid cells (CHC). CHCs are identified as the predominant cell type in circulating tumor cells (CTC) and they co-express leukocyte and tumor markers. Current studies indicate that tumor hybrids play an important role in tumor progression as they acquire genetic and phenotypic characteristics from parental cells and reportedly have enhanced motility and invasiveness, increased stem cell characteristics, and elevated resistance to chemo- and radiotherapies. It has been proposed that cellular fusion leads to the formation of these hybrids. Amongst leukocyte fusion partners, macrophages (osteoclast precursors) are identified as a frequent fusion partner with tumors. However, mechanisms and pathways that lead to fusion between macrophages and tumors are highly underexplored. To address this, we investigated macrophage fusion pathways that could be involved in regulating macrophage fusion with breast tumor. Here we report the finding of a novel hybrid cell type that is formed by in-vitro cellular fusion between osteoclast precursors/mature osteoclasts and breast tumor (hereby known as osteoclast hybrid) in the presence of osteoclastogenic cytokines that are enriched in bone metastases. Fusion was observed at all stages of osteoclast differentiation with individual macrophages fusing with tumor cells, interfusion between osteoclast hybrids, and individual tumor cells fusing into mature osteoclast hybrids. Tumor nuclei in osteoclast hybrids were found to be transcriptionally active, which may infer that osteoclast hybrids are transcriptionally different compared to regular osteoclasts. Osteoclast-derived factors were revealed to upregulate putative cell fusion genes including Mmp9 and Syna in breast cancer. Single cell sequencing study to dissect the transcriptome of osteoclast hybrids is currently under way to elucidate the mechanisms that lead to fusion hybrid formation and their potential roles in bone metastatic disease progression. Future endeavours will focus on acquiring evidence of osteoclast hybrids in-vivo.

P2-085 Chemotherapy in combination with Toll-like receptor agonism promoted antitumor immune response in triple negative breast cancer

Dotse E[1], Lim K[1], WANG M[1], Cheuk Ying T[1], CHOW K[1]
[1]City University Of Hong Kong

Background
Immunotherapy represents a promising research area for triple negative breast cancers (TNBCs), often defined by lack of estrogen and progesterone receptors and HER2 overexpression. Current treatment is limited to chemotherapy which is toxic and short lived with imminent metastatic recurrence. TNBCs are more immunogenic characterized by high number of tumor infiltrating lymphocytes (TILs), a feature which can be harnessed to increase responsiveness to immunotherapy. These TILs express pathogen recognition receptors such as toll-like receptors (TLRs) and their engagement with their ligand, activate proinflammatory cytokines and type 1 interferon production to elicit specific T-cell antitumor immunity.

Methods
Here, we evaluated the combined efficacy of intratumoral administration of TLR7/8 (Resiquimod) or TLR9 (CPG-ODN-2395) agonists with Paclitaxel in syngeneic 4T1 TNBC mouse model. Mice were treated once weekly for a total of 3 cycles. Tumor width and height were measured every other day. At endpoint, excised tumors were weighed, and the total percentage of TILs were evaluated. Single cell RNA sequencing is currently being employed to further understand the mechanisms utilized by these combined therapies to elicit the observed antitumor immunity.

Results
Chemotherapy with Resiquimod or CPG-ODN 2395 had significant tumor regression compared to chemotherapy alone. Also, Paclitaxel/Resiquimod treatment promoted influx of B-cells, pDCs, helper and cytotoxic T cells. Regulatory T cells were significantly reduced in Paclitaxel/Resiquimod treated mice compared to Paclitaxel/CPG-ODN 2395 and chemotherapy alone.

Conclusion
Overall, our findings suggest that combination therapy of TLR7/8 agonist (Resiquimod) with Paclitaxel promotes antitumor immunity and may represent a new effective treatment approach for TNBC.

P2-086 IL-27 gene therapy inhibits lethal autoimmunity in Scurfy mice

Liu J[1], Jabbari A[2], Frankel W[1], Zheng P[3], Liu Y[3]
[1]Ohio State University, [2]University of Iowa, [3]OncoC4, Inc.

Regulatory T cells (Tregs) play an indispensable role in maintaining immunological tolerance to self-antigens. Mutation of Foxp3, the master regulator of Tregs development and function, was identified to be responsible for the development of autoimmune diseases in Scurfy mice and the human immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX). IPEX is among the most severe autoimmune diseases in human with stem cell transplantation as the only available therapy. Thus, developing therapeutics that can inhibit lethal autoimmune diseases caused by dysregulated Tregs is clearly in need. We have previously shown that IL-27 gene delivery using adeno-associated virus (AAV-IL-27) strongly inhibits the development of autoimmune diseases in a few mouse models. To test if AAV-IL-27 therapy can protect mice against Treg deficiency-caused autoimmunity, we used it to treat the Foxp3 mutated Scurfy mice. We found that a single dose of AAV-IL-27 treatment given on day 10 post birth, when signs of Scurfy phenotype appeared, dramatically extended the survival of the Scurfy mice, and ameliorated immune pathology in various tissues including skin, lung, kidney, liver, and pancreatic islets. In the spleen and lymph nodes of AAV-IL-27 treated mice, AAV-IL-27 gene therapy significantly prevented naïve T cell activation as reflected by downregulation of CD62L and upregulation of CD44. AAV-IL-27 therapy promoted both IFN-γ and IL-10 production in T cells, and genetic deletion of IL-10 in Scurfy mice resulted in mice less responsive to AAV-IL-27 therapy. AAV-IL-27 therapy also induced PD-L1 expression in T cells and blocking PD-1-PD-L1 interaction exacerbated disease. Our study suggests that IL-27 inhibits lethal autoimmunity in Scurfy mice and IL-27 gene therapy may be a potentially new treatment for IPEX.

P2-087 Celastrol exhibits antitumor activity in melanoma by inhibiting the binding of CD25 through targeting IL-2

Lee J[1], Cho O[1], Jeong Y[1], Heo T[1]
[1]Laboratory of Pharmacoimmunology, College of Pharmacy, The Catholic University of Korea

Background
Interleukin-2 (IL-2) exhibits contrasting immune responses depending on its receptor subunit complex. Therefore, selective receptor binding is considered a key challenge in IL-2 cancer immunotherapy strategies. In this study, we focused on the discovery of IL-2/CD25(IL-2Rα) binding inhibitors and the investigation of the anticancer activity of the screened small molecule compounds.
Methods
Competitive ELISA screening was performed to discover IL-2/CD25 binding inhibitors. After screening, the effect of CEL on IL-2 activity was evaluated in CTLL-2, an IL-2 dependent cell line, HEK-Blue IL-2 reporter cell, and immune cells. The antitumor activity of CEL was evaluated in B16F10 tumor-bearing C57BL/6 or BALB/c nude mice.
Results
CEL was discovered by competitive ELISA screening. CEL significantly inhibited the proliferation and signaling of IL-2 dependent murine T cell and HEK-Blue reporter cells. After confirming the impacts of CEL on IL-2, we evaluated the antitumor activity in B16F10 tumor-bearing mice and found CEL significantly reduced tumor volumes by enhancing CD8+ T cells in C57BL/6 mice. We also found that CEL did not inhibit tumor growth in the T cell deficient BALB/c nude mice, suggesting that antitumor activity of CEL was T cell mediated response. Moreover, the combination therapy with low-dose CEL and a TNFR2 antagonistic antibody significantly suppressed tumor growth more than individual monotherapies.
Conclusion
These findings suggest that CEL, which acts as an inhibitor of CD25 binding by targeting IL-2, exerts antitumor activity through mediating T cell response. Therefore, CEL could be a promising partner for combination therapy for cancer immunotherapy against melanoma.

P2-088 TNFα-induced PTX3 regulates actin cytoskeleton in glioma

Kirti M[1], Umdor S[1], Gowda P[1], Patrick S[1], Suri V[2], Sen E[1]
[1]National Brain Research Centre, [2]AII India Institute of Medical Sciences

A gain-of-function mutation in isocitrate dehydrogenase 1 (IDH1) affects inflammation and cytokine release and is associated with a better prognosis in glioma and other cancers. TNFα is a proinflammatory cytokine that regulates invasion and is being studied for its prognostic values and immune significance in various tumors. TNFα induces the expression and release of PTX3 (Pentraxin 3, an innate immune molecule involved in cancer-related inflammation and extracellular matrix remodeling) in the tumor microenvironment. We have previously reported diminished TNFα and PTX3 levels in IDH1-R132H gliomas. Recent reports suggest cytoskeletal proteins as potential biomarkers and therapeutic targets for glioma treatment. These cytoskeletal proteins are not only crucial for glioma pathogenesis, but are also being investigated in detail because of their ability to interfere with rapid glioma proliferation. Interestingly, TNFα regulates actin reorganization in the cells. Given the role of TNFα in regulating actin dynamics, the possible effect of TNFα-induced PTX3 in regulating actin cytoskeleton in IDH1-R132H gliomas was investigated in this study. Here we show that glioma cells stably harboring IDH1-R132H exhibit altered actin cytoskeleton as seen by diminished expression of actin-related genes compared to the wild-type counterpart. The Cancer Genome Atlas (TCGA) analysis shows similar patterns of actin-related genes in glioma patients harboring IDH1-R132H. Site-directed mutagenesis, pharmacological and genetic manipulation of PTX3 release in the tumor microenvironment suggests its possible role in regulating actin dynamics. Drugs targeting actin cytoskeleton and PTX3 are now being looked into this study for their combinatorial effect in regulating cell death in gliomas with wild-type IDH1. As metabolic enzymes serve as a fuel for the cytoskeleton, we are currently looking into the possible effect of this PTX3-cytoskeleton axis on metabolism and vice versa in IDH1-R132H gliomas. Of clinical importance, our study suggests that this PTX3-actin cytoskeleton-metabolism regulatory circuit could be exploited for therapeutic gains.

P2-089 Elucidating the role of metabolism in regulating cytokine signaling in IDH1 mutant gliomas

Umdor S[1], Kirti M[1], Suri V[2], Sen E[1]
[1]National Brain Research Centre, [2]All India Institute of Medical Sciences

Gliomas harboring mutation in IDH1 (Isocitrate Dehydrogenase 1) gene have better prognosis than the wild type counterpart. Deregulation of fatty acid biosynthesis is a known metabolic feature in cancers and the expression of Fatty acid synthase (FASN), a key metabolic enzyme in lipid biosynthesis was reported to be upregulated in gliomas. TNFα, a pro-inflammatory cytokine is known to induce lipolysis and inhibit lipid biosynthesis by downregulating key metabolic enzymes like FASN. Pentraxin 3 (PTX3- a TNFα induced gene), exhibits an inflammatory response in various cancers including glioma and is currently being investigated as a new prognostic biomarker. Our previous published reports showed a low expression of TNFα as well as PTX3 in IDH1 mutant (IDH1R132H) glioma patients as compared to the IDH1 wild-type counterparts. While studies have highlighted the dysregulation of lipid biosynthesis in IDH1 mutant gliomas, it would be interesting to investigate this phenomenon in the context of cytokine-mediated inflammatory changes. Here, we aimed to study the mechanisms by which the lipogenic enzyme FASN and TNFα-induced PTX3 affects cancer cell survival in gliomas. This study reveals an increase in the expression of FASN enzyme in IDH1R132H-overexpressing cells compared to wild-type IDH1. The Cancer Genome Atlas (TCGA) patient data set also indicates heightened FASN expression in glioma patients harboring IDH1R132H compared to the WT counterparts. This data set also reveals the levels of FASN to be negatively correlated with that of PTX3. Since PTX3 regulates TLR4 signaling, further investigation of FASN (metabolism)-PTX3 (inflammation) crosstalk and its downstream cytokine signaling is being studied. Our experiments uses both genetic and pharmacological inhibition of FASN and PTX3 to elucidate the mechanistic alterations in TLR4 signaling. Overall, this study highlights the potential combinatorial approach targeting a metabolic (FASN) and an immune molecule (PTX3) for its clinical relevance in gliomas.

P2-090 Air Pollutant PM2.5 affects the initiation of allergic inflammation and exerts different disease outcome in naïve and asthmatic mice.

Chen T[1], Chiang B[1]
[1]Graduate Institute of Clinical Medicine, National Taiwan University

Background
Recent studies suggested that particulate matters 2.5 (PM2.5) might contribute to the prevalence of asthmatic symptoms. However, our understanding of the underlying mechanisms related to immune cells in pulmonary inflammation are still not complete.
Methods
We investigated the effect through PM2.5 exposure from Kaohsiung, Taiwan on both naïve BALB/c mice and ovalbumin (OVA)-induced asthma model. The asthma model includes a sensitization phase, which OVA with emulsified alum was given through intraperitoneal injection on Day 0, 14, and 21, and an intranasal OVA challenge phase starting from Day 28 for 3 consecutive days. According to grouping, PM2.5 was given intranasally at the exact date of OVA sensitized.
Results
The serum level of OVA-specific immunoglobulin IgE, IgG1, and IgG2a were not affected by PM2.5 exposure in either naïve mice or OVA model. Although PM2.5 alone suppressed IFNγ production in the lungs, the alternation did not disturb airway function or address histological changes. On the other hand, PM2.5 exposure in OVA-induced asthma model exacerbated the pulmonary inflammation and disturbed airway function in a dose-dependent manner. Both type 2 cytokines and Th17 cytokine IL-17 significantly increased upon PM2.5 exposure in the OVA model. Further, PM2.5 enhanced innate cytokines that are important to initiation of inflammation including TSLP, IL-12p40, Eotaxin-1, and IL-1β. However, PM2.5 exposure did not enhance lung infiltrations in OVA model but increased the CD69-expressing levels in CD4+T cells.
Conclusion
Taken together, the data revealed that PM2.5 exerted different effect in response to physiological microenvironment. The exposure had mild effect on local inflammation without disturbing the lung function in healthy condition. Meanwhile, PM2.5 exerted an adjuvant effect leading to exacerbation of symptoms in asthmatic setting. Thus, highlighting biomarkers in the initiation of allergic inflammation such as the innate cytokines are critical in the future studies on disease severity.

P2-091 Role of Interleukin-15 in the progression of liver fibrosis

Ramanathan S[1], Ilangumaran S[1], Ali Akbari S[1]
[1]Université de Sherbrooke

Introduction: Unresolved liver fibrosis (LF) can progress toward cirrhosis and promote the development of hepatocellular carcinoma. The hepatic fibrogenic response is dynamic interplay between fibrogenic cells and immune system that contributes in a progressive accumulation of extracellular matrix components and liver pathology. Interleukin-15 (Il15) is an inflammatory cytokine that promotes activation of many inflammatory cells implicated in LF. IL-15 protein expression associates with alpha-receptor subunit (Il15ra) during biosynthesis, and complex is 'trans-presented' to other cells. Therefore, in this study, we aim to elucidate the pathological role of Il-15 signaling in progression of LF.
Methodology: Liver fibrosis induced in wildtype (WT), Il15–/– and Il15ra–/– mice by feeding choline-deficient high-fat-diet ad libitum for 6 weeks (treated). Serum and liver tissues were collected. Serum levels of Alanine amino transferase (ALT) were measured and liver tissues were analyzed by histology (H&E and Fast Green/Sirius Red), immunofluorescence (α-SMA, Collagen type I, CD45, CD68) and flowcytometry.

Results: Our results indicate reduced collagen deposition and mononuclear cell infiltration in the portal area in Il15–/– and Il15ra–/– compared to WT mice following treatment. IF staining shows higher infiltration of CD45+ cells along the col1a1 deposition area in treated WT. Flow cytometric analyses of the liver tissues indicate that infiltration by CD11b+ macrophages, NK, NKT and B cells, but not T cells, contributed to the increased absolute leukocytes numbers in the treated liver tissues. Within the macrophages, significant increases were observed in inflammatory, transitioning and patrolling macrophage subsets in treated WT mice, but not in Il15–/– and Il15ra–/– mice.
Conclusion: Trans-presented IL-15 plays an important role in promoting the inflammatory response in the progression of LF.

P2-092 Altered profiles of circulating cytokines in chronic liver diseases (NAFLD/HCC): Impact of the PNPLA3-I148M risk allele?

Kirchmeyer M[1], Gaigneaux A[1], Servais F[1], Arslanow A[2,3], Casper M[2], Krawczyk M[2], Lammert F[2,4], Behrmann I[1]
[1]University of Luxembourg, [2]Saarland University Medical Center, [3]Fundació de Recerca Clínic, [4]Hannover Medical School MHH

Background: Individuals carrying the risk variant p.I148M of patatin-like phospholipase domain-containing protein 3 (PNPLA3) have a higher susceptibility to fatty liver diseases and associated complications including HCC, a cancer closely linked to chronic inflammation. Here, we assessed circulating cytokine profiles for patients with chronic liver diseases genotyped for PNPLA3.

Methods: Serum concentrations of 22 cytokines were measured by multiplex sandwich-ELISA. The cohort comprised 123 individuals: 67 patients with non-alcoholic fatty liver disease (NAFLD) without cirrhosis (57 steatosis, 10 non-alcoholic steatohepatitis), 24 NAFLD patients with cirrhosis, and 21 patients with HCC (15 cirrhosis), and 11 healthy controls. Receiver operator characteristic (ROC) analyses were performed to assess the suitability of the cytokine profiles for prediction of steatosis, cirrhosis, and HCC.

Results: HGF, IL-6, and IL-8 levels were increased in patients (highest levels in patients with cirrhosis) while PDGF-BB and RANTES showed lower concentrations compared to controls. MIF and MCP-1 were found at higher levels in NAFLD samples (maximum: NAFLD-cirrhosis) compared to healthy controls and HCC samples. In ROC analyses, MIF, IL-8, IL-6 and MCP-1 yielded high sensitivity scores for predicting non-cirrhotic NAFLD (vs. healthy). The top combination to predict cirrhosis was HGF plus PDGF-BB. MIF performed best to discriminate HCC from NAFLD; addition of MIG, RANTES, IL-4, M-CSF, or IL-17A as second parameters further increased the AUC values (> 0.9). Whereas no significant impact of the PNPLA3-I148M allele on cytokine levels was observed in this cohort, addition of the genotype as a second parameter for ROC analyses increased the HCC-predictive power for some analytes.

Conclusions: Cytokines have biomarker potential in patients with fatty liver, possibly suited for early HCC detection in patients with fatty liver.

P2-093 The effects of dietary GABA on the cytokine secretions and renal inflammation and nerve formation of von Hippel-Lindau knock-out mice

Barretto A[1], Hsu T[2], Lin B[1]
[1]Department of Biochemical Sciences and Technology, College of Life Science, National Taiwan University, [2]Graduate Institute of Biomedical Sciences, China Medical University

The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) has been postulated to have therapeutic potential in the immune system by modulation of neuronal activity. Our previous study demonstrated that dietary GABA alleviates renal inflammation in a renal tubule-specific conditional von Hippel-Lindau (VHL) gene-knockout in C57BL/6 mice (KO) that spontaneously develop renal injury, hence increasing the lifespan of KO mice. Preliminary data suggests that early treatment of dietary GABA is able to inhibit nerve formation as well as decrease levels of urinary kidney injury molecule (KIM)-1 and renal clear cell formation. To further investigate the interaction of GABA on nerve formations, five-week-old C57BL/6 mice (WT) and KO mice were fed AIN-93 with or without dietary GABA for a period of 10 weeks. The behavioral effects of GABA were assayed by Open Field Test and Marble Burying Test for anxiety-like behavior. The nerve morphology was observed by a 2 or 3-dimensional image system. The cytokine levels in the spleen, serum, urine, and brain were determined. GABA treatment resulted in a decrease of anxiety-like behaviour, nerve fiber formations, pro-inflammatory cytokines such as IL-2, IL-6, IL-17A/F, IFN-γ, and TNF-α, as well as an increased expression of anti-inflammatory cytokine IL-10. GABA supplementation may have protective effects against renal injury. While this abstract provides a brief summary, further research and more details will be presented to provide a complete picture.

P2-094 Cell-type specific transcriptional regulation of APOBEC3A highlights its role in fueling mutagenesis and modulating tumor immune microenvironment

Yesudhas D, Butler K, Chakraborty A, Lone B, Banday A[1]
[1]Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, NIH

APOBEC3s (A-H) are a family of seven cytosine deaminases that inactivate viruses as part of the innate immune response. However, APOBEC3A and APOBEC3B have been found to mediate mutagenesis in tumor genomes by deaminating ssDNA that transiently arise during transcription and DNA replication. The mechanisms that drive these enzymes to generate high mutation burdens in some cancers and affect disease progression and clinical outcomes are largely unknown. Here, we explored cell-type-specific transcriptional regulation of APOBEC3s using single-cell(sc)-RNA-seq data (>70 samples and >200,000 cells) from four tumor types–bladder, lung, breast, and kidney – that exhibit different levels of APOBEC-induced mutation burden.

Our analyses revealed that among seven members of the APOBEC3 family, APOBEC3A has a cell-type specific expression pattern. It was expressed predominantly in KRT13 and CDH12 epithelial cell types and inflamed macrophages. The APOBEC3A+ epithelial cell populations were abundant in tumors of the bladder and lung, moderate in the breast, and nearly absent in the kidney. APOBEC3A expression correlated with interferon and cytokine signaling genes in KRT13 but not in CDH12 cells. Transcription factors EST2 and ATF3 were significantly upregulated in APOBEC3A+ KRT13 cells. APOBEC3A+ cells were associated with the enrichment of anti-tumor immune infiltrates. Pseudotime analysis of scRNA-seq data revealed that cells expressing APOBEC3A are likely transitioning from epithelial to mesenchymal cell state. Lastly, we identified several gene makers that define APOBEC3A+ KRT13 and CDH12 cell types. Further data analyses with clinical correlates, cell-type specific mutations burden, and validation by in vitro functional approaches are ongoing.

Our results suggest that APOBEC3A is turned on in specific epithelial cell types by multiple pathways during tumorigenesis. Additionally, APOBEC3A likely drives mutagenesis in a cell-type-specific manner, thereby modulating tumor immune microenvironment, particularly in high mutation burden cancers.

P2-095 STAT2 MEDIATES REPROGRAMING OF LIPID METABOLISM IN COLORECTAL CANCER

Gamero A[1], Canar J[1], Alvarado A[1], Pryimych N[1]
[1]Temple University

Background: STAT2 is a key effector molecule in the transcriptional response and antitumor effects of type I interferons. Paradoxically, our early work found STAT2 operating as a tumor promoter when tested in a colitis model of colorectal cancer (CRC). This finding signaled that STAT2 has dual roles in cancer, but the molecular basis of this outcome remains unknown. The aim of our study was to elucidate molecular mechanisms by which STAT2 promotes colorectal carcinogenesis.
Methods: Apc Min/+ mice were crossed with Stat2KO mice to generate Apc Min/+ and Apc Min/+;Stat2KO mice to determine tumor burden. STAT2 expression in human colon carcinoma cell lines (HCT116 and RKO) was modified by shRNA and Crispr/Cas9 approaches and assessed by western blot analysis. Tumor xenografts were generated in Rag1KO mice and subjected to RNA-Seq and untargeted UPLC-MS lipidomic analysis. Gene expression was determined by qRT-PCR analysis. Lipid uptake was assessed using BODIPY dye and phosphatidylcholine-oleic acid as a lipid substrate. The TCGA database was analyzed for associations between STAT2 in CRC and genes associated with lipid metabolism.
Results: STAT2 loss decreased tumor burden in Apc Min/+ mice. STAT2 deficiency in tumor cell lines reduced the growth of tumor xenografts. RNA-Seq analysis revealed that STAT2 regulated cholesterol homeostasis and in particular the expression of lipase LIPG, a gene involved in lipid uptake and growth of breast cancer. Moreover, untargeted UPLC-MS lipidomic analysis revealed STAT2 deficiency altered the composition of lipids in tumors. We also demonstrated that a deficiency in STAT2 impaired lipid uptake. Interrogation of the TCGA-CRC tumor database showed high STAT2 mRNA levels was associated with poor patient survival. More importantly, we found tumors with elevated STAT2 mRNA levels correlated with increased expression of genes associated with lipid metabolism.
Conclusion: STAT2 helps promote CRC by stimulating lipid uptake and altering lipid composition in tumors.

P2-096 Role of IL-21 during Cryptosporidium infection

Haskins B[1], Cohn I[1], O'Dea K[1], Merolle M[1], Pardy R[1], Byerly J[1], Smith E[1], Buenconsejo G[1], Striepen B[1], Hunter C[1]
[1]University of Pennsylvania

Cryptosporidium is an enteric pathogen that causes a persistent infection in patients with primary and acquired defects in T cell function. IL-21 is a cytokine that is associated with the development and function of B and T cell responses, and IL-21R deficient patients are susceptible to infection with Cryptosporidium. To study the role of IL-21 during Cryptosporidium infection, IL-21R-/- mice were infected and parasite burden assessed. During the acute phase of infection IL-21R-/- mice were more resistant than WT mice but while WT mice cleared infection, the IL-21R-/- mice remained persistently infected with a low-level infection. In these chronically infected mice, the blockade of IFN-g resulted in a rapid recrudescence of parasite burden. Consistent with these data, when WT mice were treated with recombinant IL-21 every other day, there was reduced parasite burden early during infection. In contrast, administration of IL-21 to IFN-g-/- mice resulted in increased parasite burden during acute infection. This suggests that IL-21 signaling is important in fine-tuning the immune response during Cryptosporidium infection depending on the presence of IFN-g. Together these studies indicate that while the absence of IL-21 may lead to enhanced innate resistance to Cryptosporidium, during the chronic phase of this infection IL-21 signaling helps mediate clearance of this opportunistic infection.

P2-097 Creating an adjuvant bank for oral vaccines, using spore display technology.

Fortin L[1,2,3], Hamelin Morrissette J[1,3], Reyes-Moreno C[1,2]
[1]Université Du Québec À Trois-rivières, [2]Groupe de recherche en signalisation cellulaire, UQTR, [3]Ulysse Biotech

Ulysse Biotech is working on the development of oral protein subunit vaccines for animals, using Bacillus spore display technology. Oral vaccines are better than injectable vaccines at eliciting mucosal immune responses and given the fact that mucosal surfaces are the main entryways of pathogens, it is important to ensure proper mucosal response to vaccines in order to attain high efficiency. Furthermore, subunit vaccines are a safer and more economical alternative to live attenuated vaccines. However, these types of vaccines generate a weaker immune response and require the addition of adjuvants. Currently, there are very few adjuvants on the market and none that can be used in mucosal vaccines.

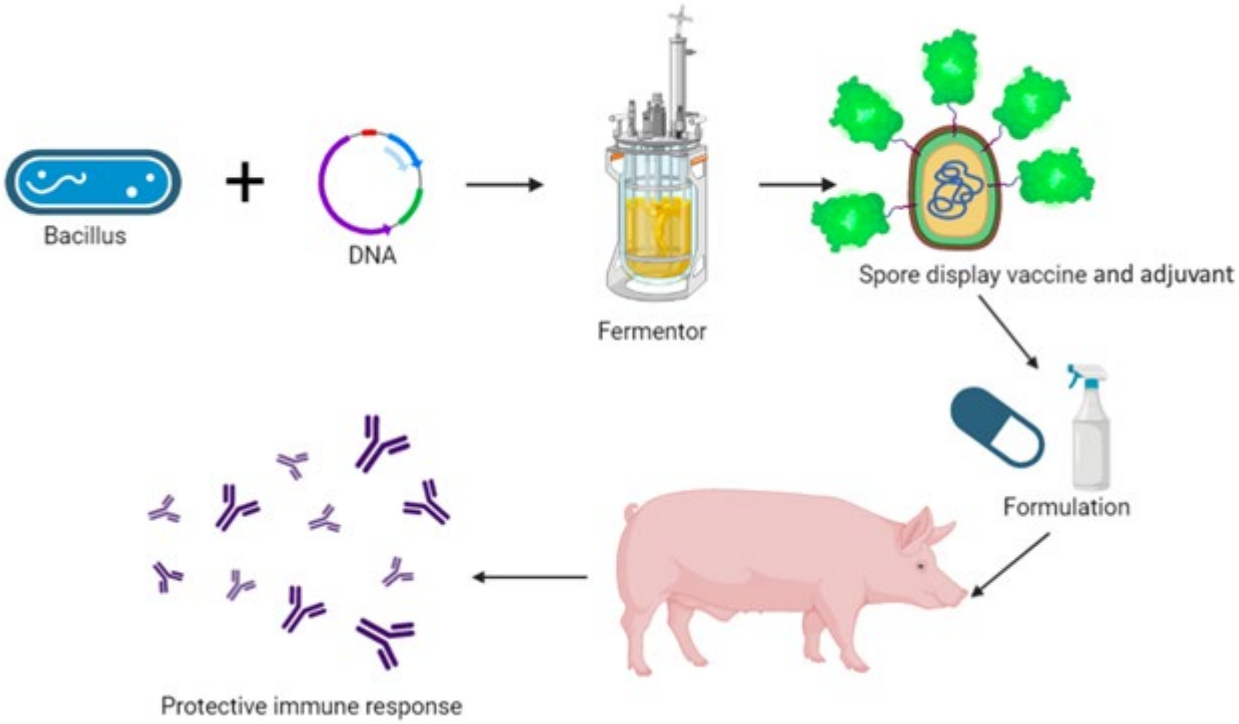
The main objective of this study is to create a bank of adjuvants that could be included in the formulation of oral vaccines. Six molecules have been selected as potential adjuvants to be expressed on the surface of Bacillus subtilis spores. The recombinant spores will be tested to verify their potential to increase the immunisation capacity of a vaccine.

Recombinant spores are produced using a Gibson-like assembly method, and transformed into Bacillus subtilis. Transformants are fermented to induce sporulation and expression of the protein on the spore surface is verified by flow cytometry.

Biological activity of the adjuvants is verified in cultured murine splenocytes first, then in mice intestinal explants. The expression of various inflammatory mediators, as well as CD40 and MHC-2 are measured in the splenocytes and explants, respectively, using western blot and immunohistochemistry.

Adjuvants showing biological activity will be tested in mice, in immunisation assays with Ulysse's oral subunit vaccine destined for pigs. The nature as well as the specificity of the induced responses will be studied to characterize the effects of the different adjuvants.

This study will allow us to gain further knowledge on the development of mucosal adjuvants.


Bacillus
DNA
Fermentor
Spore display vaccine and adjuvant
Formulation
Protective immune response

P2-098 A Longitudinal Investigation of Molecular Bacterial Vaginosis and Cytokine Relationship after Metronidazole Treatment in Women

Broedlow C[1], Basting C[1], Cherenack E[2], France M[3], Fonseca Nogueira N[4], Salazar A[4], Acosta L[4], Raccamarich P[4], Ravel J[3], Fein L[5], Holm J[3], Alcaide M[2,4,5], Klatt N[1]
[1]University of Minnesota, Department of Surgery, Division of Surgical Outcomes and Precision Medicine Research, [2]University of Miami Miller School of Medicine, Department of Public Health Sciences, [3]Institute for Genome Sciences, University of Maryland School of Medicine, [4]University of Miami Miller School of Medicine, Department of Medicine, [5]University of Miami Miller School of Medicine, Department of Obstetrics

Background:
Bacterial Vaginosis (BV) is an inflammatory condition caused by an overgrowth of anaerobic bacteria that often recurs after treatment and is associated with adverse reproductive health outcomes. Clinical criteria such as Amsel or Nugent scores are used for diagnosis, but molecular methods offer more comprehensive bacterial understanding. This six-month longitudinal study explores the relationship between vaginal microbial and cytokine profiles to understand the interplay between the microbiome and recurrent BV inflammation after treatment.

Methods:
Molecular occurrences of BV (molBV) were analyzed using samples collected longitudinally from 44 women with clinical BV (clinBV) per Amsel criteria (sexually active,18-45 years old, non-pregnant, HIV uninfected, not on hormonal contraception) and treated with seven days of oral metronidazole. Vaginal swabs and cervical vaginal lavage (CVL) samples were collected at four time points: baseline (pre-treatment), one month, and six months after treatment. In women with clinBV at one month, an additional course of metronidazole was prescribed, and samples were collected two months post baseline. MolBV was defined by the microbial community state of each sample and analyzed longitudinally with marker gene amplicon sequencing. Cytokine biomarker concentrations were measured in CVL samples through Milliplex assays.

Results:
IL-6 levels were significantly different in women who were molBV positive at three time points compared to those molBV positive two times, once, and molBV negative at all timepoint. (p=2.12E-02, p=2.87E-02, and p=2.11E-02, respectively). Linear mix effect modeling found IL-1b (p= 3.44E-03) and TNFα (p= 5.48E-03) to be positively associated with Shannon diversity. IL-1b was positively associated with A.vaginae (p=1.87E-02) P.bivia (p=1.93E-02) and P.buccalis (p= 2.64E0-2), and negatively associated with L.iners (p=7.06E-5).

Conclusion:
This study sheds light on BV recurrence mechanisms post-treatment and microbial-cytokine interplay in women with differing BV clearance rates. These findings have significant implications for novel precision-based interventions and could improve BV treatment efficacy.

P2-099 Neuroprotection of L-dopa treatment inducing IL-13 expression in cerebral ischemia

Mun S[1], Jeon E, Seo M, Lkhagva-Yondon E, Jeon M
[1]Inha University

Stroke is a leading cause of death and disability worldwide. When a stroke occurs various immune cells e.g. T cells are infiltrated, and brain cells such as neurons died. Levodopa (L-dopa), a crucial neurotransmitter for motor learning and the development of motor abilities, has recently attracted more attention since they help with motor recovery following stroke. Several clinical trials showed that the use of L-dopa showed positive outcomes. However, the molecular mechanisms are not fully investigated. Here, we measured increased amphetamine-induced rotation at one and two weeks after tMCAO (transient middle cerebral artery occlusion) which were consistent with the decreased dopamine levels in the brain. When we administered L-dopa one week after tMCAO, the amphetamine-induced rotation is reduced and dopamine levels were increased. Intriguingly we found increased IL-13 in the brain after tMCAO, which was further increased by injection with L-dopa. To investigate the function of IL-13 in tMCAO, we neutralized the IL-13 expression using anti-IL-13 antibodies and found that decreased dopamine levels and increased amphetamine-induced rotation. Furthermore, we identified that L-dopa can increase IL-13 expression in regulatory T cells using in vitro culture system. In summary, L-dopa can probably promote motor recovery by inducing IL-13 regulatory T cells.

P2-100 Characterization of STAT2 Function in Cancer

Canar J[1], Gamero A[1]
[1]Temple University

Background: Signal transducer and activator of transcription 2 (STAT2) is a key molecule in the biological effects of type I interferon (IFN-I). However, new evidence indicates that STAT2 has dual roles in inflammation and cancer by promoting inflammatory pathways and by suppressing tumor growth. We reported earlier that STAT2, paradoxically, functions as a tumor promoter in preclinical models of colon and skin cancer. The aim of this current study is to further characterize the contribution of STAT2 in cancer.

Methods: The TCGA platforms was analyzed for associations between STAT2, colorectal cancer and liver cancer. Expression of STAT2 was modified in human colon cancer cell lines (HCT116 and RKO) and human liver carcinoma PLC/PRF/5 (PLC) cells via CRISPR/Cas9 or shRNA approach. HCT116 cells overexpressing STAT2 were generated by lipofection with expression vector carrying STAT2. Interferon receptor knockout (IFNAR1 KO) HCT116 cells were generated via CRISPR/Cas9 approach. STAT2 expression and phosphorylation were assessed by western blot analysis. Tumor cell proliferation was determined by MTS assay and 3D tumor spheroid formation was done by hanging drop method. Both HCT116 and PLC cell lines were used for subcutaneous tumor xenograft models.

Results: Analysis of TCGA-colon adenocarcinoma datasets found a correlation between high STAT2 mRNA levels and overall poor survival. No clinical correlation was identified with liver cancer. STAT2 deficiency drastically suppressed while its overexpression increased the growth of HCT116 tumors. Loss of STAT2 did not affect 3D tumor spheroid formation. Meanwhile, PLC cells with STAT2 knockdown also produced very small tumors, however, these cells were defective in forming 3D tumor spheroids. Interestingly, loss of IFNAR1 did not affect the growth of HCT116 tumor xenografts.

Conclusion: Tumor cell intrinsic STAT2 signaling contributed to tumor growth in models of colon and liver cancers. IFNAR1 signaling deficiency in colon cancer cells did not affect tumor growth.

P2-101 Recombinant IL-7 administration protects host from a wide range of acute respiratory virus infection through IL-17A-producing innate-like T cells

Park S[1], Kwon D[1], Jeong Y[1], Kim Y[1], Min J[1], Lee C[1], Choi D[2], Choi Y[1], Kong H[3], Baek S[4], Lee K[1], Kang Y[1], Jeong C[1], You G[1], Oh Y[1], Im S[1], Song M[2], Kim J[1], Chang J[3], Choi D[4], Lee S[1]
[1]Pohang University of Science and Technology, [2]International Vaccine Institute, [3]Ewha Womans University, [4]Research Institute of NeoImmuneTech

Repeated pandemics and epidemics caused by influenza virus and SARS-CoV have resulted in drastic effects on global public health. Although some vaccines and antiviral drugs achieved successes to control disease spread and morbidity, their limited application to new variants emphasize the requirements for broad-spectrum therapeutics. One strategy to achieve the goal is utilizing host immune system. Upon respiratory virus infection, various tissue resident immune cells mediate antiviral response. Both innate and adaptive T cells are one of major population that participate in the antiviral response through IFN-γ and IL-17A. Generation and maintenance of T cells often requires IL-7, a T cell homeostatic cytokine. Administration of recombinant IL-7 rapidly amplify T cells and has been an attempting strategy to boost T cell response in various disease. Especially, several studies have suggested potential of IL-7 administration in protection against respiratory infection. To investigate the potential of recombinant IL-7 in protection against respiratory virus infection, we infected female C57BL/6 mice with various virus as influenza A virus, influenza B virus, SARS-CoV-2, and RSV. Surprisingly, administration of recombinant IL-7 induces protective effects against broad spectrum of viruses. To further investigate the mechanisms of IL-7-mediated antiviral effects, we used single cell RNA-sequencing paired with V(D)J sequencing and flow cytometric approaches on IAV infected mice. The mice administered with IL-7 displayed expansion of IFN-γ-producing conventional T cells and IL-17A-producing innate-like T cells. The administration of IL-7 also upregulated the expression of antiviral gene in both conventional and innate-like T cells. Cytokine depletion studies suggested that IL-17A-producing innate-like T cells, rather than IFN-γ-producing conventional T cells, are major population for the IL-7-mediated protective effects upon infection. In conclusion, we suggest the administration of recombinant IL-7 could be a potential broad-spectrum therapeutic by regulating early immune responses with IL-17A-producing innate-like T cells.

P2-102 A noncanonical RIG-I pathway triggers cancer cell-specific death

Sato S[1], Sato K[1], Takaoka A[1]
[1]Hokkaido University

The activation of RIG-I like receptors (RLRs) such as RIG-I and MDA-5 by their ligands 3pRNA and polyI:C triggers cancer cells death, and some clinical trials are ongoing. It is thought that RLRs activate cell death signaling of cancer cells in a type I IFN-dependent pathway, but it is still incompletely understood how RLR-mediated signaling leads to cell death in cancer cells. In this study, we show that treatment with RLRs ligands results in the induction of cancer cell death in vitro and in vivo. Of note, we found that not only RIG-I WT but also RIG-I T55I mutant which does not activate MAVS/IPS-1-antiviral pathway trigger cancer cell death. Our further detailed analyses have identified “a novel protein” as an interacting partner of RIG-I T55I. This protein is localized in the nuclei in non-cancer cells but its localization is altered in cancer cells. Treatment with 3pRNA, a RIG-I ligand, preferentially activates the phosphorylation of this protein in the cytoplasm of cancer cells, whereas treatment with doxorubicin activates the nucleic fraction of this protein. Intriguingly, phosphorylation of this protein is not observed in 3pRNA-treated non-immortalized cells. Finally, cytosolic localization of this protein by lacking its nuclear export signal (NES) actually suppresses melanoma growth in vivo following treatment with 3pRNA. Thus, our findings identified a novel noncanonical pathway of RIG-I to selectively activate cancer cell death in collaboration with its novel partner, which may provide a mechanistic insight for the development of novel cancer therapy.

P2-103 hetIL-15 reshapes tumor microenvironment of pancreatic ductal adenocarcinomas, introducing a new type of CD103intCD11b+ Dendritic Cells and enhancing cytotoxic cell activation

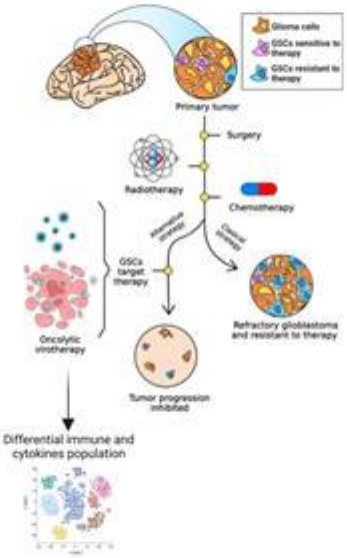
Stellas D[1,2], Karaliota S[1,3], Stravokefalou V[1,4], Guerin T[5], Kozlov S[5], Felber B[1], Pavlakis G[1]
[1]National Cancer Institute at Frederick, [2]National Hellenic Research Foundation , [3]Basic Science Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, [4]University of Thessaly, [5]Center for Advanced Preclinical Research, Frederick National Laboratories for Cancer Research

Introduction: Pancreatic ductal adenocarcinoma (PDA) is a life-threatening malignancy with limited therapeutic options, for which the mortality rate approaches the incidence rate. PDAs are immune excluded tumors, therefore immunotherapy does not provide a good therapeutic alternative. We have previously shown that hetIL-15, in addition to its role on tumor entry and activation of cytotoxic cells, also affects the recruitment of myeloid cells to sites of malignancy. We explored the effects of hetIL-15 on reshaping the tumor microenvironment using preclinical murine models of PDA.
Study design and methods: We used the Genetically Engineered Mouse (GEM) model of pancreatic cancer, KPC (KrasLSL.G12D/+;p53R172H/+;PdxCretg/+), and a syngeneic orthotopic allograft model of KPC cells. Effects of hetIL-15 on the primary tumor, metastatic disease and overall survival were evaluated. Tumor Infiltrating Lymphocytes (TIL) were analyzed by flow cytometry and immunohistochemistry (IHC). Using RNA in situ hybridization (RNAscope) we identified and characterized a novel type of CD103intCD11b+ DCs, infiltrating hetIL-15-treated PDAs. We also assessed the intratumoral necrosis and evaluated the metastatic burden.
Results: Flow analysis of TIL revealed that the NK cell population and the CD8+/CD4+ T cell ratio were increased after hetIL-15 monotherapy. Treatment increased the intratumoral infiltration of the cytotoxic cells, increasing the necrosis and thus supporting functionality of the cytotoxic immune cells. hetIL-15 treatment also reduced metastatic disease. Interestingly, analysis of the myeloid tumor-infiltrating cells identified an additional distinct DC population characterized by CD103intCD11b+ immunophenotype. We monitored this population using RNAscope, due to the unique co-expression at high levels of Mgl2, CD24a and Ccl17. IHC verified the flow observations.
Conclusions: Our results show that hetIL-15 reshaped the cytokine/chemokine milieu and promoted not only lymphoid but also myeloid cell recruitment into PDA tumors. This treatment transformed the immune-excluded PDA to an immune infiltrated tumor, providing opportunities for novel immunotherapeutic interventions.

P2-104 POTENTIAL OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV) AS A NOVEL VIROTHERAPY FOR GLIOBLASTOMA

Tur Planells V[1], Bykov Y[1], Dawodu G[1], Palacín I[2], Rius S[3], Arranz J[3], Ayuso A[2], Nistal-Villan E[3], Garcia-Sastre A[1], Cuadrado S[1]
[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, [2]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, [3]Microbiology Section, Department Pharmacological and Health Sciences, University CEU San Pablo, [4]Department of Medicine, Icahn School of Medicine at Mount Sinai, [5]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai

Despite recent advances in diagnosis and therapeutics, cancer remains one of the main cause of death worldwide. Glioblastoma (GBM) is the most aggressive malignant primary brain tumor, characterized by an immune desert tumor microenvironment (TME). GBM has shown to be highly refractory to treatments with an average of 15 months survival rate. Here, we propose the use of Infectious Bursitis Disease Virus (IBDV), an avian virus non-pathogenic in humans, as a novel treatment strategy for GBM. Experimental infections of patient-derived GBM cancer stem cells (GBM18 and GBM27), murine CT-2A, GL261 stablished GBM types, and mice-derived GBM neurospheres (H3K27-met, PDGFB and p53-/-) have demonstrated that GBM cells are highly susceptible to IBDV infection and able to support viral replication, while displaying robust stimulation of type-I interferon and pro-inflammatory responses. Furthermore, temozolomide (TMZ) resistant GMB cells displayed higher sensitivity to cell death induction upon IBDV infection, opening the possibility to combination therapies that could maximize the effect of TMZ while counteracting the emergency of resistance. In vivo, intratumoral administration of IBDV on syngeneic CT2A tumors exerted robust control over tumor growth leading to a statistically significant extension of survival when compared with PBS-treated control mice. Immunophenotyping of the TME of IBDV-treated tumors by FACS unveiled a strong effect on TME remodeling characterized by a significant increased on CD8+/Treg ratio.

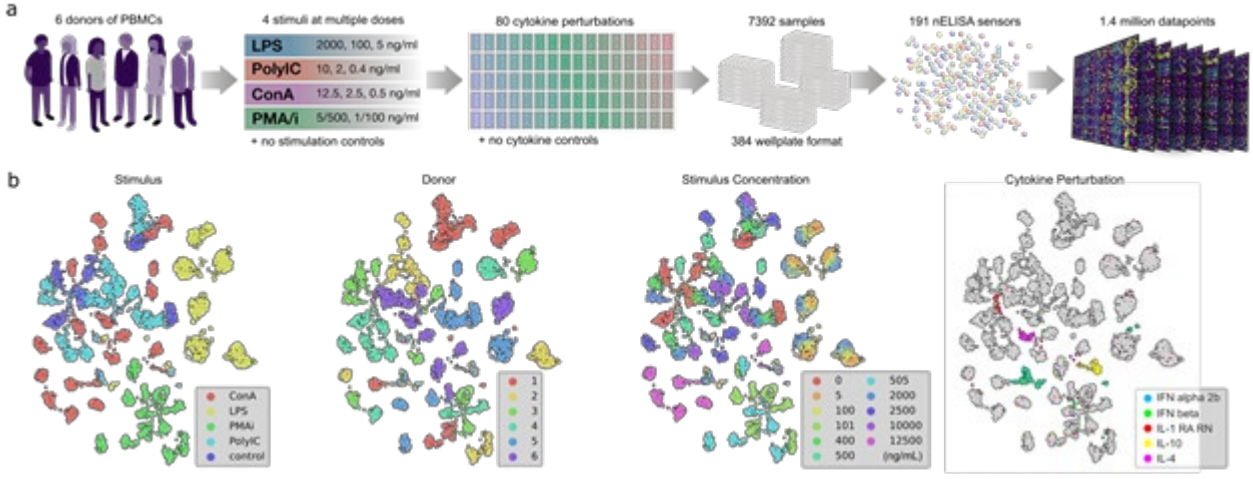

Glioma cells
GSCs sensitive to therapy
GSCs resistant to therapy
Primary tumor
Surgery
Radiotherapy
Chemotherapy
GSCs target therapy
Oncolytic virotherapy
Refractory glioblastoma and resistant to therapy
Tumor progression inhibited
Differential immune and cytokines population

P2-105 Cytokine-Specific Phenotypes in PBMCs Revealed by nELISA High-Throughput Proteomics

Robichaud N[1], Ongo G[1], Rao W[1], Teahulos I[1], Munzar J[1], Dagher M[1]
[1]Nomic Bio

The complex networks of interactions between cytokines, the cells that produce them and those that respond to them, have been challenging to map. In part, this is due to the lack of proteomics tools with sufficient cytokine coverage, throughput, and cost-efficiency to meaningfully capture the variety of signals and responses of interest. Here, we introduce the nELISA, a massively-parallelized and miniaturized ELISA that overcomes cross-reactivity limitations of multiplexing. We built a 191-plex cytokine/chemokine panel amenable to high-throughput screens, and demonstrated its applicability to mapping cytokine interactions.

To achieve this, we ran the largest PBMC secretome screen to date, in which >7000 PBMC samples were treated with various inflammatory stimuli, and further perturbed with a selected library of 80 recombinant protein “perturbagens”. 191 secreted proteins were profiled in all samples, resulting in ~1.4M datapoints. The nELISA profiles were able to capture phenotypes associated with specific stimulation conditions, individual donors, and potent cytokine perturbagens. By compensating for stimulation and donor differences, we clustered perturbagens according to their effects on PBMC secretomes, identifying well-established cell responses such as Th1 or Th2. Novel phenotypic effects were also identified, such as distinct responses to the near identical CXCL12 alpha and beta isoforms. Interestingly, we observed important similarities between PBMC responses to the cytokine drugs IFN beta and IL-1 Receptor antagonist, supporting the use of anakinra as a replacement for IFN beta in certain indications, such as multiple sclerosis. We will highlight these findings and discuss the broad applicability of the nELISA for drug discovery, including phenotypic screening, target deconvolution, target identification, hit/lead characterization, and discovery of markers of target engagement and off-target effects.



P2-106 The drug retention rates between the drugs for urate-lowering therapy in Korean patients with gout: data from the Health Insurance Review & Assessment claims database

Jeong H[1], Hong S[2], Kim S[1], Son C[3]
[1]Department of Rheumatology, Keimyung University Dongsan Hospital, [2]Department of Rheumatology, Kyung Hee University Hospital, Kyung Hee University, [3]Department of Rheumatology, Uijeongbu Eulji Medical Center, Eulji University School of Medicine

Background : In South Korea, febuxostat has become first-line urate lowering therapy since 2016. This study aimed to compare drug retention rate according to drug types between allopurinol and febuxostat in patients diagnosed with gout for the first time.
Methods : We analyzed gout patients aged 19 years or older who were registered with the Health Insurance Review and Assessment Service from January 2017 to December 2018 and had not received a prescription for uric acid-lowering drugs in the previous 6 months. Persistence was defined as more than 80% of prescriptions within the prescription period. One-year drug retention rates were analyzed for two years.
Results : A total of 839,706 cases were included in the analysis. The drug retention rate was higher for allopurinol than febuxostat (26.51% vs 17.36%). For both drugs, the retention rate of women was higher than that of men, and it was higher with increasing age. In patients receiving treatment at tertiary hospitals, the drug retention rate was higher than those of primary and secondary hospitals for both drug. The retention rate of medication was higher in patients receiving treatment from internal medicine compared to patients treated in other departments for both drug. Analysis of the factors affecting the retention rate of the two drugs showed that the age of 65 years or older and the treatment at the internal medicine department were associated with a high drug retention rate.
Conclusion : The drug retention rate of allopurinol was higher than that of febuxostat in Korean gout patients. The elderly and patients receiving internal medical care were associated with higher drug retention rates.

P2-107 Sex-based differences are driven by microbiome composition in a model of CD-like ileitis

Wargo H[1], Russo L[3], Williams J[1], Vidmar K[1], De Salvo C[1], Goodman W[1], Del Pinto R[2], Pizarro T[1]
[1]Case Western Reserve University, School of Medicine, [2]University of L'Aquila, Department of Life, Health and Environmental Sciences, [3]Gilmour Academy

Inflammatory bowel disease (IBD), i.e., ulcerative colitis and Crohn's disease (CD), is a chronic, relapsing inflammatory disorder of the gastrointestinal tract, whose etiology is currently unknown. It is well-recognized, however, that IBD results from dysregulated immune responses in genetically susceptible individuals in response to environmental triggers, including components of the gut microbiome. Sex-based differences have been reported for IBD patients, with prior work from our group supporting these findings in ileitis-prone SAMP1/YitFc (SAMP) mice, showing earlier onset and more severe disease in SAMP females (SAMP-F) vs. -M. The goal of this study was to determine whether the gut microbiome plays a role in sex differences observed in the SAMP model of CD-like ileitis. To test this hypothesis, 10- and 20-wk-old SAMP-M and -F raised under either specific pathogen-free (SPF) or germ-free (GF) conditions were histologically evaluated for ileitis and draining mesenteric lymph nodes immunophenotyped by FACS analysis. Feces from SPF-SAMP and -AKR (control) mice were analyzed by 16s, and fecal microbiota transplantation (FMT) performed into either same or opposite sex GF-SAMP recipients exposed to dextran sodium sulfate (DSS) to induce colitis. Cytokine profiles were determined using the Luminex multiplex platform. Our results showed increased richness and opposing β-diversity trends in microbiome composition comparing SAMP to AKR, with divergence in β-diversity between SAMP-F and -M overtime. Notably, while SAMP still developed ileitis under GF conditions, inflammation was dramatically attenuated with disease onset significantly delayed. Interestingly, sex bias in ileitis and increased frequency of CD4+Foxp3+ Tregs no longer existed in GF-SAMP, while significant differences in cytokine profiles were observed in GF- vs. SPF-SAMP. Importantly, FMT using SPF-SAMP-F vs. -M donors into DSS-treated GF-SAMP showed more severe colitis, independent of recipient sex. Together, these results suggest the existence of the microsexome during CD that may contribute to sex-based differences in IBD.

P3-001 Durable cytokine-mediated reprogramming of antifungal immunity during chronic inflammation

Iliev I[1]
[1]Weill Cornell Medicine

Gastrointestinal fungal dysbiosis is a hallmark of several inflammatory diseases marked by systemic immune activation. Whether persistent colonization with pathobionts in the face of impaired gut barrier function and dramatic immune alterations has a durable impact on host immunity is currently unknown. We investigated the effects of gut mycobiota dysbiosis and intestinal Candida overgrowth on the immune system of severe COVID-19 patients.

We found increased levels of circulating anti-Candida albicans IgG antibodies (ACAL IgG) and systemic neutrophilia in severe COVID-19 patients who suffered from gut mycobiota dysbiosis and Candida overgrowth. Further analysis revealed enduring transcriptional changes in granulocyte myeloid progenitors (GMP) that persisted for up to a year in severe COVID-19 patients who tested positive for ACAL IgG. Intestinal colonization of mice with C. albicans strains isolated from COVID-19 patients resulted in lung neutrophilia and pulmonary NETosis in a mouse model of COVID-19, which was largely resolved upon antifungal treatment. The persistent effects on proinflammatory immunity after colonization with C. albicans were attributed to IL-6 mediated immune activation. Therapeutic intervention in severe COVID-19 patients guided by these findings led to a successful rescue of this long-lasting immune phenotype. These findings suggest that gut fungal pathobionts might contribute to lasting immune alterations during inflammatory diseases, and mycobiota-immuno-based approaches could help identify patients at risk for severe COVID-19 who may suffer long-lasting immune alterations.

P3-002 Machine Learning-Guided Design of Naturally Occurring and Synthetic Cytokines for Precise Cellular Targeting and Controlled Responses

Abedi M[1], Exposit M[1], Jane S[1], Krishnakumar A[1], Baker D[1]
[1]Institute for Protein Design, University of Washington

Despite the growing excitement surrounding the engineering of native cytokine functions, the inherent challenges in modifying these proteins have prompted us to explore an alternative approach. Instead of attempting to engineer existing cytokines, we set out to design them from the bottom up by generating cytokine receptor binders and rigidly fusing them to form de novo designed agonists with a precise spatial arrangement of the two receptors, thus facilitating controlled signal transduction. Such precise control over cytokine-receptor interactions allowed us to tune properties of these complexes such as: Emax and EC50 to achieve desired cellular responses in a targeted manner. To achieve this, we have incorporated machine learning models into a design pipeline that enables the rapid and efficient design of high-affinity binders against cell surface receptors, yielding high affinity binders that are subsequently combined through a rigid fusion to form agonists.

Moreover, our method can be further expanded to facilitate the discovery of novel signaling receptor complexes that are not formed by naturally occurring cytokines. By deploying these deep-learning based techniques, we established a high-throughput screening platform capable of interrogating over a thousand distinct cytokine receptor pairs. Through this platform, we identified new receptor combinations that signal despite lacking a known endogenous cytokine, potentially uncovering exciting opportunities for precise cellular interventions. Our current focus involves deciphering the functional implications of these newly engineered natural and synthetic signaling complexes. We envision that these designer cytokines will open avenues for innovative biological programming, unlocking novel opportunities for therapeutic intervention and enabling the development of targeted treatments.

In conclusion, we present a new approach for cytokine engineering from the bottom up, culminating in a high-throughput and precise method for rapidly designing agonists that activate natural or synthetic receptor combinations to facilitate controlled cellular responses, including cellular targeting, cytokine stability, and signaling strength.

P3-003 The two faces of Ninj1: exacerbating Inflammation and attenuating infections

GUPTA R[1]
[1]In between jobs

Ninj1 was identified as a regulator of Plasma membrane rupture (PMR) - a post cell death phenomena in different modalities of cell death, viz- apoptosis, pyroptosis and necroptosis (1). In hepatocellular injury mice model (apoptosis induction with TNF and D-galactosomine), the Ninj1 sufficient mice showed higher serum levels of alanine aminotransaminase (ALT), aspartate aminotransferase (AST), LDH, HMGB1 and IL-18 in comparison to the Ninj1 deficient mice (2). Ninj1 oligomerization blocking antibody (clone D1) attenuated the PMR event along with cargo release of large inflammatory mediators while maintaining the balloon morphology – after cell death (2). Similar observations were noted with other hepatic insults model too, like ConA, anti-Fas agonistic antibody and ischemia –reperfusion injury (2).
Intriguingly, Ninj1 homozygous deficient mice was found to be more susceptible to Citrobacter rodentium (1) and Yersinia pseudotuberculosis (3) infection in comparison to wild type mice. Similarly, Ninj1 heterozygous deficient mice was more prone to systemic inflammation and pancreatic insulitis with enhanced immune cell infiltration and pro-inflammatory cytokine production, with respect to wild type mice (4). A plausible explanation of this susceptibility can be due to liberation of HMGB1 during PMR, which helps in containment of bacterial growth by disruption of biofilm (5).
Ninj1 being an adhesion molecule and expressed in epithelial cells and neurons (6), the migration of different myeloid lineages and the T cells from neuronal niche could play a role in regulating inflammation and controlling infections in a context dependent fashion.
Bacterial infections, hepatic insults and tissue damages are few integral features of sepsis phenomena. Understanding the antithetical role of Ninj1 in inflammation and infection biology, might prove to be helpful in sepsis management.

References:
1) https://doi.org/10.1038/s41586-021-03218-7
2) https://doi.org/10.1038/s41586-023-06191-5
3) https://doi.org/10.1371/journal.ppat.1009967
4) https://doi.org/10.1073/pnas.1711814114
5) https://doi.org/10.1172/JCI140527
6) DOI: 10.1016/s0896-6273(00)80166-x

P3-004 The Involvement of PBRM1 in Alveolar Macrophage Signaling and Immune Function

Mandias H[1,2], McRae H[1], Hargreaves D[1,2]
[1]The Salk Institute for Biological Studies, [2]University of California, San Diego

Alveolar macrophages (AMs), the respiratory system's first line of defense, communicate with epithelial and adaptive immune cells via cytokine crosstalk to inform immune tolerance in the lungs. Tissue-specific signals are required for the differentiation and function of resident AMs. Derived from circulating blood monocytes, recruited AMs function similarly to resident AMs while exhibiting subtle differences in their gene signatures, chromatin accessibility, and cytokine secretion. Their plastic response to the pulmonary microenvironment is regulated by epigenetic modifications, such as DNA methylation, nucleosome remodeling, and transcription factor recruitment. The importance of AMs is further emphasized by their dysfunction in lung diseases, such as SARS-CoV-2 and Influenza A virus. Lower respiratory infections are the leading cause of communicable deaths worldwide, resulting in more than 2.4 million deaths per year. During IAV infection, AMs play a critical role in preventing tissue damage and mortality.
Previous studies indicate that SWI/SNF chromatin remodeling complexes are mediators of gene expression within CD8+ T cells and other myeloid-derived cells. To investigate the involvement of SWI/SNF variant PBAF toward regulating AM populations in vivo, I employed mice harboring myeloid-specific deletion of PBAF's core subunit, Pbrm1. Adult knockout mice exhibit a reduction in AMs. We excluded the possibility that this was a consequence of perturbed development or proliferation, and rather identified that the AM reduction was a result of increased cell death. Bulk RNA-sequencing indicated dysregulation of several scavenger receptor genes (Marco and Colec12) and cytokine signaling genes (Prkacb and Il2rg) in knockout AMs. Influenza-infected Pbrm1-deficient mice exhibit higher rates of weight loss and death compared to control mice. Altogether, this data suggests that Pbrm1 is involved in orchestrating AM immune processes. By assessing the epigenetic, transcriptional, and functional effects of Pbrm1-loss in AMs, this study establishes Pbrm1's previously unappreciated role in orchestrating AM signaling and function.

P3-005 Neurotropic vesicular stomatitis virus infection induces changes in the transcriptome of CNS resident myeloid cells that are critical for leukocyte recruitment

Waltl I[1], Bošnjak B[2,3], Pavlou A[1], Mulenge F[1], Förster R[2,3], Kalinke U[1,3]
[1]Institute for Experimental Infection Research, TWINCORE, Centre for Experimental and Clinical Infection Research, a joint venture between the Hannover Medical School and the Helmholtz Centre for Infection Research, [2]Institute of Immunology, Hannover Medical School, [3]Cluster of Excellence – Resolving Infection Susceptibility (RESIST), Hannover Medical School

Virus infection of the central nervous system (CNS) can cause potentially life-threatening disease and long-term sequelae in affected patients. Some neurotropic viruses infecting the nasal epithelium have the ability to migrate via olfactory sensory neurons to the CNS, where they induce inflammation and tissue disruption. Recently, we have shown that upon intranasal instillation of mice with vesicular stomatitis virus (VSV), microglia play a key role in orchestrating local anti-viral responses that are type-I interferon-dependent and regulated by neurons and astrocytes. Furthermore, microglia have been shown to be crucial for the proper function of infiltrating virus-specific T cells and consequently for VSV clearance. Here, we sought to investigate microglia during acute anti-viral responses in the olfactory bulb as well as their interaction with infiltrating cells by performing single-cell RNA-sequencing of previously sorted CD45+ cells. We detected drastic changes in the transcriptome of microglia relative to uninfected controls. Expression profiles of microglia from the virus-infected brain differed strikingly from those reported in models of sterile brain inflammation. Additionally, we found a cell subset that emerged during infection that showed a distinct transcriptomic signature with genes that are involved in antigen-presentation, T-cell activation and differentiation. We also found that monocytes and NK cells rapidly accumulated at 3 days post infection (dpi) within the olfactory bulb, while T cells were the predominant leukocyte population at 6 dpi. Expression profiles of chemokine receptors such as CCR5, CXCR3, and CXCR6 were strongly upregulated suggesting that these were involved in T-cell recruitment during VSV-infection. High expression levels of CXCL9 and CXCL10 in VSV-activated microglia and infiltrating monocytes underline their crucial role for T-cell migration into the infected CNS. Hence, our data further highlights the strong impact of CNS resident cells on T-cell recruitment and migration.

P3-006 Sirt6 suppress type 1 IFN response through IRF3 deacetylation

Park S[1], Gu S[2], Lee S[1]
[1]Chungbuk National University, [2]Jeonbuk National University

The innate immune response provides the first line of defense against viruses and other pathogens by responding to pathogen-associated molecular pattern (PAMP). Sirtuin6 (Sirt6) is an NAD+-dependent histone deacetylase belonging to the Sirtuin family. Sirt6 is previously shown to act as an important regulator in innate immune response, especially inflammation. However, it's role in toll like receptor 3(TLR3) mediated antiviral immune response is still unclear. In this study, we aimed to explore the extent of Sirt6's involvement in immune responses triggered by TLR3 activation. We conducted a comparative analysis of cytokine secretion in vitro and in vivo using bone marrow derived dendritic cells (BMDCs) from both Sirt6 wild-type (WT) and knockout (KO) mice, treated with poly (I:C). Our findings revealed that Sirt6 KO BMDCs exhibited elevated levels of pro-inflammatory cytokines, such as interleukin-6 (IL6), tumor necrosis factor alpha (TNF-α), and type I interferon IFNβ, in comparison to WT BMDCs. This suggested a possible role of Sirt6 in the TLR3 signaling pathway, thereby influencing the secretion of type I interferon (IFNβ). Next, we investigated the interaction between Sirt6 and crucial transcription factors, IRF3 and IRF7, which are critical for IFNβ synthesis. Remarkably, our results revealed that Sirt6 contributes to the regulation of IRF3 and IRF7 by enhancing their ubiquitination, consequently leading to reduced protein levels. These findings underscore the potential of Sirt6 as a promising target for combating viral infections.

Keywords: Sirt6, Type I interferon, IRF3, IRF7, Poly (I:C)

P3-007 Study of the Role of Genetic Mutations and Cancer Associated Macrophages in Development of Colorectal Cancer Metastases

Chigvinadze N[1], Pantsulaia I[1], Lezhava T[1], Jokhadze T[1], Buadze T[1], Gaiozishvili M[2], Sigua T[2], khvichia N[2]
[1]Tbilisi State University, [2]Tbilisi State Medical University

The colorectal cancer is a complex disease where there are involved both, genetic factors and immune system. Mutation of the p53, and pRB genes are one of the commonest genetic changes in the development of human colorectal cancer.
It is shown that the system of the ribosomsal cistrons involved in the neoplastic vulnerability of tumors. This facts shows the importance of studying the variability of the genes pRb and p53 products in carcinogenesis and the effectiveness of ribosomal in the cells of patients with CC.
Immune cells have substantial place in the microenvironment of the tumor, one of the subjects of the most recent researches are the tumor associated macrophages playing either positive or negative role in development of cancer.
The study material was the cells of stimulated peripheral blood lymphocytes from colon cancer patients and healthy donors. P53 and PRB gene products and macrophages have been evaluated by the ELISA method. The activity of ribosomal genes was studied on chromosome preparate derived from peripheral blood lymphocyte cultures. The method of silver impregnation to reveal active nuclear organizers.
As a result of the analysis show: the patients with CC is characterized general instability of genome. p53 and pRB, gene products change of according to individuals, and therefore the role of mutations of these genes can be varied in case of specific tumors. In addition, as the chromosomal fragility test indicates, changed the distribution of damaged chromosomes by groups, what should be a specific feature for a tumor of this type. Against the background of general high instability, the genome of patients with CC is characterized by the presence of specific areas of the greatest vulnerability (damageability). t should be also noted that CD68 associated cells restrict tumor growth at the first stage while CD163 associated cells – on the contrary.

P3-008 Uncovering novel mechanism of pathogenesis in Hidradenitis suppurativa (HS)

Raman C[1], Kashyap M[1], Mishra B[1], Jin L[1], Sinha R[1], Deshane J[1], Mukhtar S[1], Elewski B[1], Elmets C[1], Athar M[1]
[1]University of Alabama at Birmingham

Hidradenitis suppurativa (HS) is a chronic and debilitating inflammatory skin disease characterized by deep-seated lesions in the apocrine gland areas commonly within skin fold regions of the body. The immunopathogenic mechanisms underlying disease onset and progression are poorly defined and current therapies provide limited benefit. We performed single cell RNAseq analysis of HS tissue and discovered novel roles for NKT and NK cells in disease pathogenesis. NKT cells and NK cells were greatly expanded in HS skin in comparison to healthy skin that we confirmed by immunohistochemistry. High resolution confocal images showed that cytolytic classical NK cells (CD56dim) expressing high levels of perforin and granzyme A were enriched within the epidermis and sinus tracts and frequently juxtaposed with cells undergoing apoptosis in HS skin. In contrast, NKT cells (CD3+CD56bright) expressing high levels of granzyme A, low levels of perforin, were adjacent to α-SMA expressing fibroblasts within fibrotic regions of sinus tracts. The stimulatory adhesion associated molecule, CD2, was expressed at high levels on NKT cells and NK cells and they interacted with keratinocytes expressing CD58 (LFA-3), the counter-receptor for CD2. NKT and NK activating cytokines (IL-12, IL-15, IL-18) expressed by keratinocytes and macrophages were expressed highly in HS skin along with several chemokines associated with NKT/NK recruitment. Micro-RNA (miRNA) regulome in HS revealed a pattern critical for NK cell differentiation and cytotoxicity. The expression of these cytokines and chemokines along with HS associated gene signature were mostly reversed in HS skin organ cultures treated with an antagonist CD2 mAb. In epithelial cells, the epigenetic switch reshapes the progenitor cell signatures, which drives inflammatory signaling via S100A7/8/9 enhancers. Altogether, we report novel epigenetic and NKT/NK cell pathogenesis of HS and propose that CD2 blockade can be a highly effective therapy.

P3-010 Collaborative Cross screen identifies quantitative trait loci (QTL) for rapid coronavirus clearance.

Menachery V[1], Kalveram B[1], Gralinski L[2], Bubier J[3]
[1]University Of Texas Medical Branch, [2]University of North Carolina, [3]The Jackson Laboratory

While the COVID-19 pandemic has had an undeniable impact on the world, its disease burden was not uniform across the human population. Specific factors including age and host genetics contributed to differential outcomes for COVID-19 and other respiratory virus infections. Yet, little is understood about the interplay between host genetics and age during infection, or their combined impact on host immunity and susceptibility to infection. Therefore, in this study, we used the diverse collaborative cross mouse resource to examine the role of host genetics on susceptibility to SARS-CoV infection in both young and aged animals. Building on a prior study, we used an F2 cross to define a quantitative trait loci (QTL) associated with rapid clearance of virus from the lung. We go on to show that the allele, associated with a wild-derived founder, was dominant with low/undetectable viral titers in heterozygous mice. Importantly, this phenotype was preserved in both young and aged mice despite a range of disease. Finally, we have used established prioritization approaches to define high priority targets in the PARP family likely mediating the phenotype. Additional work seeks to define the mechanism of action and establish additional QTL associated with age-dependent susceptibility and/or resistance.

P3-011 Lipid nanoparticle induced IL-27 promotes vaccineelicited memory CD8+ T cells

Phan A[1], Aunins E[1], Cruz-Morales E[1], Alameh M[1], Weissman D[1], Hunter C[1]
[1]University Of Pennsylvania

Lipid nanoparticle (LNP)-mRNA vaccines have shown remarkable efficacy in promoting adaptive immunity. Recent studies have demonstrated that a single dose of LNP-mRNA vaccine induces the production of inflammatory molecules and antigen necessary to induce T cell responses. However, the specific cellular and molecular mechanisms that elicit the T cell response remain relatively unexplored. LNP-mRNA induced IL-6 is critical for the development of T follicular helper cells and the antibody response, but how it and related family members impact the induction of CD8+ T cell responses following immunization remain unclear. Expression of IL-27, a member of the IL-6 family of cytokines, is rapidly induced in monocytes and cDC1s in the draining lymph node following intramuscular immunization with LNP-mRNA vaccines. Immunization of IL-27-deficient mice revealed a striking defect in antigen-specific CD8+ T cell expansion in comparison to wildtype mice demonstrating that IL-27 is necessary for the optimal expansion of the antigen-specific CD8+ T cell response. Following LNP-mRNA vaccination cDC1s rapidly present antigen on MHC-I and express high levels of IL-27 suggesting a critical role in promoting CD8+ T cell responses in line with their role in responses to infection and malignancy. Surprisingly, cDC1-deficient mice immunized with LNP-mRNA exhibited largely intact antigen-specific CD8+ T cell responses despite the loss of a population of IL-27 producing and antigen presenting cells. In contrast, antigen-specific CD8+ T cell responses in mice where monocytes and macrophages were deficient in MHC-I expression were significantly impaired suggesting that antigen-presentation by macrophages and monocytes is more critical for CD8+ T cell responses to LNP-mRNA immunization. Thus, LNP-mRNA induced CD8+ T cell responses exhibit unique requirements for antigen-presentation and IL-27 signaling via monocytes and macrophages.

P3-012 Bacillus species Regulates Lipid Accumulation and Inflammation in High Fat Diet-induced Obesity Mice

Chae J[1], Suh I[1], Lee S[1]
[1]College of Veterinary Medicine, Chungbuk National University

Prolonged consumption of a high-fat diet leads to fat accumulation and ultimately obesity. Obesity, in turn, is closely linked with hypertension, type 2 diabetes mellitus, cardiovascular diseases, and persistent low-grade inflammation. Researchers are actively investigating the therapeutic potential of lactobacillus-based probiotics as a means to counteract obesity and address associated metabolic disorders. Simailarly, Bacillus species, beneficial bacteria commonly found in fermented foods like cheonggukjang and natto, have also been associated with various health benefits. However, research on the anti-obesity effects of Bacillus species is limited. This study aims to explore the potential of two strains of Bacillus species (BS01 and BS02) in preventing high-fat diet-induced obesity and inflammation. Daily oral administration of BS01 or BS02 reduced body weight and epididymal fat weight and improved blood glucose levels. It also decreased excessive insulin and leptin concentrations, suppressing insulin resistance. Moreover, there was a significant reduction in blood lipid levels. Not only were the size of adipocytes in epididymal adipose tissue reduced, but also the number of lipid droplets in liver tissue. Bacillus species exerted an influence on the expression of genes involved in lipid metabolism, effectively suppressing fat accumulation, leading to alleviate inflammation.
These findings highlight the ability of Bacillus species to alleviate inflammation by reducing liver lipid accumulation, improving lipid metabolism in adipose tissue, and suppressing obesity, suggesting their ability as a potential probiotics to prevent obesity and metabolic disorders.

P3-013 Electroporation-mediated delivery of mRNA vaccine induces protective immune responses

Kim M[1], Kim Y[1], Chae J[1], Park S[1], Seo Y[2], Kim M[2], Jung H[2], Lee Y[3], Lee S[1]
[1]College Of Veterinary Medicine, Chungbuk National University, [2]Department of R&D, Hulux, [3]Department of R&D, Live Cell Instrument (LCI)

In vivo Electroporation (EP) has proven to be a highly effective method for enhancing the delivery and efficacy of DNA vaccines. While DNA vaccines have received substantial attention, several research groups have also proposed the potential of mRNA delivery through EP. The present study was designed to develop electroporation strategy for naked mRNA vaccine delivery and evaluate antigen specific immune responses in mice. By using luciferase-encoding mRNA we showed that intramuscular EP of mRNA induces high levels of luciferase at the injection site. Following intramuscular injection and electroporation with SARS-CoV-2 spike mRNA at optimum pulse conditions, humoral and cellular immune responses specific to spike protein were efficiently induced. Notably, this approach conferred protective immunity against lethal SARS-CoV-2 infection in mice. Similarly, B16-OVA murine melanoma model, EP mediated delivery of naked OVA mRNA significantly reduced tumor growth and prolonged survival. Taken together, our findings serve as compelling evidence that EP-facilitated administration of "naked" mRNA holds considerable promise as a strategy to prevent infectious diseases and cancer development.

P3-014 Recombinant SARS-CoV-2 Spike Protein stimulates secretion of Chymase, Tryptase and IL-1β from human mast cells, augmented by IL-33

Tsilioni E[1], Theoharides T[2]
[1]Department of Immunology, Tufts University School of Medicine, [2]Institute of Neuro-Immune Medicine, Nova Southeastern University

Background: SARS-CoV-2 infects cells via its spike (S) protein binding to its surface receptor Angiotensin Converting Enzyme 2 (ACE2) and results in production of multiple pro-inflammatory cytokines, especially in the lungs, leading to what is known as COVID-19. However, the cell source and the mechanism of secretion of such cytokines have not been adequately characterized.
Methods: Human LADR mast cells were stimulated with recombinant full-length SARS-CoV-2 S (1-10 ng/mL; Abcam), or RBD (1-10 ng/mL; Abcam), and/or preincubated with the following: 1) anti-TLR2 Antibody (2 µg/mL, 1 h; InvivoGen), 2) anti-TLR4 Antibody (2 µg/mL, 1 h; InvivoGen) and 3) anti-ACE2 Antibody (2 µg/mL, 1 h; InvivoGen). Substance P (SP) (2 µM, Sigma-Aldrich), Interleukin-33 (IL-33) (30 ng/mL, R&D Systems) and their combination were used as positive controls.
Results: Recombinant SARS-CoV-2 full-length Spike protein (1-10 ng/mL), but not its receptor-binding domain (RBD), stimulates human LADR mast cells to secrete pro-inflammatory cytokine IL-1β as well as the proteolytic enzymes chymase and tryptase. IL-33 significantly augments the ability of SARS-CoV-2 Spike protein to stimulate secretion of chymase, tryptase and IL-1β from human mast cells. This effect is mediated via toll-like receptor 4 (TLR4) for IL-1β, but via ACE2 for chymase and tryptase. However, the mechanism via which activation of ACE2 leads to degranulation and secretion of chymase and tryptase is not presently known.
Conclusion: These results provide evidence that SARS-CoV-2 spike protein contributes to inflammation by stimulating mast cells through different receptors. Mast cells can, therefore, contribute to COVID-19 and possibly also to Long-COVID. Preventing or minimizing the detrimental effects of the spike protein could lead to novel targeted treatment approaches.

P3-015 TGF-β producing Innate Lymphoid regulatory Cells (ILCregs) contribute in towards immunopathology of Lepromatous Leprosy

Saini C[1], Sapra L[1], Tarique M[3], Sharma A[3], Ramesh V[2], Puri P[4], Srivastava R[1]
[1]Department of Biotechnology, All India Institute of Medical Sciences, [2]Department of Dermatology, ESI college and hospital, , [3]Department of Biochemistry, All India Institute of Medical Sciences, [4]Department of Dermatology, SJH

Introduction
Lepromatous leprosy (LL) is a generalized disease of skin caused by Mycobacterium leprae. LL reflects T cell anergy and dysregulation between T helper cells, particularly Th17 and Tregs, which have been postulated in earlier investigations to be responsible for LL progression. ILCs (innate lymphoid cells) are predominantly present in the skin and are known to modulate Th17 and Tregs cells. Thus, understanding the pathogenic involvement of ILCs in leprosy would offer fresh perspective on the intervention and therapy of lepromatous leprosy.
Methods
The study investigated 20 Lepromatous Leprosy (LL) and 20 Borderline leprosy (BT) patients as control. We analysed both IL-17 producing ILC3s and IL-10 producing ILCregs, in the stimulated PBMCs as well as in skin biopsy of leprosy patients by flow cytometry. In-situ gene expression of cytokines, such as IL-10, IL-17, IL-22, and TGF-β, was further examined using real-time PCR (q-PCR) in the skin lesions of leprosy patients.
Results
LL patients showed significantly ($p<0.002$) higher percentages of TGF-β producing CD3-CD25+CD127+FOXP3- (ILCregs; regulatory) than BT leprosy patients in skin lesions. Surprisingly, CD3-CD25+CD127+FOXP3- cells had no significant difference between LL and BT leprosy patients in stimulated PBMCs. On the other hand BT patients had significantly ($p<0.0001$) higher of CD3-CD19-CCR6+IL17A+ (ILC3s; inflammatory) as compared with LL patients in skin lesions. Moreover, inflammatory cytokines (IL-17 and IL-22) mRNA expression were significantly ($P<0.01$) low in LL patients skin lesions as compared to BT patients. On the other hand TGF-β and IL-10 were significantly ($p<0.03$) upregulated in the skin lesions of LL as compared to BT patients.
Conclusion
Altogether, our results in LL leprosy showed dysregulated “ILC3s- ILCreg” cell balance in polar form of leprosy patients in the skin. Importantly, TGF-β producing ILCreg are involved in the immunopathology of generalized lepromatous leprosy.

P3-016 A Feeder-Free Workflow for the Expansion and Gene Editing of Human Blood Natural Killer Cells

Romera Hernandez M[1], Le Fevre T[1], Ang E[1], Nguyen Truong D[1], Bodykevich G[1], Antonchuk J[1], Ewen C[1], Kokaji A[1], Eaves A[1,2], Louis S[1], Tabatabaei-Zavareh N[1]
[1]Stemcell Technologies Inc, [2]Terry Fox Laboratory, BC Cancer

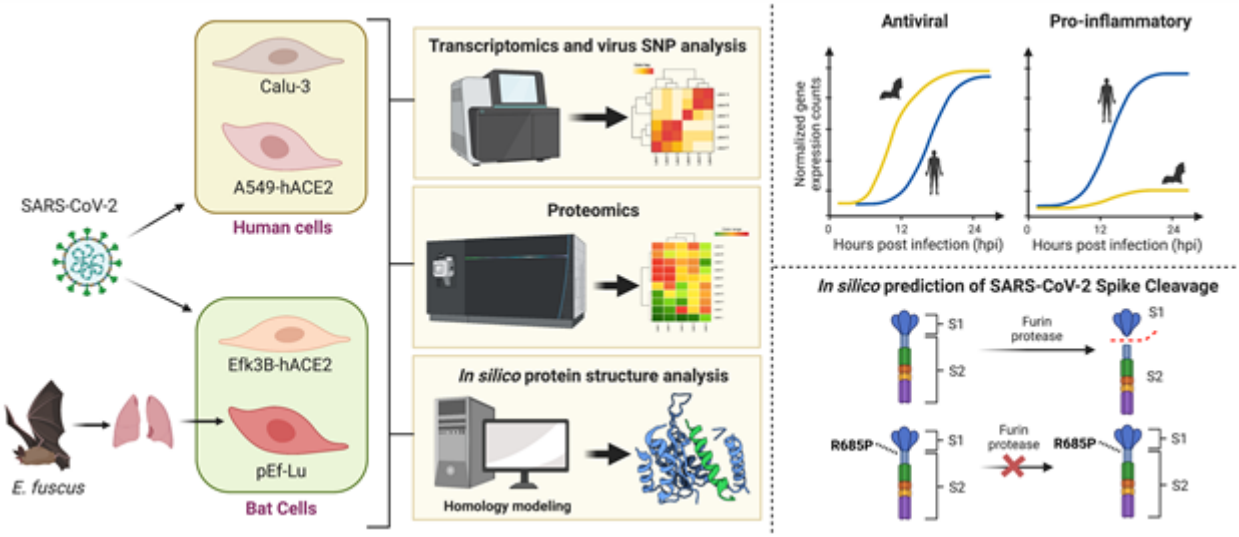
Natural killer (NK) cells are promising cells for immunotherapies against cancers that escape recognition by T cells. However, generating relevant numbers of functional NK cells for clinical applications is a major challenge. We have developed a serum-, xeno-, and feeder-free culture system that enables the expansion and genetic manipulation of NK cells. Peripheral blood NK cells were cultured in ImmunoCult™ NK Cell Expansion Medium and harvested on day 14. CD56+CD3- NK cells expanded 66 ± 9.8-fold (mean ± SEM, n = 78) with an average frequency of 90 ± 0.7%. 75 ± 2.1% of NK cells were CD16+. ArciTect™ CRISPR-Cas9 targeting of CD45 or TIGIT achieved editing efficiencies of 91 ± 3% and 88 ± 3%, respectively (n = 4). IFN-γ expression was observed in 49 ± 3.7% (n = 15) of NK cells cultured with K562, and in 32 ± 4.1% (n = 7) of NK cells cultured with anti-HER2-coated SK-BR-3 cells. TNFa expression and the degranulation marker, CD107a, were also detected in both co-cultures. Finally, NK cell killing was detected by using target cells treated with a fluorescent-based caspase-3/7 substrate to measure apoptosis. An effector:target ratio of 1:1 resulted in 50 ± 2.6% (n = 11) killing of K562 cells, and 60 ± 2.4% (n = 7) killing of anti-HER2-coated SK-BR-3 cells. The work presented here highlights the suitability of our NK cell expansion system to expand and edit human NK cells to aid in the development of immunotherapies.

P3-017 Comparative cytokine response in human and insectivorous bat cells infected with SARS-CoV-2

Baid K[1], Luc J[2], Richard D[3], Aguiar J[2], Machado Y[4,5,6], Siwak K[7], LeBlanc E[7], Aicher S[8], Khatooni Z[1], Nguyen H[1], Stacey H[11,12,13], McArthur A[11,13], Miller M[11,12,13], Wilson H[1,14], Capellini T[3], Faure P[15], Liu Q[1,14], Mubareka S[16], Menachery V[17,18,19], Mossman K[11,12,20], Endres N[9,10], Müller M[9,10], Drosten C[9,10], Jouvenet N[8], Colpitts C[7], Overall C[4,5,6], Doxey A[2,20], Banerjee A[1,2,14,16,20,21]
[1]Vaccine And Infectious Disease Organization, University Of Saskatchewan, [2]Department of Biology, University of Waterloo, [3]Department of Human Evolutionary Biology, Harvard University, [4]Department of Biochemistry and Molecular Biology, University of British Columbia, [5]Centre for Blood Research, Life Sciences Institute, University of British Columbia, [6]Department of Oral Biological and Medical Sciences, Faculty of Dentistry, University of British Columbia, [7]Department of Biomedical and Molecular Sciences, Queen's University, [8]Institut Pasteur, Université de Paris; CNRS UMR3569, Virus Sensing and Signaling Unit, [9]Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Virology , [10]German Center for Infection Research (DZIF), partner sites Giessen and Charité , [11]Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, [12]McMaster Immunology Research Centre, McMaster University, [13]Department of Biochemistry and Biomedical Sciences, McMaster University, [14]Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, [15]Department of Psychology, Neuroscience and Behaviour, McMaster University, [16]Department of Laboratory Medicine and Pathobiology, University of Toronto, [17]Department of Microbiology and Immunology, University of Texas Medical Branch, [18]Institute for Human Infection and Immunity, University of Texas Medical Branch, [19]Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, [20]Department of Medicine, McMaster University, [21]Department of Biochemistry and Molecular Biology, University of British Columbia

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the largest known coronavirus pandemic. The disease severity in COVID-19 patients is driven by a dysregulated immune response, which includes a dampened or delayed innate antiviral response, along with an exaggerated pro-inflammatory response. SARS-CoV-2 is speculated to have evolved in an insectivorous bat, likely from the genus Rhinolophus. Coronaviruses from the Sarbecovirus lineage circulate widely in different bat species, but little is known about how bats tolerate infections with these viruses that cause severe and often fatal disease in spill over mammalian species, such as humans.
Methods: We investigated SARS-CoV-2-host interactions using cells derived from humans and Eptesicus fuscus, an insectivorous bat species used widely in bat coronavirus research. We used transcriptomic and proteomic assays to identify global host responses in human and bat cells. In addition, we performed in silico characterization of a single nucleotide polymorphism (SNP) in SARS-CoV-2 spike gene observed in E. fuscus cells.
Results: Our transcriptomic and proteomic data demonstrate that bat cells mount a more robust and early antiviral response to SARS-CoV-2 relative to human cells. In contrast, pro-inflammatory responses were dampened in bat cells. Further analysis of SARS-CoV-2 sequences within infected cells identified a viral SNP in E. fuscus cells resulting in R685P amino acid substitution. Data from our computational modelling and docking studies predict a disruption of E. fuscus furin-spike interaction as a result of the R685P substitution.
Conclusion: Our study sheds light on the evolution of coronavirus-host interactions in reservoir and spillover species. We demonstrate that insectivorous bat cells mediate a differential innate antiviral

response against SARS-CoV-2 which might contribute to reduced immunopathology and virus tolerance.



P3-018 Fractalkine/CX3CR1 axis contribute to renal fibrosis induced by unilateral ureteral obstruction

Iwahashi Y[1], Ishida Y[2], Kuninaka Y[2], Nosaka M[2], Kimura A[2], Mukaida N[2], Hara I[1], Kondo T[2]
[1]Wakayama Medical University, department of Urology, [2]Wakayama Medical University, department of Forensic Medicine

Background
Obstructive nephropathy is a common clinical case due to urinary stone or ureteropelvic junction obstruction and lead to renal fibrosis. Innate immune cells are main contributors to kidney fibrosis. Infiltration of the kidney by leukocytes is caused by chemokine. CX3CR1 is ubiquitously expressed in most tissues on mononuclear and circulatory leucocytes. Though CX3CR1+ cells and its ligand fractalkine expression are evident in many renal diseases, the role of fractalkine/CX3CR1 axis in unilateral ureteral obstruction (UUO), model of renal fibrosis, has not been clarified yet.
Methods
8-12-week male Cx3cr1 KO mice and C57BL/6 background wild-type (WT) were used. Unilateral ureteral obstruction (UUO) was performed through a flank incision. The left ureter was tied with 4-0 silk suture at two points and ligated. Mice were sacrificed at 1, 3, and 7 days after UUO to obtained the kidney samples for histological and immunohistochemical analysis, real-time RT-PCR, and Flow cytometry.
Results
Gene expression of Cx3cr1 in the kidneys of WT mice increased over the time course of the experiment. Cx3cr1 KO mice showed markedly reduced renal fibrosis compared to WT mice after 7 days of UUO by Masson trichrome staining. Inflammatory cytokines such as IFN-γ and IL-6 in Cx3cr1 KO mice were significantly reduced after UUO compared to WT mice. Double immunofluorescence analysis revealed that both F4/80+ macrophages and CD45+vimentin+ fibrocytes infiltrating the kidney after UUO expressed CX3CR1. In addition, flow cytometric analysis showed that both CX3CR1-expressing Ly6-ChighMHC class II low macrophages and CD45+collagen 1a1+ fibrocytes in WT mice on day 7 after UUO were significantly increased compared to WT on day 0.
Conclusion
The fractalkine/CX3CR1 axis promotes renal fibrosis by promoting migration of Ly6-ClowMHC class II high macrophages and fibrocytes in the UUO model. Regulation of fractalkine/CX3CR1 axis may have therapeutic aspect to reduce renal fibrosis.

P3-019 The NF-kB binding site in the Ifnb1 promoter is indispensable for resistance to pathogenic viruses and defines a predominant role of Ifnb1 over Ifna genes

R Melo-Silva C[1], Heath N[1], Tang L[1], Ramage H[1], Sigal L[1]
[1]Thomas Jefferson University

Type I interferon (IFN-I) signaling through the IFN-I receptor promotes the transcription of hundreds of interferon-stimulated genes (ISG) that protect from viral infections. Most mammals encode multiple IFN-I isoforms comprised of one IFN-β and multiple IFN-αs (14 in mice, 13 in humans). It is unknown why there are so many IFN-I isoforms. The promoter of Ifnb1 (encoding IFN-β) binds the transcription factors AP-1, IRF3, and NF-κB, which are constitutive, and IRF7, which is an ISG. The promoter of Ifna4 (encoding IFN-α4) binds IRF3 and IRF7, while the promoters of all other IFN-α genes (non-α4) bind only IRF7. To test whether individual IFN-I differentially or redundantly protect against viral infections, we used the CRISPR/Cas9-based iGONAD method to inactivate one or multiple IFN-I genes in C57BL/6 (B6) mice. Ifnb1-/- and to a greater extent ifna4,b1-/- (deficient in Ifnb1 and Ifna4) mice were significantly more susceptible than wild-type (WT) B6 mice to lethal infection with the Orthopoxvirus ectromelia virus and the highly virulent NY2000 or the attenuated Kunjin strains of the flavivirus West Nile virus (WNV). On the other hand, Ifna4-/-, Ifna1,5,7-/- (deficient in three IRF7-only regulated IFN-α) and Ifna16-6-/- (deficient in six IRF7-only regulated IFN-α) suffered increased viral loads, but were as resistant to lethal ECTV and WNV infections as B6 mice. Thus, the various IFN-I isoforms are non-redundantly protective against ECTV and WNV, but only IFN-β is uniquely critical to resist their lethality. Because NF-κB is essential to resist ECTV infection, we also made Ifnb1 PRDII mice, with a deletion of the NF-κB binding site (PRDII) in the Ifnb1 promoter and found that they were more susceptible to ECTV and WNV infection than B6 mice. These data indicate that by regulating Ifnb1, NF-κB is the master regulator of IFN-I for anti-viral protection.

P3-020 Establishment of a Cynomolgus Macaque Model for Acute SEB Intoxication

Hwang E[1], Hur G[2], Baek S[1], Oh T[1], Kim G[1], Woo Y[1,3], Kim Y[1], Koo B[1], Shim K[1,3], Park J[4], Hong J[1,3]
[1]Korea Research Institute Of Bioscience And Biotechnology, [2]Agency for Defense Development, [3]KRIBB School of Bioscience, Korea University of Science & Technology (UST), [4]Seoul National University

Background
Staphylococcal enterotoxin B (SEB) is a potent bioterrorism agent that primarily stimulates CD4+ T cells and antigen-presenting cells (APCs) to release proinflammatory cytokines, causing toxic shock syndrome. However, animal models have difficulty reproducing symptoms in humans due to lower binding affinity of SEB to MHC class II compared to humans.
Method
In this study, we aimed to establish a SEB acute toxicity model in nonhuman primates, specifically cynomolgus macaques, which have a similar MHC class II region to humans. We used the respiratory route, which is applicable to bioterrorism in humans. Through this approach, we observed CD4+ T cell-related responses, including cytokine release and pulmonary pathology, consistent with SEB intoxication in humans.
Results
In cynomolgus monkeys intoxicated with SEB, the plasma SEB levels peaked at 6 hours post-exposure and gradually decreased thereafter. Notably, a reduction in lymphocytes was observed in the blood at 6 hours. CD4+ T cells were activated in the spleen, and proinflammatory cytokines and chemokines, including CD4+ T cell-related cytokines, were elevated in the plasma during the early stages of intoxication. Histopathologic examination revealed pulmonary edema, infiltration of immune cells, and depletion of germinal centers.
Conclusion
Our findings demonstrate that the cynomolgus macaque SEB acute intoxication model effectively replicated key aspects of SEB intoxication in humans, including CD4+ T cell hyperactivation, massive cytokine release, and lung and spleen pathology. Therefore, the establishment of this suitable SEB intoxication model in cynomolgus monkeys could serve as a crucial platform for evaluating potential treatments and vaccines against SEB intoxication, providing valuable insights into the mechanisms of SEB toxicity and facilitating the development of novel therapeutics.

P3-021 Adenovirus mediated expression of porcine epidemic diarrhea virus (PEDV) S1-ferritin nanoparticles induced significant neutralizing antibody response against PEDV in mouse models

Tennakoon M[1], Ryu J[2], Shin H[1]
[1]College of Veterinary Medicine, Chungnam National University, [2]Research Institute of Veterinary Medicine, Chungnam National University

Porcine epidemic diarrhea virus (PEDV) which belongs to the family Coronaviridae is a highly contagious virus among pig populations and it causes severe diarrhea and mortality in neonatal piglets. The development of rapid vaccine strategies is an essential requirement as long as the vast economic loss due to the virus spreading. Since spike protein is the major antigenic molecule that contains a plethora of neutralizing epitopes against PEDV, it has been established diverse vaccine platforms employing the monomeric spike protein or its elements. Manifolding antigenic molecules on the self-assembling nanoparticles is one of the best strategies for the induction of robust immunogenicity in subunit vaccine development. Moreover, recombinant adenovirus (rAd) based gene delivery is also a promising platform for the efficient introduction of genetic materials into host cells. Currently, it has not been reported a ferritin-based nanoparticle vaccine or a combining approach of adenoviral gene delivery and subsequent generation of self-assembling nanoparticles as vaccine candidates for PEDV. Here, S1 subunit of the spike protein was linked with the N-termini of the human ferritin heavy chain (hFTHC). Thereafter, rAds for PEDV S1 and PEDV S1-ferritin were generated in 293A cells using a commercial adenoviral vector system and the expression of the recombinant proteins was ascertained in-vitro. Vaccine efficacy of the generated rADs was assessed in female Balb/C mice. Three doses of both S1 and S1-ferritin rAds (8×106 PFU/mL) were administered intramuscularly with an interval of two weeks. Antigen-specific and PED virus-specific IgG titer was evaluated at 14 and 40 days after the final boost and both specific IgG titers were significantly higher in the S1-ferritin immunized groups. Furthermore, serum neutralization assays were performed at both day points thus, it was revealed that PEDV S1-ferritin has significantly higher virus neutralization activity than PEDV S1 itself.

P3-022 An IL-23 reporter strain reveals the developmental identity and functional regulations of the IL-23-producing gut cDC2s that mediate mucosal host defense against infectious pathogens

Ohara D[1], Takeuchi Y[1], Watanabe H[1], Lee Y[1], Mukoyama H[1], Ohteki T[2], Kondoh G[1], Hirota K[1]
[1]Kyoto University, [2]Tokyo Medical and Dental University (TMDU)

Interleukin (IL)-23 is a pivotal inflammatory cytokine that mediates immune responses by Th17 cells and ILC3s (type 17 immunity), which are critical for mucosal host defense against infectious pathogens. However, the identities, localization, and developmental pathways of IL-23-producing mononuclear phagocytes (MNPs) remain poorly understood due to the highly heterogenous nature of MNPs and technical challenges in visualizing IL-23-producing cells.
Our study addresses this gap using an IL-23 reporter strain, which demonstrates that EpCAM+ DCIR2+ CD103- cDC2s among MNPs are the primary source of IL-23 at steady state and specifically expand in gut-associated lymphoid tissues (GALTs) after Citrobacter rodentium or flagellin challenge. Furthermore, we propose a two-step model for the development of IL-23-producing cDC2s, which involves Notch2 signaling for the development of EpCAM+ DCIR2+ cDC2s preceding IL-23 expression, and retinoic acid signaling for the maturation of these cells into IL-23-producing cDC2s.
Our findings provide novel insights into how the development and function of GALTs-associated IL-23-producing cDC2 are controlled in mucosal host defense during pathogen infection.

P3-023 CD160 serves as a co-inhibitory receptor in bacterial sepsis by regulating the iNKT-NK cell axis

Ha H[1], Kim J[1], Kim J[1], Kim T[1], Park Y[1], Lee H[1], Hwang Y[1], Sim Y[1], Lee K[1]
[1]Korea University

Sepsis is a life-threatening condition caused by a systemic inflammatory response to bacterial infection, resulting in organ damage or death. CD160 is a glycophosphatidylinositol immunoglobulin(Ig) domain protein found on the surface of T cells, NK cells, and iNKT cells that compete with B and T lymphocyte attenuator (BTLA) to bind with herpesvirus entry mediator (HVEM). While the role of BTLA in LPS-induced sepsis has been investigated in mice models, the function of CD160 remains unknown. This study aimed to investigate the role of CD160 in bacterial sepsis using CD160-deficient mice and LPS-induced sepsis or cecal ligation and puncture (CLP) models. Results showed that CD160-/- mice had increased mortality and cytokine secretion compared to WT mice, with increased IFN-γ secretion from NK cells as the main factor contributing to the mortality. However, in mixed bone marrow chimera of WT and CD160-/- mice, CD160-/- NK cells had reduced IFN-γ expression, indicating that the increased activation of NK cells in CD160-/- mice was due to extrinsic factors. Furthermore, iNKT cells, which express CD160 constitutively, had increased IL-17 secretion and CD69 activation marker expression at early time points in CD160-/- mice. These findings suggest that CD160 serves as an important co-inhibitory receptor in sepsis by maintaining cytokine balance and preventing overactivation of the iNKT-NK cell axis. This study sheds new light on the role of CD160 in sepsis and provides a basis for further investigation into the mechanism of CD160 regulation in the iNKT-NK interaction.

P3-024 Regulatory function of PTP4A2 on cytokine and chemokine expression in lung epithelial cells in response to viral infection

Weerasooriya M[1], Ke J[2], Lee I[2], Chan A[3], Ting O[1], Yongliang Z[1]
[1]National University Of Singapore, [2]Raffles Institution, [3]Australian International School Singapore

Background
During viral infections, Pattern Recognition Receptors (PRRs) play an important role in the regulation of the innate immune system. Binding of ligands to these PRRs activate a variety of intracellular signalling pathways involving protein phosphorylation and ubiquitination. Previous studies in our lab had identified PTP4A2 as one of the host proteins that interact with the SARS-COV-2 spike protein. PTP4A2 belongs to protein tyrosine phosphatase (PTP) family of proteins that have shown to regulate various cellular processes such as cell cycle regulation, however the role of PTP4A2 during viral infection and host response remains unclear.
Methodology
In this study, we over-expressed PTP4A2 in the human lung carcinoma cell line A549. The control vector and PTP4A2 over-expressed cells were used to study the effect of PTP4A2 towards anti-viral immune response. The cells were first stimulated with Poly I:C (a synthetic dsRNA molecule) and the pro-inflammatory cytokine expression levels were measured using qPCR and ELISA. Following this, the cells were also infected with influenza PR8 and SARS-CoV-2 pseudo virus, and cytokine expression was measured through qPCR and ELISA, and changes in protein expression was detected using western blot.
Results
In response to Poly I:C, protein levels of the pro-inflammatory cytokines such as TNF-α, IL-6 and IFN-β were reduced when PTP4A2 was over-expressed. When the cells were infected with influenza PR8, we observed a similar trend for the pro-inflammatory cytokines and western blot results showed reduced pERK and pIRF3 when PTP4A2 is over-expressed. Infection with SARS-CoV-2 pseudo virus also showed reduced MCP-1 in the over-expressed cells.
Conclusion
The results indicate that PTP4A2 may negatively regulate the production of these pro- inflammatory cytokines, possibly through involving other upstream proteins such as IRF-3 and MAPKs which are commonly associated with controlling the signaling cascades during viral infections.

P3-025 Positive regulation of Innate Immune signaling by TRIM65 through the facilitation of IRF3 recruitment to chromatin and increase ability to bind to promoters

Fonseca D[1], Miorin L[1], Pisanelli G[1], Garcia-Sastre A[2]
[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, [2]Department of Microbiology, Global Health and Emerging Pathogens Institute, and Department of Medicine, Icahn School of Medicine at Mount Sinai

Viral infection triggers a fast and effective cellular response mediated primarily by the production of interferon β (IFNβ) that induces an anti-viral state through complex signaling 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 still remain poorly investigated.
In this study we used Antiviral Assays, Luciferase Assays, RT-qPCR, Western blots, Co-immunoprecipitations, Immunofluorescence, Subcellular fractionations and Chromatin Immunoprecipitation assays (ChIP) in cells overexpressing TRIM65 as well as in TRIM65 transient knock down (KD) and CRISPR knock out (KO) A549 and 293T cells.
Previous work in our lab revealed that an unprecedented number of TRIMs play critical roles as enhancers in the regulation of innate immune signaling pathways. We have unveiled a new stimulatory role for TRIM65 through its interaction with IRF3 downstream the interferon induction pathway. We showed that its overexpression strongly increased not only 2CARD-RIG-I but also IRF3-mediated activation of the IFNβ and ISRE reporters. Consequently, IFNβ as well as interferon stimulated genes (ISGs) mRNA levels are decreased in TRIM65 KD and KO cells upon infection compared to control cells. Re-constitution assays in TRIM65 KO cells reverted the inhibition of IFNβ and ISGs confirming that TRIM65 is specifically the protein responsible for the phenotype observed. Mechanistically, TRIM65 does not affect IRF3 phosphorylation, stability, or nuclear translocation, but rather facilitates its recruitment to chromatin and subsequent binding to the IFNβ promoter with a concomitant enhancement of IFNβ and ISGs production, hence facilitating the control of viral infection.
A better understanding of IFN positive regulatory networks will provide new knowledge that will help to design more effective therapeutics.

P3-026 Interleukin 26 induces acute inflammation.

Okamura Y[1], Hendricks M[1], Dinu A[1], Hansen J[2], Savan R[1]
[1]School of Medicine, University of Washington, [2]U.S. Geological Survey, Western Fisheries Research Center

IL-26 is an inflammatory cytokine that belongs to the IL-10 cytokine family. Its genetics and expression are significantly related to various inflammatory diseases, such as ulcerative colitis and psoriasis. However, the biological function of IL-26 is still unclear, and studying its role in vivo is challenging since rodents do not have the IL26 gene. To address this issue, we used zebrafish as a model organism to study the function of IL-26. Our research showed that IL-26 activates the JAK-STAT pathway via the IL-20RA/IL-10RB receptor complex. We conducted RNA-seq analysis on IL-26-treated cells to identify the gene programs induced by IL-26 in human keratinocytes. Our results show that IL-26 mediates rapid but transient transcriptional responses in cells, with significant variance in our dataset being driven by IL-26 responses at 3 and 6 hours post-treatment. We observed approximately 750 differentially expressed genes, of which ~64% of genes were induced and 34% of genes were downregulated. In addition to the activation of the JAK/STAT pathway, we also observed activation of the MAPK and NF-κB pathways, as well as an increase in genes associated with acute inflammation, such as IL1B, IL8, IL6, and CXCL1. Interestingly, the differentially expressed genes returned to baseline levels by 12 and 24 hours post-treatment. We found similar results in zebrafish gut epithelial cells when treated with zebrafish recombinant IL-26. To test the in vivo roles of IL-26, we generated il26-/- and il20RA1-/- zebrafish using CRISPR-Cas9. While we did not observe any abnormalities in the developmental stages of these zebrafish, we are currently investigating the role of IL-26 in fungal infections.

P3-027 IL-1β exacerbates the lung pathology of mice with pulmonary infection of Mycobacterium abscessus by increasing IL-17 production

Jung D[1], Ahn E[1,2], Kim D[1], Lee T[1], Kim Y[1], Lee Y[1], Seo I[1], Cho Y[1], Kim W[1], Shin S[3], Park J[1]
[1]Laboratory Animal Medicine, College of Veterinary Medicine and Animal Medical Institute, Chonnam National University, [2]Biomedical Research Institute, Seoul National University Hospital, [3]Department of Microbiology, Institute for Immunology and Immunological Diseases, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine

Background
Mycobacterium abscessus (MAB) is one of the non-tuberculous mycobacteria (NTM) that cause chronic pulmonary inflammation in humans. IL-1β is a representative pro-inflammatory cytokine and plays an important role in host defense against bacterial infections, but its excessive production leads to harmful effects in the host by causing tissue damage. Although NLRP3 contributes to IL-1β production by macrophages in response to MAB, it is unclear the in vivo role of NLRP3 and IL-1β in host defense against MAB infection.

Methods
To determine the effect of MAB infection on macrophages in vitro, we isolated bone marrow-derived macrophages (BMDMs) from wild-type, Nlrp3-/-, Caspase1-/-, Asc-/-, and Nlrc4-/-, mice on a C57BL/6 background and intracellular bacterial infection assay was conducted. And we also performed co-culture model using splenocytes and BMDMs from wild-type and Il1b-/- mice. In the in vivo experiment, wild-type, Nlrp3-/-, and Il1b-/- mice were infected with MAB via the intranasal route and bacterial burden and histopathology were evaluated and various cytokines were measured from the lung homogenates.

Results
In this study, we revealed that the MAB infection in BMDMs triggers the secretion of IL-1β via the activation of the NLRP3 inflammasome, but not the NLRC4 inflammasome. An in vivo experiment showed that deficiency of IL-1β attenuated the lung pathology in MAB-infected mice, although the bacterial clearance was not affected. The production of IL-17, but not IFN-γ and IL-4, was decreased in the lung homogenates of IL-1β-deficient mice, as compared with WT mice. An in vitro study using co-culture system with BMDMs and splenocytes showed that an IL-1R antagonist anakinra reduced MAB-induced production of IL-17 in a dose-dependent manner. Finally, we showed that treatment with anti-IL-17 antibody reduced the MAB-induced lung pathology.

Conclusion
Our results suggest that IL-1β may contributes to the lung pathology induced by MAB infection by upregulating IL-17 production.

P3-028 Cellular Senescence in Lung and Nasal Epithelial Cells Result in Elevated Pro-inflammatory Cytokine Production in Human Monocyte/Macrophages.

Song W[1], Choi S[1], Lee S[1], Kang Y[1,2], Kim H[1], Lee W[1,2]
[1]Laboratory of Inflammation and Autoimmunity (LAI), Dept. of Biomedical Sciences and Dept. of Microbiology and Immunology, Seoul National University College of Medicine, [2]Institute of Infectious Diseases, Seoul National University College of Medicine

Background:
The COVID-19 pandemic has caused an enormous public health crisis, resulting in the deaths of over 6.9 million people worldwide. The elderly population is especially vulnerable to severe health impacts, including aberrant cytokine up-regulation. However, underlying mechanisms remain poorly understood.
Methods:
We first re-analyzed public data on single-cell RNA sequencing to identify 19 differentially expressed genes (DEGs) whose expression changes depending on age and COVID-19 severity. Human lung and nasal epithelial cell lines (A549 and RPMI 2650) were treated with doxorubicin or irradiation to induce senescence and activated with poly(I:C), a TLR3 agonist to mimic viral infection. The expression of previously identified genes was examined by qPCR and ELISA. Additionally, the supernatant of A549 and RPMI 2650 was collected and incubated with human monocyte cell line THP-1 and human monocyte-derived macrophage (HMDM) to investigate the impact of cytokines, chemokines, and SASPs on human immune system.
Results:
Our results showed elevated expression of ACE2 in senescent and poly(I:C) treated A549 and RPMI 2650 in both mRNA and protein levels, which implies the susceptibility to COVID-19 infection may increase with age. We found no change in response to TLR4 agonist LPS treatment, suggesting that this effect is only limited to viral infection. Senescence induction and poly(I:C) treatment increased the expression of pro-inflammatory cytokines and chemokines including TNF-α, CXCL2, CXCL3, and CXCL8. Treatment of THP-1 and HMDM with supernatant from senescence-induced and poly(I:C)-treated A549 and RPMI 2650 cells resulted in elevated pro-inflammatory signature genes. These findings suggest a potential link between cellular senescence and the immune response in COVID-19 patients.
Conclusion:
Our study illustrates the senescence-associated changes in gene expression in the lung microenvironment may contribute to the higher severity of COVID-19 in the elderly population. These findings highlight the need for further investigation to mitigate disease risk in this vulnerable population.

P3-029 IFN responses induced by fractionated viral RNA replication products of influenza A virus, Zika virus and SARS-CoV-2

Jiang M[1], Laine L[1], Skön M[1], Julkunen I[2], Österlund P[1]
[1]Finnish Institute for Health and Welfare, [2]Institute of Biomedicine, University of Turku

Many viruses, especially RNA viruses generate different types of viral RNAs (vRNAs) in the host cells during the virus replication, including genomic vRNAs, aberrant small or long vRNAs and dsRNA intermediates. These foreign RNAs are recognized by different RNA receptors of the host (such as RLRs, TLRs and NLRs, etc.) and followed by innate immune responses. In our study, we used Field Flow Fractionation (FFF), µMACS technology or gel cutting methods to fractionate vRNAs from the host cells infected with three different types of RNA viruses: influenza A virus (IAV), Zika virus (ZikV) and SARS-CoV-2. We investigated the ability of different fractionated vRNAs to induce IFN responses. Small RNAs separated from IAV infected macrophages or SARS-CoV-2 infected Vero E6-TMPRSS2 cells induced moderate IFN responses after being transfected back into macrophages, while medium-sized RNAs, including IAV genomic vRNA segments or SARS-CoV-2 intermediate vRNAs, induced pronounced IFN responses. Similar results were also found with RNAs fractionated from ZikV infected Vero E6 cells. However, as expected, mRNA mimicking genomic +ssRNAs of either ZikV or SARS-CoV-2 failed to induce IFN responses in human macrophages or monocyte-derived dendritic cells. Further RNA sequencing will be applied to get more information on the structure and sequence of vRNAs from fractionated RNAs with highest IFN inductivity.

P3-030 Cytoplasmic ATM mediates manganese-dependent phosphorylation of TBK1 and enhances innate immune response

Sui H[1], Hao M[1], Chang W[1], Imamichi T[1]
[1]Frederick National Laboratory

Background
Ataxia-telangiectasia mutated (ATM) is a serine/threonine protein kinase and is best known for its role in the DNA damage response after double-strand breaks. We previously reported that manganese (Mn) significantly enhances DNA/RNA-mediated innate immune response through inducing the phosphorylation of TANK-binding kinase 1 (TBK1). However, a precise mechanism regarding how Mn phosphorylate TBK1 remains unknown.
Methods
Different cell lines (HEK293, 293T, HeLa, and A549), ATM KO A549, ATM KO 293T cells, and human primary cells (macrophages and CD4+ T cells) are used in this study. The cells are treated by MnCL2 at different concentrations, and the cell lysate is prepared for western blot to detect the expression of ATM and TBK1. DNA/RNA-mediated interferon induction is measured by real-time RT-PCR or ELISA assay. siRNA and ATM inhibitors are also used in this study to inhibit ATM activity. The cellular distribution of ATM and TBK1 are detected by Immunofluorescence microscopy.
Results
Mn dose-dependently (0~500 µM) induced phosphorylation of TBK1 with the presence of ATM and this Mn-TBK1 phosphorylation pathway directly contributed Mn-enhanced innate immune response. This correlation was demonstrated in all tested cell lines and human primary cells. The inhibition of ATM using a specific inhibitor AZD6738, or siRNA suppressed phosphorylation of TBK1 and Mn-enhanced interferon inductions. Additionally, the Mn-induced phosphorylation of TBK1 was completely abolished in ATM KO cells and Mn treatment was not able to enhance DNA-mediated innate immune response.
Conclusion
The current result hammered the essential role of ATM in Mn-induced phosphorylation of TBK1 signaling pathway, and ATM mediates Mn-enhanced innate immune response. Our ongoing study will further elucidate how ATM interacts with TBK1 and induces phosphorylation of TBK1. The findings from this study will highlight the regulatory role of cytoplasmic ATM in innate immune response and therefore offers potential new therapeutic options.

P3-031 Multi- “omic” analysis reveals dynamic relationships between the metabolome, cytokine profile, and gut barrier integrity in multiple COVID-19 cohorts

Guerrero C[1,2], Basting C[2], Murray K[1], Broedlow C[2], Velez A[2], Schroeder T[2], Langat R[2], Southern P[3], Yang O[4], Schifanella L[5,2], Klatt N[2]
[1]University of Minnesota, Center for Metabolomics and Proteomics, [2]University of Minnesota, Department of Surgery, Division of Surgical Outcomes and Precision Medicine Research, [3]University of Minnesota, Department of Microbiology and Immunology, [4]University of California, Los Angeles, Department of Medicine, [5]National Institutes of Health, National Cancer Institute, Center for Cancer Research, Vaccine Branch, Animal Models and Retroviral Vaccines Section

Background: COVID-19 increases production of proinflammatory cytokines which leads to inflammation and subsequent metabolic dysregulation in infected patients. Studies have suggested that upon infection with SARS-CoV-2, routine mechanistic pathways for metabolites such as tryptophan can be altered, leading to downstream cellular unrest. In addition, connections between perturbed metabolome and cytokine production and gut barrier integrity are not fully understood. Here we investigate the dynamic relationships between the metabolome, cytokine production, and gut barrier integrity from 3 COVID-19 infected patient cohorts from around the world, including outpatients, hospitalized patients, and healthy controls.

Methods: Plasma samples were analyzed using MILLIPLEX assays for cytokine quantification, single target ELISAs for gut barrier markers, and targeted LC-MS/MS using biocrates’ MxP500 kit for metabolomic profiling.

Results: Outpatients infected with COVID-19 that were closer to symptom onset had higher concentrations of glycine-conjugated bile acid, glycochenodeoxycholic acid (GCDCA) and TNF-α compared to patients who were further from symptom onset (p=0.004 and p=0.013 respectively). Additionally, the gut barrier integrity biomarker Zonulin negatively correlated with the ratio of triglycerides to fatty acids (Pearson correlation, p=0.01). Hospitalized patients had significantly higher concentrations of IL-6 and lipopolysaccharide binding protein compared to healthy controls (p=0.007 and p=0.003 respectively) and the pro-inflammatory cytokine IL-12 was negatively correlated with the short-chain fatty acid butyrate (Spearman correlation, p=0.014). We are currently performing metabolomics on a remaining cohort.

Summary: COVID-19 has been demonstrated to work in a cascade motif altering protein abundances of proinflammatory cytokines, as well as the metabolome. Integration of multi- “omics” applications for unveiling mechanistic relationships between the metabolome and cellular inflammation in COVID-19 cases will be integral for future precision treatment with patients with COVID-19. This study aims to highlight the dynamic relationships between cytokine production, metabolites, and gut barrier integrity in COVID-19 infection.

P3-032 Regulation of antibody responses by the transcription factor TFEB

Song H[1], Yu J[1], Kim J[1], Park S[1], Jung W[1], Lee J[1], Kim Y[1]
[1]Korea Advanced Institute Of Science And Technology

Transcription factor EB (TFEB) binds to the specific DNA sequence called the coordinated lysosomal expression and regulation (CLEAR) element and upregulates the expression of lysosomal and autophagy-associated genes. Recently, the immunoregulatory role of TFEB has been investigated in highly phagocytic cells such as macrophages and dendritic cells, whose cellular functions are critically regulated by the lysosomal activity and autophagy machinery. However, the role of TFEB in lymphocytes are not well understood.
In this study, we found that TFEB is highly expressed in B cells and the activation of B cell receptors (BCRs) induces rapid dephosphorylation and nuclear localization of TFEB in a Syk-dependent manner, suggesting that BCR signaling promotes the TFEB-mediated transcriptional regulation in B cells. Indeed, the transcriptome analysis revealed that a number of genes were differentially expressed in wild type (WT) and TFEB-deficient B cells, among which AhR was one of the most significantly downregulated gene in TFEB-deficient B cells. We showed that the BCR activation rapidly induces AhR expression in a TFEB-dependent manner and TFEB directly binds to the AhR promoter. Previous studies reported that AhR negatively regulates antibody responses. Accordingly, immune phenotyping of B cell-specific TFEB knock-out (TFEB△B) mice revealed significantly higher serum antibody titers and increased numbers of bone marrow-resident antibody secreting cells compared to WT mice both at steady state and after antigen immunization. In addition, increased fecal antibody titers were detected along with elevated germinal center responses in Peyer's patches. Moreover, TFEB-deficient B cells showed more efficient in vitro plasma cell differentiation compared to wild-type B cells. Because these phenotypes are opposite of what have been observed in autophagy-deficient mice, we believe that TFEB-mediated regulation of antibody response is independent of autophagy.
In conclusion, this study demonstrates that TFEB is a novel, key factor that regulates antibody responses in a B cell-intrinsic manner.

P3-033 IL-4-induced IgE isotype switching is enhanced by IRG1/itaconate in B cells

Park S[1], Han Y[1]
[1]Konyang University College of Medicine

The enzyme IRG1 is responsible for producing itaconate and has been recognized as a new immune metabolic regulator in inflammation and infection. However, its specific function in B cells is unknown. This study aims to investigate the impact of IRG1/itaconate on B cells by examining its expression and effects on Ig isotype switching and antibody production in mouse B cell cultures in vitro. We observed that itaconate enhances IL-4-induced IgE/IgG1 isotype switching and production, as well as the expression of the IL-4-induced circle transcript ε-γ1, a molecular marker for sequential switching to IgE via IgG1. On the other hand, IRG1 deficiency reduces IL-4-induced IgE/IgG1 isotype switching and production by B cells. Additionally, itaconate increases IL-4-induced STAT6 phosphorylation, a crucial transcription factor for IgE isotype switching, while IRG1 deficiency decreases STAT6 phosphorylation. These results suggest that IRG1/itaconate selectively enhances IL-4-induced IgE isotype switching through STAT6 phosphorylation, leading to elevated IgE production by B cells.

P3-034 Profiling of Immune Infiltrates in Relapsing-Remitting Experimental Autoimmune Encephalomyelitis Reveals Dynamic Changes in Myeloid Cells

Benitez A[1], Devlin J[1], Au Yeung E[1], Swiecki N[1], Adler C[1], Lim W[1], Atwal G[1], Sleeman M[1], Orengo J[1], Haxhinasto S[1]
[1]Regeneron Pharmaceuticals, Inc.

Background
Relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE) is characterized by a pattern of relapses and spontaneous recovery occurring over a period of weeks to months. This pattern is very similar to the clinical signs of disease observed in multiple sclerosis (MS) patients over many years. The immune response in MS is primarily driven by autoreactive T lymphocytes that recognize myelin peptides. However, the mechanisms involved in the induction of the disease and clinical relapses are not well understood.

Methods
SJL mice were immunized with PLP139-151/CFA, CFA, or naïve and disease scores were monitored. Single-cell suspensions of spinal cord infiltrates were collected and processed into RNA libraries using Chromium Single Cell 3' Library, Gel Beads and Multiplex Kit (10X Genomics). Paired-end sequencing was performed on Illumina NextSeq500. Sample demultiplexing, alignment, filtering, and UMI counting were performed on Cell Ranger Single-Cell Software Suite and analyzed using version 2 of the Seurat R package.

Results
This line of experimentation revealed drastic changes in immune cell composition over time with unique cellular compositions during the different disease phases (Figure 1). Further analysis uncovered marked changes in the number and the activation status of myeloid cells such as macrophages, dendritic cells, and monocytes and respective subsets during each disease phase. The infiltrating myeloid cells had distinct transcriptional signatures with macrophages expressing high levels of the inflammatory cytokine Il1β and the T-cell chemoattractant Cxcl16, which might define their role throughout the phases of the disease. In addition, a subset of microglia was found to express very high levels of interferon stimulated genes (ISGs) such as Ifit2, Ifit3, and Isg15 suggesting a role for interferon signaling during the disease.

Conclusion
Our comprehensive analysis of immune infiltrates identifies functional heterogeneity of key myeloid cell subsets that in concert with other cells could be contributing to disease pathogenesis.

P3-035 IL-15 promotes self-renewal of progenitor exhausted CD8 T cells during persistent antigenic stimulation

Lee K[1], Lee J[1], Im S[1]
[1]Sungkyunkwan University School of Medicine

Background: In chronic infections and cancer, TCF1+PD-1+ progenitor exhausted CD8 T cells (Tpex) can self-renew and give rise to Tim-3+PD-1+ terminally differentiated CD8 T cells that retain effector functions. Tpex cells are essential to maintaining a pool of antigen-specific CD8 T cells during persistent antigenic stimulation, and only they respond to PD-1-targeted therapy. Despite their potential as a crucial therapeutic target for immune interventions, the mechanisms controlling the maintenance of virus-specific Tpex cells remain to be determined.
Methods: IL-15R expression on CD8 T cells from chronic LCMV infection and human renal cell carcinoma (RCC) were analyzed by flow cytometry. Proliferation of virus-specific CD8 T cells from chronic viral infection was examined by CTV dilutions and in vivo BrdU incorporation. Proliferation of CD8 TILs from human RCC was assessed by Ki-67 expression. scRNA-seq were performed after IL-15 stimulation of chronic infected mice.
Results: Similar to memory CD8 T cells, Tpex cells underwent self-renewal in the lymphoid organs, prominently the bone marrow, during chronic LCMV infection. ex vivo treatment with IL-15 preferentially induced the proliferation of Tpex cells rather than the terminally differentiated subsets. The exogenous administration of IL-15 to chronically LCMV-infected mice also significantly increased self-renewal of Tpex cells in the spleen and bone marrow. The expansion of the Tpex subset of PD-1+ CD8 TILs from RCC patients upon ex vivo IL-15 treatment was significantly higher than that of the terminally differentiated subset. scRNA sequencing analysis of LCMV-specific exhausted CD8 T cells after ex vivo IL-15 treatment compared with those before treatment revealed upregulation of ribosome-related genes and downregulation of genes associated with the TCR signaling pathway and apoptosis in both Tpex and Ttex subsets.
Conclusion: These results show that IL-15 could promote self-renewal of Tpex cells, which has important therapeutic implications.

P3-036 Interferon-Induced Transmembrane Protein 3 (IFITM3) Provides an Innate Immune Barrier to Influenza Virus Interspecies Infection and Adaptation

Denz P[1], Speaks S[1], Kenney A[1], Eddy A[1], Bowman A[1], Yount J[1]
[1]The Ohio State University, Department of Microbial Infection & Immunity

Influenza virus pandemics involving zoonotic virus strains have occurred sporadically in recent history, but outbreaks of new viruses could be considered rare given the routine contact between humans and animal reservoirs of diverse influenza virus strains. Here, we examine roles of the host innate antiviral immunity protein Interferon-Induced Transmembrane Protein 3 (IFITM3) in interspecies virus infection and adaptation. We found that IFITM3 deficient human lung cells, macrophages, and fibroblasts, with or without interferon treatment, were universally infected at a higher rate by swine and avian influenza viruses compared to WT cells. Accordingly, we found that IFITM3 raises the minimum infectious dose threshold for achieving a productive infection with avian influenza viruses in vivo. To examine interspecies virus adaptation, we serially passaged human-isolated influenza viruses through WT or Ifitm3-/- mice. H3N2 and H1N1 viruses passaged 10 times in WT mice were minimally increased in their ability to replicate while viruses passaged in Ifitm3-/- mice showed significant adaptation with increased ability to replicate and induce inflammation in WT mouse lungs. Together, this work is the first to demonstrate the following fundamentally important new concepts: 1) innate immunity dependent on IFITM3 is responsible for preventing influenza virus lung infections when virus doses are below a minimal threshold, 2) IFITM3 deficiency allows extremely low dose influenza virus infections to occur, and 3) IFITM3 limits adaptation of influenza viruses to a new host species. Our findings provide proof-of-principle evidence that IFITM3 deficiencies known to exist in the human population represent a vulnerability for emergence of new pandemic viruses.

P3-037 Mechanism of type I interferon repression by the epigenetic reader SP140

Witt K[1], Abraham P[1], Lee O[1], Lee A[2], Dhaliwal H[2], Gaidt M[3], Vance R[4]
[1]Division of Immunology and Molecular Medicine, Department of Molecular and Cell Biology, University Of California, Berkeley, [2]Cancer Research Laboratory, University Of California, Berkeley, [3]Research Institute of Molecular Pathology, [4]Howard Hughes Medical Institute, University of California, Berkeley

Type I interferons (IFN-I) induce a potent antiviral response, but must be tightly regulated to prevent host pathology. Here we describe a novel mechanism by which the epigenetic reader SP140 represses Ifnb1 expression. SP140 is conserved among vertebrates, and is associated with susceptibility to bacterial infections and autoimmune disorders. However, the molecular function of SP140 is unclear. We found that SP140 represses Ifnb1 levels at late timepoints post-induction in macrophages. We profiled SP140-regulated genes with ATAC-seq, Cut&Run, and RNA-seq and found that SP140 does not bind the Ifnb1 locus, but instead binds and represses an uncharacterized gene that we name Raid (Regulator of Antiviral Immune Defense). Similar to Ifnb1, Raid was upregulated in cells lacking SP140. Moreover, CRISPR-Cas9 mediated disruption of Raid abrogated elevated Ifnb1 in Sp140–/– macrophages. We hypothesize that in the absence of SP140, RAID enhances Ifnb1 mRNA stability. Interestingly, SP140 localizes to nuclear complexes called nuclear bodies (NBs), and is homologous to NB proteins such as the anti-viral protein SP100 which represses viral gene expression in the nucleus. Viruses such as HSV-1 encode effector proteins (e.g., ICP0) to degrade SP100 and other NB proteins. We found that ICP0 also degrades SP140. However, there is no evidence that SP140 is itself antiviral. Instead, we propose that SP140 may act as a sensor that derepresses Raid and IFNB1 when it is degraded by viral effectors that attack nuclear bodies. Taken together, our work 1) establishes a major function of SP140, an understudied epigenetic reader, 2) identifies RAID as a novel regulator of IFN-I, and 3) suggests a novel strategy for IFN-I induction in response to viruses.

P3-038 A unique subpopulation of naïve CD4+ T cells exhibits distinct effector function in response to cytokine stimulation

Yoon J[1,2], Cho S[1,2], Cho M[1,2], Jeong H[1,3], Choi J[1,2,3]
[1]Department of Life Science, College of Natural Sciences, Hanyang University, [2]Research Institute for Natural Sciences, Hanyang University, [3]Hanyang Institute of Bioscience and Biotechnology, Hanyang University

Naïve CD4+ T cells are generally considered as a quiescent and homogenous population requiring cognate antigen stimulation to perform an effector function. However, recent studies have shown that naïve CD4+ T cells acquire a phenotypic heterogeneity while they receive the consecutive strength of self-antigen stimulation during the thymic development. Although the heterogeneity of naïve CD4+ T cells has been reported, distinct subpopulation functions remain unclear. In this study, we found 4 distinct subpopulations in the steady-state splenic naïve CD4+ T cells by scRNA-sequencing. We identified a unique subpopulation that still maintains a resting state but expresses relatively high levels of cytokine receptors and Ly6C. Moreover, we examined whether they have a sensitive response to Th1-related cytokines such as IL-18 with IL-12, demonstrating significantly stronger Th1/CTL-like function to metastatic melanoma cells in the lung. Furthermore, we found these populations are enriched in the peripheral mucosal tissues and could be generated by peripheral germs, especially in the lung based on label-transfer analysis of single cell/ATAC-seq data. Finally, we explored these populations in human naïve CD4+ T cells in patients with multiple sclerosis or COVID-19 revealing significant quantitative relevance with disease severity. Collectively, our findings demonstrate a novel subpopulation of naïve CD4+ T cells possibly generated by peripheral germs have much favor to performing sensitive Th1/CTL-effector function, which may explain efficient immune surveillance in the homeostatic condition.

P3-039 Effective Mucosal Adjuvantation in an Intranasal Enterovirus A71 Vaccine with Fungal Zymosan

Chin C[1], Lin Y[2], Cheng P[2], Chiang B[1,2,3]
[1]Graduate Institute of Immunology, College of Medicine, National Taiwan University, [2]Department of Medical Research, National Taiwan University Hospital, [3]Department of Pediatrics, National Taiwan University Hospital

Background
Human enterovirus A71 (EV-A71) has caused the hand, foot, and mouth disease (HFMD) and is recently associated with an increased prevalence of neurological symptoms, cardiopulmonary complications, and acute mortality. Due to the critical role of the airway in defending the initial invasion of EV-A71, intranasal vaccines have been suggested to provide local primed immunity to prevent its infection. However, the major challenge of developing protective intranasal vaccines is limited immune responses. To promote vaccine-specific responses, adjuvants could be adopted in the vaccine formula as additives, but safe and effective intranasal adjuvants are still lacking. Zymosan, a yeast cell wall component, has been identified to interact with dendritic cell-associated C-type lectin 1 (dectin-1) and toll-like receptor 2 (TLR2) to induce synergistic activation of downstream signaling pathways and to shape adaptive immune responses.

Methods
In this study, we aimed to investigate the effect of zymosan on the immune responses both in vitro and in vivo. We isolated bone marrow-derived dendritic cells and cultured with zymosan to analyze the surface molecules expression and cytokine profiles. In addition, we also used zymosan as the adjuvant for EV-A71 vaccine immunization.

Results
Herein, we confirmed the potential of zymosan in innate activation in vitro, and demonstrated the capacity of zymosan as an intranasal adjuvant in promoting immunogenicity after triple vaccination with inactivated EV-A71 in vivo. Furthermore, we validated the adjuvanticity of zymosan in improving the efficacy of the nasal vaccine in a neonatal mouse model of EV-A71 infection.

Conclusion
In conclusion, these results expatiate an ameliorative intranasal EV-A71 vaccine formulation with zymosan adjuvantation, providing a feasible strategy in preventing EV-A71 infection with severe complications and contributing to the development of nasal spray vaccination.

P3-040 Trogocytic molting of T cell microvilli upregulates T cell receptor surface expression and promotes clonal expansion

Park J[1], Kim J[1], Soh W[1], Kim N[1], Lee K[1], Kim C[1], Chung I[2], Lee S[1], Kim H[3], Jun C[1]
[1]Gwangju Institute Of Science And Technology, [2]Chonnam National University Medical School, [3]National Cancer Center

While T cell activation is known to involve the internalization of the T cell antigen receptor (TCR), there is a lack of understanding regarding the release of TCRs after T cell interaction with cognate antigen-presenting cells. In this study, we aimed to investigate the physiological implications of TCR release. Our findings show that T cell activation results in the shedding of many TCRs through T cell microvilli, which involves a combined process of trogocytosis and enzymatic vesiculation, leading to the loss of membrane TCRs and microvilli-associated proteins and lipids. Surprisingly, unlike TCR internalization, this event leads to the rapid upregulation of surface TCRs and significant metabolic reprogramming of cholesterol and fatty acid synthesis to support cell division and survival. These results suggest that the loss of external microvilli components, including TCRs, plays a crucial role in clonal expansion, which contradicts the traditional view emphasizing the importance of TCR internalization.

P3-041 SARS-CoV-2 ORF6 protein targets TRIM-25 mediated ubiquitination of RIG-I to mitigate Type I Interferon Signalling

Khatun O[1], Sharma M[1], Narayan R[1], Tripathi S[1]
[1]Indian Institute Of Science

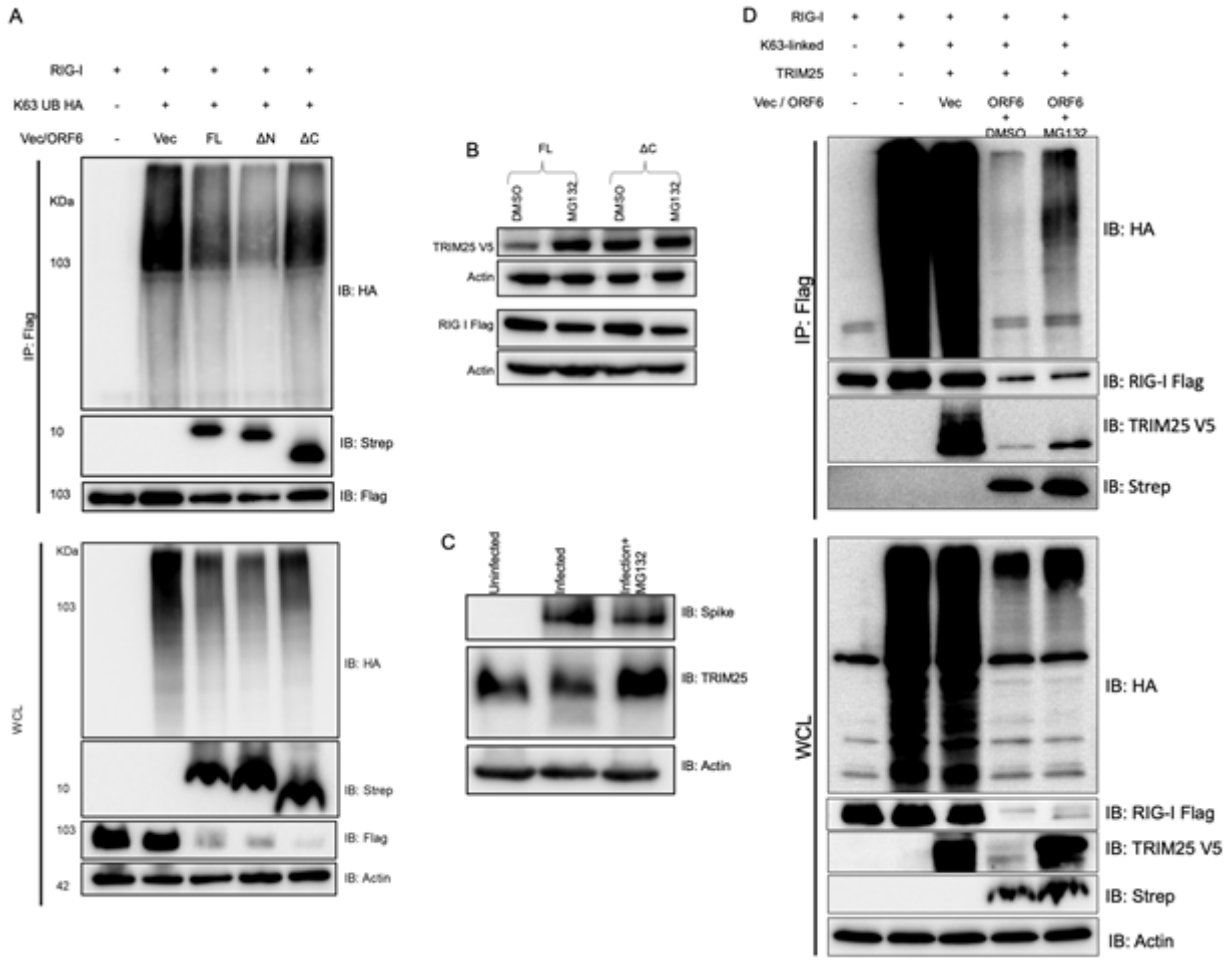
Background
SARS-CoV-2 ORF6, a small protein consisting of 61 amino acids, inhibits the interferon (IFN) induction and signalling. Its primary mode of action has been described as inhibition of the nuclear import of transcription factors like IRFs, and STATs. These factors are downstream of viral detection by RIG-I. The action of ORF6 on the upstream event of viral detection by RIG-I has not been described and the molecular basis of the same was unclear.

Methods

We screened the Type I IFN-antagonistic ability of SARS-CoV-2 proteins using IFN beta promoter-driven dual-luciferase assay which revealed ORF6 as one of the most potent inhibitors of both IFN induction and signalling. The IFN-antagonistic activity of ORF6 was mapped through domain deletion and mutagenesis studies. Next, immunoprecipitation was performed to examine the interaction of ORF6 with key mediators of the IFN pathway. Furthermore, the impact of ORF6 on RIG I ubiquitination through TRIM-25 was examined.

Results
ORF6 directly interacts with RIG I and blocks its ubiquitination. More specifically, ORF6 restricts K63-linked ubiquitination of RIG I, which regulates its activation and stability. It does so by targeting TRIM25 E3 ligase for proteasomal degradation and the C-terminal cytoplasmic tail of ORF6 is crucial for this activity.

Conclusion
SARS-CoV-2 ORF6 inhibits K63-linked ubiquitination of RIG I by E3 ligase TRIM25, which leads retards the downstream signalling leading to type I IFN induction. This activity is mapped to the C-terminal cytoplasmic domain of ORF6.


A
RIG-I + + + + +
K63 UB HA - + + + +
Vec/ORF6 - Vec FL ΔN ΔC
KDa
103
IP: Flag
IB: HA
10
IB: Strep
103
IB: Flag
WCL
KDa
103
IB: HA
10
IB: Strep
103
IB: Flag
42
IB: Actin
B
FL
ΔC
DMSO
MG132
DMSO
MG132
TRIM25 V5
Actin
RIG I Flag
Actin
C
Uninfected
Infected
Infection+ MG132
IB: Spike
IB: TRIM25
IB: Actin
D
RIG-I + + + + +
K63-linked - + + + +
TRIM25 - - + + +
Vec / ORF6 - - Vec ORF6 + DMSO ORF6 + MG132
IP: Flag
IB: HA
IB: RIG-I Flag
IB: TRIM25 V5
IB: Strep
WCL
IB: HA
IB: RIG-I Flag
IB: TRIM25 V5
IB: Strep
IB: Actin

P3-042 Using a Fibroblast Zoo to Interrogate Intrinsic Barriers in Cross-Species Viral Replication

Mickelson C[1], Neal T[1], Matchett W[1], Pross L[1], Langlois R[1]
[1]University Of Minnesota

The global virome is immense, and understanding how viruses have the potential to drive novel pandemics is largely unknown. Climate Change and its effects continue to force the migration of species into new habitats, and it's known that the frequency of novel cross-species interactions is rising. With each interspecies exchange, endemic pathogens will encounter increasing opportunities to extend their host range. Using retrospective epidemiological and ecological data, some studies have attempted to predict viruses that are likely to cross species barriers; however, these experiments are subject to sampling biases and can only capture events that are successful. Moreover, most studies addressing viral spillover have been highly anthropocentric and fail to address interspecies transmission among multiple animal species, which is a key part of viral emergence. To overcome these hurdles and capture the full breadth of cross-species replication potential, we built a library of primary mammalian dermal fibroblasts from over 40 species across 14 orders. Using these primary cultures, we assessed the replicative success of both live positive sense RNA viruses and their in vitro transcribed genomes. One of the major barriers these viruses will face is the innate antiviral immune system. To specifically address this, we assessed viral replication in fibroblasts primed with Interferon-α (IFNα) or suppressed through Janus kinase pathway (JAK) inhibition. Through fold expression comparisons, we will be able to scalably interrogate the role of antiviral sensitivity and replication competency that serve as the barriers to multiple viruses across the biodiversity of Mammalia. By incorporating host and virus features into computational models that utilize these data, we can uncover virus-host interactions needed for successful spillover. These findings will expand our understanding of how viruses overcome intrinsic barriers that typically limit virus range.

P3-043 Lysophosphatidylcholine modulates the inflammatory immune cell response to favor Leishmania major infection in vivo

Tounsi N[1], Djerdjouri B[1]
[1]Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene

Background
Leishmaniasis is a disease caused by flagellated protozoans of the genus Leishmania. The obligate intracellular parasite of mononuclear phagocytes is transmitted to humans through the bite of infected sandflies. Lysophosphatidylcoline (LPC), an important lipid mediator in inflammatory diseases, can play both pro-inflammatory and anti-inflammatory roles. The aim of this study was to investigate the immunomodulatory effects of LPC on the immune response to Leishmania.
Methods
The air pouch provides a localized environment for the study of inflammation and cellular response. It is formed by the subcutaneous injection of sterile air into the back of a mouse. Metacyclic promastigotes of Leishmania major collected at stationary phase were used for BALB/c mice infection. The parasite burden expressed as the number of intracellular viable parasites was obtained by a dilution limiting assay. The inflammatory response was assessed by IL-6, TNF-α and nitric oxide (NO) levels and arginase 1 activity.
Results
The results showed that LPC stimulation failed to further enhance IL-6, TNF-α and NO levels compared to the infected group. In addition, LPC favored Leishmania proliferation (45%, p 0.01) by sustaining arginase 1 activity at the expense of nitrite levels (123% vs. 114%). We conclude that LPC modulates the early immune cell response and boosts Leishmania infection through increased polyamine production, limited NO and pro-inflammatory cytokine production.
Conclusion
Further studies are needed to explore the mechanisms underlying the LPC effects on Leishmania infection and inflammatory cell response.

P3-044 Distinct type I interferon subtypes differentially stimulate T cell responses in HIV-1-infected individuals

Karaköse Z[1,2], Schwerdtfeger M[1], Karsten C[2], Esser S[2,3], Dittmer U[1,2], Sutter K[1,2]
[1]Institute for Virology, [2]Institute for Translational HIV Research , [3]Department of Dermatology and Venerology

Type I interferons (IFNs) are induced as one of the immediate host responses during most viral infections. The type I IFN family consists of one gene encoding for IFNβ, IFNε, IFNτ, IFNκ, IFNω, IFNδ, and IFNζ but numerous highly conserved genes encoding for IFNα. Although these IFNα subtypes were initially believed to act interchangeably, their discrete biological properties are nowadays widely accepted. These biological properties cover a wide range of distinct subtype-specific antiviral, immunomodulatory, and anti-proliferative activities which were explained by differences in receptor affinity, downstream signaling events, and individual IFN-stimulated gene (ISG) expression patterns. Type I IFNs and increased IFN signatures potentially linked to hyperimmune activation of T cells are critically discussed for chronic HIV (human immunodeficiency virus) infection. Here, we aimed to analyze the broad immunological effects of IFNα2, IFNα14, and IFNβ on T and NK cell subsets during HIV-1 infection in vitro and ex vivo by flow cytometry and multiplex bead arrays. We could show that the stimulation with IFNα14 and IFNβ but not IFNα2 significantly increased frequencies of degranulating (CD107a+) gut-derived CD4+ T cells and blood-derived T and NK cells. However, frequencies of IFNγ-expressing T cells were strongly reduced after stimulation with IFNα14 and IFNβ. Furthermore, phosphorylation of downstream molecules was not only IFN subtype-specific; also, significant differences in STAT5 phosphorylation were exclusively observed in both healthy PBMCs and PBMCs of people living with HIV (PLWH), but not in healthy LPMCs assuming cell and tissue specific discrepancies. In conclusion, we observed distinct IFN subtype-specific potencies in stimulating T and NK cell responses during HIV-1 infection and gained new insight in downstream activities upon IFN subtype-specific stimulation which might be considered in immunotherapy against HIV-1 infection.

P3-045 Modulation of Friend Virus-specific CD4+ T cell responses by different IFNα subtypes

Schwerdtfeger M[1], Adamczyk A[2], Lang K[3], Guo K[4], Santiago M[4], Westendorf A[2], Dittmer U[1], Sutter K[1]
[1]Institute for Virology, [2]Institute for Medical Microbiology, [3]Institute of Immunology, [4]University of Colorado School of Medicine

As key mediators of the innate and adaptive immunity, interferon (IFN) α subtypes harbor crucial functions for the defense against viral infections by modulating a variety of cellular responses like proliferation, and cytokine expression. In the past different immunomodulatory activities for IFNα subtypes have been observed in various viral infections. However, the complex system of type I IFN responses and their regulation is still not sufficiently investigated.
To investigate the impact of IFNα subtypes on CD4+ T cell proliferation, differentiation, and effector function during a retroviral infection, the well-established Friend retrovirus (FV) mouse model was used.

For this purpose, an in vitro FV-specific proliferation assay was established. Murine bone marrow-derived DCs were loaded with FV-specific peptides and co-cultivated with FV-specific naive CD4+ T cells in the presence and absence of different IFNα subtypes. The supernatants and cells were collected three days post stimulation for RNA isolation, flow cytometric analysis, and ELISA. In addition, IFNAR1-/- DCs were used to investigate direct or indirect effects of the IFNα subtypes on FV-specific CD4+ T cells.

Using the established FV-specific proliferation assay, the impact of the different IFNα subtypes on the proliferation of CD4+ T cells could be investigated. IFNα2 and IFNα5 showed the most prominent antiproliferative effect. Interestingly, after stimulation with these two IFNα subtypes an increased expression of TH17-related cytokines was detected. Contrary, the subtypes with less potent antiproliferative effects showed significantly higher expression of TH1-related cytokines. Interestingly, the IFN-mediated effects on CD4+ T cell proliferation were indirect, whereas the cytokine expression of FV-specific CD4+ T cells was directly affected by IFNα subtypes.

We observed opposing effects of IFNα subtypes on naive CD4+ T cells. Our results might help for a better understanding of the role of type I IFNs in virus-specific CD4+ T cell differentiation, activation, as well as effector functions.

P3-046 Reprogramming inflammatory myeloid cells by SIK2 inhibition

McMillan L[1], Fenton-May A[1], Kelly M[1], Strickland M[1], Richmond N[1], Bennett N[1], Makrecka-Kuka M[1], Barbeta G[2], Felwick R[3], Sanchez-Elsner T[2], Cummings F[3], Madden J[1], Kilty I[1]
[1]Sitryx Therapeutics, [2]Faculty of Medicine, University of Southampton, [3]University Hospital Southampton NHS FT

Salt-Inducible Kinases (SIKs) are a family of 3 Serine/Threonine kinases and a subset of the AMPK-related kinase family. Their catalytic activity is highly regulated in response to changes in cellular homeostasis, including inflammation. SIKs are positively phosphoregulated by the metabolic sensor LKB1 and upon activation drive changes in downstream gene expression through their key substrates class IIA HDACs and CRTCs. During inflammation, SIK activation results in the upregulation of proinflammatory cytokines such as TNFα to drive an inflammatory response. Conversely, negative phosphoregulation by PKA inactivates SIKs resulting in proinflammatory cytokine downregulation and the concomitant upregulation of anti-inflammatory IL-10. Inhibition of SIK activity thus shows potential in the resolution of inflammation and presents a promising therapeutic target.
Selective pharmacological inhibition of SIKs has thus far proved challenging - a number of pan-SIK inhibitors have been developed but typically show poor kinome-wide selectivity resulting in non-desirable side effects, underlining the need for improved selectivity.
Of the three isoforms, SIK2 is the most active isozyme in bone marrow-derived macrophages and is thought to be the key driver of macrophage IL-10 production through its substrate CRTC3. Using selective small molecules, we show that SIK2 inhibition modulates inflammatory and immunometabolic pathways in human myeloid cells including monocytes and monocyte-derived macrophages. Furthermore, SIK2 inhibition drives a tolerogenic phenotype in dendritic cells, as demonstrated by suppression of a broad range of proinflammatory cytokines as well as downregulation of activation markers; functionally these modulated DCs can suppress Th1 CD4 T cell IFNγ production. Importantly, cytokine modulation by SIK2 inhibition translates across to treated ex vivo biopsies from ulcerative colitis patients. Together, we demonstrate that SIK2 inhibition reprograms myeloid cells within an inflammatory environment to drive them into a pro-resolution state, supporting the robust therapeutic potential of SIK2 inhibition in the treatment of chronic inflammatory conditions.

P3-047 Dynamic regulation of Caspase-5, a functional protein in the inflammatory response in the in vitro co-culture pneumonia model.

Jeznach A[1], Sidor K[1], Hoser G[1], Skirecki T[1]
[1]Centre Of Postgraduate Medical Education

Acinetobacter baumannii is one of the major etiologic factors of ventilator-associated pneumonia in Europe and relatively little is known about the local immune response to this pathogen. In order to gain insight into the mechanisms of the regulation of early inflammatory response to A. baumannii, particularly activation of the inflammasome during lung infection, we used in vitro human cell co-culture system. To model the intercellular interactions during lung infection, primary monocytes isolated from PBMCs of healthy donors were differentiated into macrophages and were co-cultured together with air-liquid interphase differentiated human lung epithelial cells (HBEC-3KT). Then, cells were treated with LPS, live bacteria or isolated outer membrane vesicles (OMVs). Four hours after stimulation with bacterial components (LPS, OMVs) or live bacteria, the level of Capase-5 transcripts in the co-culture of macrophages together with the HBEC-3KT cells increased. While, in cultured macrophages, Caspase-5 increased only after stimulation with live bacteria. Interestingly, in the epithelial cells only, the expression of pro-inflammatory genes (NLRP3, IL-18, IL-1β, Casp-4, Casp-1) increased significantly, while Caspase-5 did not increase. We determined the levels of pro-inflammatory cytokines in supernatants after stimulation. Finally, to confirm the involvement of the non-canonical inflammasome in the inflammatory response to multidrug-resistant bacteria we silenced the expression of the Caspase-5 gene using the siRNA method. Taken together, our in vitro model of pneumonia showed requirement of macrophage-epithelial interaction in the activation of the non-canonical inflammasome in response to A. baumannii lung infection.

P3-048 Generation and Function of the soluble IL-2Rα/CD25

Lokau J[1], Dahl S[1], Khalilova J[1], Ogunsulire I[2], Rose-John S[2], Scheffold A[2], Garbers C[1]
[1]Otto-von-Guericke University Magdeburg, [2]Kiel University

Interleukin-2 (IL-2) is one of the main regulators of immune responses through modulating a variety of immune cell functions, most importantly the homeostasis of regulatory T-cells. Signaling of IL-2 is mediated via a receptor complex consisting of IL-2Rα (CD25), IL-2Rβ and IL-2Rγ. Of those, IL-2Rβ and IL-2Rγ are mandatory for signal transduction whereas the IL-2Rα is not directly involved. Instead, IL-2Rα expression increases the affinity of the receptor complex, and thus the whole cell, for IL-2. High IL-2Rα expression is found primarily on regulatory T-cells and, but also transiently on other T-cell types upon activation of the T-cell receptor. In addition to the membrane-bound form, there is also a soluble (s)IL-2Rα that is reported to be mainly produced by activated T-cells. Of note, blood sIL-2R levels are elevated in a variety of diseases and discussed as a clinical marker. However, the exact mechanism(s) of sIL-2Rα generation and function of the soluble receptor remain elusive.
Here, we analyzed the contributions of different proteases to constitutive and induced production of sIL-2Rα and identified the related metalloproteases ADAM10 and ADAM17 as the most likely sheddases of the IL-2Rα. We confirmed that ADAM17 is mainly involved in induced IL-2Rα shedding from primary T-cells upon activation of the T-cell receptor. In contrast, ADAM10 is responsible for constitutive IL-2Rα shedding in vitro as well as in vivo. Furthermore, we found that the sIL-2Rα acts as an antagonist of IL-2 signaling in T-cells. Notably, our data showed that the antagonistic properties of the sIL-2Rα are influenced by expression of the membrane-bound IL-2Rα.
In summary, we identify ADAM10 and ADAM17 as sheddases of the IL-2Rα and show that constitutive and induced generation of sIL-2Rα are executed by different proteases. Further, we unravel differential functions of the sIL-2Rα. These results reveal new opportunities to modulate IL-2 function.

P3-049 Comparison Between the Human and Avian IFN-Induced Immune Response to Influenza

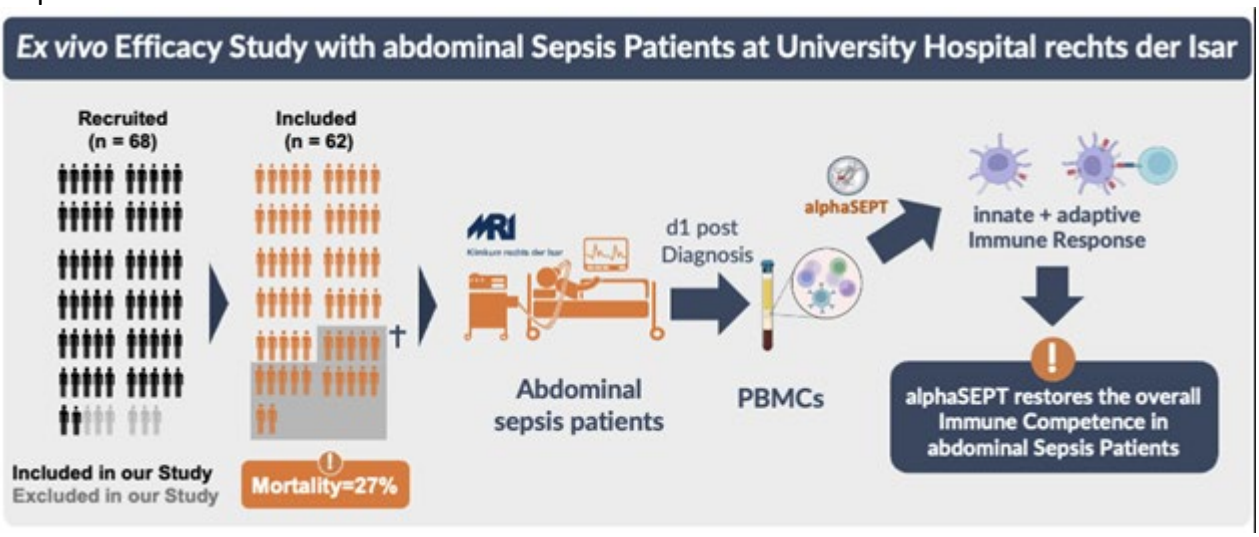
Barkhymer A[1], Becker J[1], Langlois R[1]
[1]University Of Minnesota

Influenza is a virus capable infecting and transmitting between a wide variety of species. Waterfowl, such as ducks, make up influenza's natural reservoir. Transmission between wild birds and domestic poultry, as well as zoonotic transmission from birds to humans, are global threats to public health. Such spillover events can cause large disruptions in the agricultural industry and severe morbidity and mortality in humans infected with avian influenza. Despite these occurrences, influenza transmission between birds and humans is rare. This is due to a number of obstacles the virus must overcome in order to establish an infection in a human host. One of these is the cell's intrinsic innate immunity. One important aspect of this is Type I interferons (IFN). IFN is an integral part of the early innate immune response to viruses and protects cells by inducing the expression of IFN-stimulated genes (ISGs). ISGs can restrict viruses in a wide variety of ways and are subject to high rates of positive selection. As such, orthologs of the same gene may have different functions in different species. We generated a list of duck and chicken ISGs and determined the sequence divergence between humans, ducks, and chickens. We found that a significant proportion of chicken and duck ISGs shared less than 50% nucleotide identity with the human orthologs. We therefore hypothesize that ISGs that are divergent between birds and humans prevent the cross-species transmission of influenza. We are currently screening divergent ISGs for antiviral function in chickens, ducks, and humans.

P3-050 Preclinical Validation of alphaSEPT, an engineered human Cytokine, as a Next-Generation Immunotherapy

Laschinger M[1], Held J[2], Holzmann B[1], Feige M[2], Behnke J[1]
[1]Klinikum Rechts der Isar | Technical University of Munich, [2]Technical University of Munich | Department of Chemistry

Sepsis is an indication with an enormous unmet medical need. In Western societies more people die of sepsis than of most common cancers combined. No treatment is available yet. To address this, we develop alphaSEPT. alphaSEPT is a rationally engineered human immune signalling molecule. It has immunomodulatory functions that are key in indications caused by an immune system out of balance. Sepsis is such an indication: the immune dysbalance in sepsis involves both, overreaction to an infection and extensive functional defects leading to immune paralysis. Patients die either of multiorgan dysfunction due to hyperinflammation or of secondary infections due to immune paralysis. The immunobalancing functions of alphaSEPT provide a new mode of action (MoA) that addresses both mortality drivers. Studies with the mouse protein in mice demonstrated a reduction of sepsis mortality of up to 70%. Our studies with abdominal sepsis patients at the Klinikum rechts der Isar, the hospital of the Technical University of Munich, demonstrated that alphaSEPT can precisely restore the immune competence of immune cells isolated from sepsis patients. Importantly, alphaSEPT modulated the immune responses that are key for survival of sepsis patients. With this novel MoA alphaSEPT achieves the potential of precision medicine via active immunomodulation. We now aim to perform indication extension studies and to bring alphaSEPT as a next generation biopharmaceutical into the clinics. Further, we have developed a platform biotechnology to overcome developability bottlenecks during development and production of next-generation biopharmaceuticals in a non-standard format.



P3-051 Pro- and anti-viral responses of cytomegalovirus-exposed human monocyte-derived dendritic cells

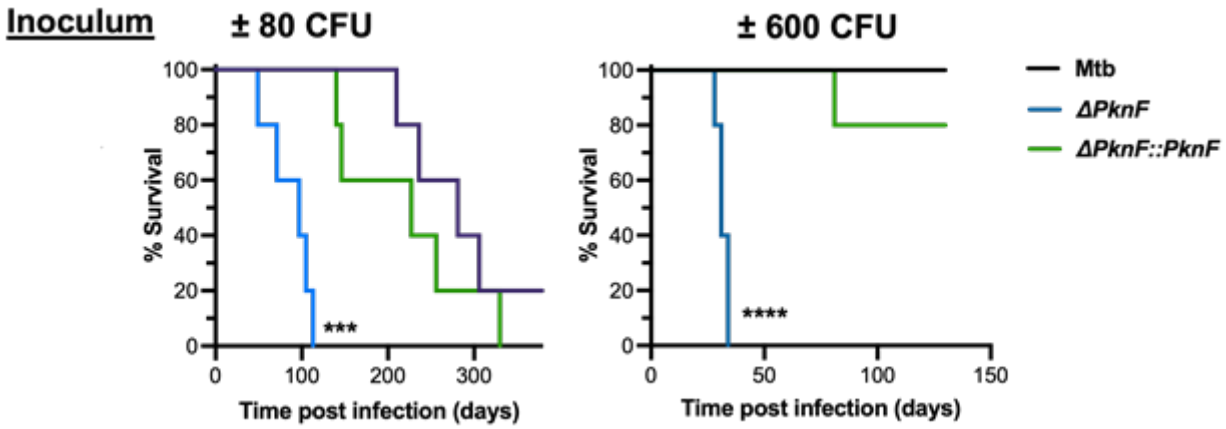
Costa B[1], Becker J[1], Mulenge F[1], Pavlou A[1], Gern O[1], Cicin-Sain L[2], Erhard F[3], Kalinke U[1]
[1]TWINCORE, [2]HZI, [3]University of Würzburg

Human cytomegalovirus (HCMV) is a widespread human pathogen with a 40–99% seroprevalence among the global population. Although infection of healthy individuals typically is sub-clinical, it's considered a life-threatening disease in immunocompromised hosts. During viral infections, monocytes are recruited to sites of inflammation where they differentiate into macrophages and/or dendritic cells (DC) to further coordinate anti-viral immunity. Previous studies reported that upon HCMV exposure, only 2-30% of the monocyte-derived DC (moDC) become productively infected. Here, we found that most HCMV-exposed moDC got infected, whereas only a fraction initiated immediate early (IE) gene expression. Upon HCMV entry, Cyclic GMP-AMP synthase/ Stimulator of interferon genes (cGAS/STING) sensing induces IFN-β and ISG responses. In some cells, these responses were sufficient to block the continuity of the viral life cycle resulting in latent and/or abortive infection. However, in certain cells, HCMV exploited STING-dependent NF-κB activation to exacerbate IE gene expression which culminated in productive infection and IFN-β and ISG inhibition. Interestingly, this inhibition didn't apply to IFN-λ1. Additionally, several candidates with strong pro- and anti-viral profiles were identified. Analysis of nascent RNA indicated that upon HCMV exposure, ISGs were initially induced in productively infected cells. However, HCMV-encoded viral modulators efficiently shut off ISG expression. ISG shut-off correlated with the expression of virion-associated transcripts and with STAT2-induced proteasomal degradation.
Thus, this work showed that although most moDC are infected with HCMV the mechanisms regulating IE gene expression are deeper than previously anticipated. We identified a non-canonical role of STING that exacerbates virus gene expression as well as potential pro- and anti-viral factors that support or suppress productive infection. Finally, we could also show that early in infection ISG induction is solely conferred by bystander cells.

P3-052 The serine/threonine protein kinase PknF of Mycobacterium tuberculosis represses bacterial virulence and host cell NLRP3-inflammasome activation in mice via unrelated pathways

Rastogi S[1], Torres-Juarez F[2], Baker P[2], Mayer-Barber K[2], Briken V[1]
[1]University of Maryland, [2]National Institute of Allergy and Infectious Diseases

Mycobacterium tuberculosis (Mtb) has evolved strategies to evade host innate immunity and persist inside host cells. We previously demonstrated that in vitro Mtb inhibits NLRP3 inflammasome activation and subsequent IL-1β production in macrophages and dendritic cells via the serine/threonine kinase PknF, as the pknF deletion mutant (MtbΔPknF) induces higher IL-1β production and enhanced pyroptosis compared to Mtb-infected cells. However, the role of PknF during in vivo infection has not yet been established. Here, we examined how pknF deletion affects the pathogenicity of Mtb in wild-type (WT) and nlpr3-/- C57BL/6 mice. We show that MtbΔPknF infection induces more IL-1 in the lungs of WT but not nlpr3-/- mice when compared to Mtb-infected mice. Nevertheless, in WT and nlpr3-/- mice the infection with MtbΔPknF results in higher bacterial loads in the lungs, bronchoalveolar lavage fluid, and spleens on days 28 and 120 post-infection compared to Mtb-infected mice, suggesting that PknF can repress bacterial virulence in vivo via mechanisms independent of Nlrp3 inflammasome regulation. Histopathological analysis of mouse lungs on days 28 and 120 post-infection shows an increased accumulation of immune cells, in particular foamy macrophages, in MtbΔPknF-infected mice compared to Mtb-infected mice which correlates with an increase in cell death. Finally, we investigated the virulence of Mtb compared to MtbΔPknF in a susceptible mouse model using sst1-/- mice. Survival studies revealed that the MtbΔPknF strain kills the mice more rapidly than the Mtb strain. In conclusion, our results using two different mouse models show that PknF limits the virulence of Mtb during in vivo infection via NLRP3 inflammasome independent mechanisms.



P3-053 Protective effects of Heat-killed Lactobacillus sakei CVL-001 on DSS-induced colitis

Kim D[1], Lee T[1], Jung D[1], Kim Y[1], Lee Y[1], Seo I[1], Kim W[1], Cho Y[1], Song E[2], Jang A[2], Park J[1,2]
[1]Chonnam National University, [2]NODCURE, Inc

Background
Inflammatory bowel disease (IBD) is a type of gastrointestinal immune disease caused by an excessive immune response that occurred in gastrointestinal tract. The exact cause of IBD is not yet fully understood, but it is believed to involve a combination of genetic, environmental, and immune system factors. Recent studies have demonstrated that lactic acid bacteria, which known as beneficial gut microbiota, can provide protection against IBD through a variety of mechanisms.

Methods
We isolated CVL-001 from Korean traditional food, baechu kimchi. CVL-001 was heat killed (HK) at 100 ℃ for 30 minutes and administered at 109 CFU/mouse during the experiment period. IBD animal model was established by treating 2 % DSS (w/v) ad libitum. Immunologic analysis was performed using colonic tissue.

Results
Administration of HK CVL-001 exhibited a protective effect against DSS colitis, but not in supernatants. Colonic tissue exhibited decreased mRNA expression level of pro-inflammatory cytokine, however, those of anti-inflammatory cytokine was increased. In flow cytometry analysis, regulatory t cell population was increased compared to PBS-treated group. Nucleotide-oligomerization domain 2 (NOD2) is deeply related to development and pathogenesis of IBD. We revealed that HK CVL-001 induced a NOD2-dependent regulatory phenotype in mouse bone marrow derived dendritic cells (BMDCs) by upregulated-expression of CD103. BMDCs-treated CVL-001 favored the expansion of regulatory T cell. In vivo studies, NOD2-/- mice did not showed protective effect against DSS colitis. However, DC transfer mice by intact BMDC injection on NOD2-/- mice exhibited a protective effect against DSS-induced colitis. Also, colon administered with HK CVL-001 favored the expansion of mucosal CD103 DCs.

Conclusion
Administration of HK CVL-001 contribute to protect mice against DSS-induced colitis. HK CVL-001 induced tolerogenic DCs through NOD2-mediated pathway and promotes the differentiation of regulatory T cell. This result indicated that HK CVL-001 might be used therapeutic agent and strategy in IBD.

P3-054 Maturation of cerebral organoids dictates permissiveness to Enterovirus-D68 infection and subsequent IFIT1 expression

Vazquez C[1], Han B[1], Negatu S[1], Bannerman C[1], Bongiovanni A[1], Lanoce E[1], Ming G[1], Jurado K[1]
[1]University Of Pennsylvania

The central nervous system (CNS) has classically been considered “immune-privileged”. However, emerging viruses with the capacity to enter the CNS have challenged what we consider immune privilege. Specifically, neuropathogenic Enterovirus-D68 (EV-D68) strains have been associated with a polio-like paralysis called acute flaccid myelitis (AFM) in some children. AFM remains a rare infection outcome, suggesting that host immune responses may limit neurological disease. Studies of such immune responses remain poorly understood. Human stem cell-derived cerebral organoids have emerged as three-dimensional in vitro systems, displaying the heterogeneity of cell types present in our brains. Thus, to better dissect differences between non-neuropathogenic and neuropathogenic EV-D68 strains, I generated and infected early and late cerebral organoids, analyzed viral titer using plaque assays, and visualized viral and immune proteins using immunofluorescence microscopy. I found that cerebral organoids were more permissive to neuropathogenic EV-D68 infection compared to non-neuropathogenic EV-D68 in early organoids, mimicking disease as children are more likely to develop AFM and viral encephalitis in general. Surprisingly, non-neuropathogenic EV-D68 replicated to the level of neuropathogenic EV-D68 in late organoids. Neural rosettes, accumulated hubs of neural progenitors, are highly expressed in early organoids and decrease as organoids age. Interestingly, these neural rosettes were not infected with EV-D68, suggesting that they may be immune signaling hubs, limiting virus infection. Further, I found that during non-neuropathogenic EV-D68 infection in late organoids, IFIT1 was produced in bystander cells surrounding infected cells, but activated as a consequence of neuropathogenic EV-D68 infection. These data suggest that tight control of immune responses may limit neuropathogenesis and also highlight the utility of using cerebral organoids to examine host immune responses to neurotropic virus infection. Future studies will implement single-cell RNA sequencing of EV-D68-infected organoids to define immune signatures of neuropathogenic infection.

P3-055 The role of cell-to-cell communication in modulating combinatorial TLR responses

Salvador Rocha E[1], Miller-Jensen K[1]
[1]Yale University

Simultaneous engagement of the MyD88- and TRIF-dependent signaling pathways results in synergistic inflammatory responses in macrophages. However, the role of cell-to-cell communication upon activation of multiple Toll-like Receptor (TLR) signaling pathways remains poorly understood. To explore the role of cell-to-cell communication, we cultured bone-marrow derived macrophages (BMDMs) and challenged them with single or dual stimulations of Pam3CSK4 (TLR2 agonist) and poly-IC (TLR3 agonist) at varied time points while blocking certain key intracellular and paracrine signaling pathways (i.e., TBK1, IFNAR, TNFR). In addition, we tested if paracrine communication was sufficient to establish synergistic responses by co-culturing TLR2 KO and TLR3 KO BMDMs and stimulating them with the single or dual ligands. We assayed the transcriptional response of specific cytokines (e.g., TNF, IFNb, IL-6, CXCL10) through RT-qPCR. We also assayed the secretion response with a multiplexed bead-based immunoassay of 32 cytokines/chemokines (C/C) and by ELISA. Our results show that at early timepoints TLR-dependent activation of key proinflammatory C/Cs (i.e., TNF and IL-6) exhibits synergy at the level of both transcription and secretion in a TNFR- and IFNAR-independent manner. Additionally, the primary response gene TNF remains synergistic despite blocks to protein synthesis or secretion but did not maintain synergy in the TLR KO co-culture suggesting that individual cells require both TLR signaling pathways for synergy a process that is independent of paracrine communication. In contrast, secondary response genes such as IL-6 and interferon-dependent genes (i.e., IFITM3 and MX1) show modulation of their synergy in a paracrine manner. The TBK1 signaling pathways is central for IL-6 synergy but not for TNF suggesting that seemingly similar synergistic effects are mediated by different intracellular pathways. Future experiments will determine the global transcriptional response and cell-to-cell heterogeneity. We expect these studies to provide insights on the role of cellular communication in establishing the early proinflammatory immune response.

P3-056 Inflammatory profile induction of BMDM after co-/superinfection of Influenza virus and S. pneumoniae.

Arranz Herrero J[1,2,3], Rius S[2,3], Miranda-Bedate A[4], Pinelli E[4], Tur-Planells V[2,3], Inchausti I[2,3], Fernandez P[3], Ochando J[1,5], Nistal-Villan E[2,3]
[1]Instituto de Salud Carlos III, [2]Fundacion Universitaria San Pablo Ceu, [3]Institute of Applied Molecular Medicine (IMMA), [4]National Institute for Public Health and the Environment, [5]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai

Influenza virus causes thousands of respiratory deaths yearly. Most infections by Influenza A virus (IAV) generally cause mild or moderate symptoms in healthy adults. However, approximately a fifth of them are co-diagnosed with bacterial coinfections, which frequently worsen their prognosis. Among them, Streptococcus pneumoniae (Spn) is the most common pathogen in human respiratory coinfections. Despite the many described mechanisms used by Influenza viruses to neutralize the immune response of the host, specific antagonistic activities in macrophages require a more detailed description. To better understand the cooperation between Influenza virus and Spn, we performed transcriptomic analysis (RNA-Seq) of GM-CSF differentiated bone-marrow-derived macrophages (BMDM) infected with Influenza A/PR/8/34 at an MOI of 1 and coinfected (simultaneous) or superinfected (after 48h of influenza infection) for 8h with Streptococcus pneumoniae (ATCC®6301). Coinfection presented similar pattern than bacterial infection but showing a synergistic induction in the expression of specific cytokines, chemokines, and IFN-related genes. We identified 445 genes with significant increased expression in coinfection, including inflammatory chemokines CXCL2 and CCL3, and others under NF-κB regulation pathway. In contrast, a synergistic inflammatory response effect can be observed in the superinfection model with similarities to the viral infection alone. Superinfection showed that 227 genes were synergistically upregulated. Most of them were related to IFN-γ, TNF-α via NF-κB, IFN-α and inflammatory response, highlighting the antiviral response. Further proteomics analysis confirmed the synergistic effects in the expression of inflammatory proteins. Regarding bacterial transcripts in both coinfection and superinfection, results showed that Influenza virus induces a generalized bacterial transcriptional shut-off inside macrophages during superinfection 8h after Spn but not in coinfection. Our results link in vitro infection of Influenza virus and Spn to dynamic transcriptomic changes in the three actors. The results were validated by RT-qPCR and proteomics, highlighting genes that are putatively responsible for the severity of complications.

P3-057 Macrophage activation by Zymosan: a new insight.

Levy M[1], Turgeman O, Kaempfer R
[1]Hebrew University Jerusalem Israel

The inflammatory response is indispensable for protective immunity, yet virulent pathogens, whether bacteria or viruses, often elicit greatly excessive inflammation, 'cytokine storm', harmful to the host. Zymosan is a glucan derived from yeast and is an agonist of TLR2/6 on macrophages. Until now it was thought that induction by Zymosan alone is enough to initiate secretion of inflammatory cytokines IL-1α and IL-1β, indicating macrophage activation. However, our results showed that in addition to Zymosan, the presence of T cells is required as well in order to induce optimal IL-1α/β secretion and thus to fully activate macrophages. By depleting human Peripheral Blood Mononuclear Cells (PBMC) from CD3+ T cells and activating the T-cell depleted PBMC with Zymosan we could show a significant reduction in IL-1α/β secretion, indicating that full activation of macrophages is dependent on the presence of T cells. To answer the question whether macrophages are dependent on cytokines expressed by T cells or whether they are are dependent on direct contact through receptors, T cells were treated with GolgiStop to block cytokine secretion and added to the macrophages. After induction with Zymosan there was a significant reduction of IL-1α/β secretion, suggesting that full activation of macrophages is dependent on cytokines from T cells. A wide screening of cytokines expressed by T cells revealed IFN-γ as a likely candidate. When adding αIFN-γ blocking antibodies to PBMC and inducing them with Zymosan, we showed a significant reduction of IL-1α/β secretion, and by adding soluble IFN-γ together with Zymosan to T-cell depleted PBMC, we could show a near-full restoration of IL-1α/β secretion. Hence, induction of macrophages with Zymosan through TLR2/6 depends on IFN-γ from T cells.

P3-058 Association of inflammatory cytokines with lung function, chronic lung diseases and COVID-19

Rontogianni M[1], Gill D[2], Bouras E[1], Asimakopoulos A[1], Tzoulaki I[1,2], Karhunen V[2,3,4], Lehtimäki T[5,6,7], Raitakari O[8,9,10], Wielscher M[2], Veikko S[11], Jalkanen S[12,13,14], Salmi M[12,13,14], Timonen M[15,16], Yarmolinsky J[17,18], Chen J[19], Tobin M[19,20], Izquierdo A[21], Herzig K[16,22,23], Ioannides A[24], Jarvelin M[2,3,25,26], Dehghan A[2,27,28], Tsilidis K[1,2]

[1]Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, [2]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, [3]Research Unit of Population, Life-course Epidemiology, Faculty of Medicine, University of Oulu , [4]Research Unit of Mathematical Sciences, University of Oulu, [5]Department of Clinical Chemistry, Faculty of Medicine & Health Technology, Tampere University, [6]Finnish Cardiovascular Research Center Tampere, Faculty of Medicine & Health Technology, Tampere University, [7]Fimlab Laboratories, [8]Centre for Population Health Research, University of Turku and Turku University Hospital, [9]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, [10]Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, [11]Finnish Institute for Health and Welfare, [12]MediCity Research Laboratory, University of Turku, [13]Institute of Biomedicine, University of Turku, [14]InFLAMES Fiagship, University of Turku, [15]Research Unit of Population Health, University of Oulu, [16]Medical Research Center (MRC) and University Hospital, [17]MRC Integrative Epidemiology Unit, University of Bristol, [18]Population Health Sciences, Bristol Medical School, University of Bristol, [19]Genetic Epidemiology Group, Department of Health Sciences, University of Leicester, [20]National Institute for Health Research, Leicester Respiratory Biomedical Research Centre, Glenfield Hospital, [21]Dept of Health Sciences, University of Leicester, [22]Research Unit of Biomedicine and Internal Medicine, Faculty of Medicine, University of Oulu, [23]Pediatric Gastroenterology and Metabolic Diseases, Pediatric Institute, Poznan University of Medical Sciences, [24]Department of Primary Care and Public Health, School of Public Health, Imperial College London, White City Campus, [25]Unit of Primary Care, Oulu University Hospital, [26]Department of Life Sciences, College of Health and Life Sciences, Brunel University London, [27]Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, [28]UK Dementia Research Institute at Imperial College London

Background: Inflammation plays a role in the pathophysiology of chronic respiratory diseases and COVID-19. We aimed to causally identify the exact inflammatory proteins involved, performing a two-sample Mendelian randomization (MR) analysis.
Methods: We used genetic instruments for 47 circulating inflammatory cytokines from a meta-analysis of genome-wide association studies (GWAS), and selected cis-genetic variants in the vicinity of the coding gene as instrumental variables. Outcome data were obtained from multiple GWAS for COVID-19 outcomes (including any COVID, hospitalization and severe COVID), asthma (atopic and non-atopic), chronic obstructive pulmonary disease (COPD) and lung function indices. The primary analysis utilized inverse variance-weighted MR with colocalization applied to assess MR assumptions. We used false discovery rate to correct for multiple comparisons.
Results: After colocalization analysis, we found an inverse association between genetically-predicted macrophage colony-stimulating factor (MCSF), soluble intercellular adhesion molecule 1 (sICAM) and soluble vascular cell adhesion molecule-1 with risk of COVID-19 outcomes. sICAM was positively associated with risk of atopic asthma, while tumour necrosis factor alfa showed an inverse association. A positive association was shown between interleukin-18 (IL-18) and COPD risk, while an inverse association was shown for interleukin-1 receptor antagonist (IL1-ra). IL-1ra and monocyte-specific chemokine were positively associated with increased lung function indices, whereas inverse associations were shown for MCSF and IL-18.

Conclusions: Several inflammatory cytokines were shown to associate with lung function, chronic lung diseases and COVID-19. Further research is needed to examine these cytokines as potential therapeutic targets.

P3-059 Bypassing the induction of IFN-γ-secreting cells correlated with an increased risk of HIV-1 infection via intrastructural help

Damm D[1], Rojas-Sánchez L[2], Theobald H[1], Überla K[1], Epple M[2], Temchura V[1]
[1]Institute Of Clinical And Molecular Virology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, , [2]Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen

During the last decade antigen-functionalized nanoparticles have become a major focus in the field of HIV-1 vaccine research. The efficacy of an HIV-1 vaccine, however, depends on the presence of vaccine-induced immune mechanisms that may increase the susceptibility for HIV-1 infection. In particular, the number of HIV-specific IFN-γ-secreting cells induced by vaccination correlates with an increased risk of infection. In this study, we designed biodegradable calcium phosphate (CaP) nanoparticles with stabilized HIV-1 envelope trimers (Env) coupled to the surface (Env-CaP). To avoid the induction of HIV-1 specific CD4+ T helper (Th) cells we recruited heterologous T cell responses previously induced by a licensed vaccine via the intrastructural help (ISH) mechanism. To this end, we encapsulated universal helper peptides of Tetanus Toxoid in the core of Env-CaPs. The incorporation of T helper epitopes of heterologous (non-HIV) proteins in Env-CaP nanoparticles elevated the magnitude of anti-Env specific IgG antibody responses by ISH without significant induction of Env-specific IFN-γ-secreting Th cells in a small animal model. This suggests that the ISH strategy for nanoparticle-based HIV-1 vaccines may allow to bypass the induction of HIV-1-specific IFN-γ-secreting Th cells suspected to enhance the susceptibility for HIV-1 infection.

P3-060 Cell type-specific cross-talk of type I and III interferon pathways determines the prioritization of the interferon response

GALANI I[1], Triantafyllia V[1], Eleminiadou E[1], Vasilogianni M[1], Andreakos E[1]
[1]Biomedical Research Foundation of the Academy of Athens

Interferons (IFNs) are cytokines that are readily produced upon viral infection and are largely responsible for orchestrating the host anti-viral response. Understanding thus the mechanisms that regulate IFN production during viral infection is of high importance. The state-of-the-art supports a compartmentalization of the IFN response, in such way that type III IFNs or IFN-λ play an important role at mucosal surfaces, while leucocyte-derived type I IFNs are important for combating viral infections beyond epithelial barriers. By employing the Ifnl2Egfp reporter mouse, we monitored IFN-λ production by immune cells in several settings both in vitro and in vivo. Moreover, by crossing the Ifnl2Egfp mouse with mice lacking functional receptors for type I or III IFN signaling, we observed the amplification of the IFN-λ response by type I IFN signaling in immune cells, which has not been previously described. Of note, this is in contrast to the epithelial-derived IFN-λ, which is not affected by type I IFN signaling, suggesting a differential regulation of the IFN response at barrier surfaces versus systemic infections. Finally, by generating bone-marrow chimeras between WT and ifnl2-/- ifnl3-/- mice, we decipher the contribution of immune cell- versus epithelial cell-derived IFN-λ production during viral infection.

P3-061 IL-23 is dispensable for differentiation of Th1* and maintenance of Th1* and Th17 memory T cells

Puchan J[1], Philippot Q[3,4], Bohlen J[3,4], Mele F[2], Latorre D[1], Bustamante J[3,4,5,8], Puel A[3,4,5], Boisson-Dupuis S[3,4,5], Casanova J[3,4,5,6,7], Sallusto F[1,2]
[1]Institute of Microbiology, ETH Zürich, [2]Institute for Research in Biomedicine, Università della Svizzera italiana, [3]Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Santé et de la Recherche Médicale (INSERM) U1163, Necker Hospital for Sick Children, [4]University Paris Cité, Imagine Institute, [5]St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller University, [6]Howard Hughes Medical Institute, [7]Department of Pediatrics, Necker Hospital for Sick Children, [8]Study Center for Primary Immunodeficiencies, Necker Hospital for Sick Children, AP-HP

Cytokines are important cues that guide differentiation of naïve T cells in response to different pathogens. IL-23 has been implicated in the differentiation and maintenance of the human helper T cells subsets Th1* and Th17, which develop in response to mycobacteria and fungi, respectively. Its exact function in this process is however still unclear.

To understand the role of IL-23 in human helper T cells, we therefore studied patients with different mutations that impair or fully abrogate IL-23 signaling. These patients develop infections with weakly virulent mycobacteria, and in some cases fungal infections. Through naïve T cell priming with mycobacteria, we found that IL-23 signaling is neither required for development of Th1* cells expressing appropriate chemokine receptors nor for optimal cytokine production in primed cells in response to restimulation.

We also found that abrogation of IL-23 signaling did not impair the functionality of the memory helper T cell compartment of these patients, measured by frequency and cytokine production of antigen-specific Th1* and Th17 cells. This may explain why the patients, which have deficits in their innate immune response to mycobacteria, do not develop recurrent infections.

Our results imply that IL-23 signaling is dispensable or at least redundant for the development and function of Th17 and Th1* cells and raise the question which signals are essential to differentiate naïve T cells into these subsets.

P3-062 Evaluation of interferon signature as a biomarker in human biofluids by multiplexed immunoassay

Ahmed R[1], Baines S[2], Gilliam B[1], Saporita A[1], Qiang X[1]
[1]MilliporeSigma, [2]Merck

Interferons (IFNs) are widely expressed cytokines that are recognized as essential component of Innate immunity and host defense. IFN proteins provide the frontline defense against viral infection primarily by interfering with viral replication and playing critical roles in providing antitumor immunity. Different viral infections, chronic inflammatory and autoimmune diseases known to have unique IFN response signature, which makes them intriguing biomarker candidates. The ability to simultaneously measure IFNs in biological samples by multiplexed immunoassay provides an ideal tool to evaluate IFN signature across different diseases. We recently developed a customizable MILLIPLEX® Human Interferon Panel (Cat. No. HIFN-130K) that allows simultaneous detection of 9 biomarkers relevant to IFN biology, including four type I interferons (IFNα2, IFNβ, IFNε, IFNω), the type II interferon IFNγ, three type III interferons (IFNλ1/IL-29, IFNλ2/IL-28A and IFNλ3/IL-28B), and a soluble receptor IFNγR1 (CD119). Here, we report key assay characteristics of this novel multiplex assay including dynamic range, sensitivity, specificity, inter- and intra-assay precision, accuracy (spike recovery) and linearity of dilution/parallelism for each of the 9 biomarkers. We investigated sample detectability using serum and plasma sample from healthy donors (n = 16), observing detectability in at least 50% of the evaluated samples for all biomarkers. Further, these IFNs were measured in 7 psoriasis and 13 rheumatoid arthritis serum and plasma samples. Finally, we compared the IFN profile of plasma samples from individuals recovered (PCR-negative) from COVID-19 (n = 5) and healthy donors. This latter experiment was extended to include MILLIPLEX® Human Cytokine/Chemokine/Growth Factor Panel A and Panel B (Cat.No. HCYTA-60K and HCYTB-60K, respectively), comprising 96 biomarkers across the two panels. Overall, our results highlight the assay performance characteristics of the MILLIPLEX® Human Interferon Panel and demonstrate the utility of MILLIPLEX® immunoassays for establishing an immunological profile in human biofluids.

P3-063 A sequential network of functionally linked death complexes triggers pyroptosis and IL-1β release in response to pathogen blockade of immune signaling

Schwartz Wertman R[1], Saller B[4], Tummers B[2], Green D[2], Groß O[4], Monack D[3], Brodsky I[1]
[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, [2]Department of Immunology, St. Jude Children's Research Hospital, [3]Department of Microbiology and Immunology, Stanford University School of Medicine, [4]Institute of Neuropathology, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg

The innate immune system provides protection against bacterial pathogens by initiating conserved cell death responses following the detection of pathogen associated molecular patterns. Pathogenic Yersinia species inject a virulence protein, YopJ, thereby inhibiting host inflammatory gene expression. YopJ activity triggers caspase-8-dependent cell death that mediates the activation of caspase-1 via a poorly defined mechanism that does not require known inflammasome components. Here we demonstrate that caspase-1 activation by caspase-8 requires caspase-8 dimerization, auto processing, and catalytic activity. Intriguingly, caspase-1 catalytic activity is also required for its own processing downstream of caspase-8, indicating that both caspase-8 enzymatic and scaffolding activity mediate caspase-1 activation. Although the inflammasome adaptor protein ASC is dispensable for caspase-1 activation during Yersinia infection, IL-1β maturation and release in the setting of YopJ-induced cell death requires ASC and occurs via NLRP3 activation downstream of caspase-8-dependent Gasdermin D processing. Notably, active caspase-8 and ASC form sequentially and spatially distinct death complexes during infection. Altogether, this work demonstrates that a network of functionally interconnected, but distinct death complexes mediate cell lysis and IL-1β release in response to pathogen blockade of innate immune signaling.

P3-064 Discovery and characterization of novel peptides in inflammation and immunity

Malekos E[1]
[1]University Of California, Santa Cruz

Next generation sequencing technologies have greatly expanded the size of the known transcriptome. Many newly discovered transcripts are classified as long noncoding RNAs which are presumed to influence phenotype through sequence and structure and not via translated protein products, despite the vast majority of them harboring short open reading frames (sORFs). Recent advances have demonstrated that the noncoding designation is incorrect in many cases and that micropeptides translated from these transcripts and other “untranslated” regions are important contributors to diverse biological processes including inflammation, cell viability, and cancer surveillance. However, the extent to which the majority of sORFs are translated, and whether the resultant peptides are biologically active remain open questions. In this work ribosome sequencing data from a wide range of mouse immune cells was analyzed and evidence of translation was documented for thousands of “noncoding” RNAs (adjusted p-value < 0.1) with additional evidence of translation of hundreds of putative peptides further supported by tandem mass spectrometry in macrophages. Many of these peptides are predicted to contain known protein domains including transmembrane domains and signal peptides, hinting at novel actors in cell signaling pathways. Furthermore, translation of many of the predicted peptides are altered following stimulation with the inflammatory ligand lipopolysaccharide in macrophages implicating some of them in roles related to inflammation. Work is currently underway to establish the molecular function of these micropeptides using high throughput CRISPR/Cas9 screens in both naive and LPS stimulated macrophages. Simultaneously, top candidates have been selected and are being functionally characterized with targeted experiments. I hypothesize that some of these peptides will reveal important aspects of cell signaling and the inflammatory response.

P3-065 Investigating how soluble Interferon-alpha Receptor 2 affects interferon signaling

Joyner O[1]
[1]University Of Colorado, Boulder

Interferon-alpha Receptor 2 (IFNAR2) signaling is crucial for mediating the type I interferon (IFN) response in the defense against pathogens. Most research on IFNAR2 has focused on its canonical full-length isoform, but human cells express multiple isoforms produced by alternative splicing of terminal exons. Currently, the biological consequences of these alternative isoforms on human innate immunity remain unclear. We are investigating one isoform, IFNAR2-soluble, which is generated by skipping exon 8, causing an early frameshift and generates a soluble IFN receptor. The IFNAR2-soluble transcript is selectively expressed in specific tissues and is detectable in human serum, but the functional impact of IFNAR2-soluble on human IFN signaling is largely unknown. Recently, IFNAR2-soluble was analyzed in blood serum of patients who experienced a severe SARS-CoV-2 response. Patients with a more robust response had higher expression of IFNAR2 soluble suggesting a protective role, but the mechanism of action is unknown. To investigate this further, we are carrying out genetic and functional experiments to characterize the impact of IFNAR2-soluble in human cells. We are also investigating how disease-associated variants within IFNAR2 affect the splicing of alternative isoforms such as IFNAR2-soluble. These experiments aim to provide more insight into how IFNAR2-soluble influences human antiviral responses, such as with SARS-CoV-2.

P3-066 A three-step process for regulatory T cell development in thymus

Zhang D[1], Patino-Molano L[1], Lim Y[1], Jin W[1], Chen W[1]
[1]NIH, NIDCR

The mechanism of regulatory T cell generation in the thymus (tTreg cells) remains incompletely understood. Here we show that the development of tTreg cells requires three-step process involving distinct yet not mutually exclusive functions of TGF-β, IL-2 and glucocorticoid-induced tumor necrosis factor receptor (GITR) in the context of TCR engagement. Specifically, TGF-β signaling was the key to induce the expression of foxp3 in both CD4+CD8-CD25+Foxp3- and CD4+CD8-CD25- Foxp3+ tTreg precursors. On the other hand, IL-2 primarily protected tTreg cells and tTreg precursors from death, and the major function of GITR was to promote proliferation of tTreg cells. At the molecular level, we uncovered that TGF-β increased the expression and activation of Stat5, and enhanced the expression of Foxo1 and Foxo3. Thus, we conclude that TGF-β induces, IL-2 maintains and GITR expands tTreg cells in the thymus and provide the missing link of TGF-β signaling to the expression and function of Stat5 and Foxo1 and Foxo3 during the development of tTreg cells.

P3-067 Type I interferons promote natural killer cell-mediated inhibition of early anti-influenza antibody responses

Samelis V[1], Rappe J[1], Davidson S[1], Trapani J[2], Wack A[1]
[1]The Francis Crick Institute, [2]Cancer Immunology Program, Peter MacCallum Cancer Centre

Type I interferons (IFN-I) are vital for influenza virus control. However, IFN-I can also have pathogenic effects towards the host. Although several studies have suggested a stimulatory role of IFN-I on B cell function and antibody production, others have shown that these responses may be negatively regulated by IFN-I. Moreover, whether IFN-I promotes or inhibits antibody responses against influenza virus is unclear, and mechanistic insights to explain the conflicting observations are lacking. As IFN-I signal through the IFN-I receptor (IFNAR), we have compared mice lacking IFNAR to wt mice in the 129 background which is known to show strong IFN responses in influenza. We have found that IFNAR KO mice show increased total influenza-specific antibodies early in infection both in serum and the bronchoalveolar space, including a prominent increase in IgA. These differences are transient, and later timepoints show a reversal of this phenotype, suggesting time-dependent effects of IFN-I on antibody responses. We also find that IFNAR KO mice show a decrease in the frequency of natural killer (NK) cells and their cytotoxic profile, suggesting a role of NK cells in inhibiting, at least in part, these early antibody responses. We demonstrate that depletion of NK cells in wt mice enhances total influenza-specific antibody responses, driven by an increase in IgA, in the bronchoalveolar space. This may be caused by a direct cytotoxic effect of NK cells, as inhibition of perforin causes an increase in IgA-producing plasmablasts in the lung-draining mediastinal lymph node. We aim to determine the mechanism by which NK cells cause inhibition of anti-influenza IgA responses, including identification of the direct cellular targets of NK cells and the activating versus inhibitory receptors engaged on NK cells.

P3-068 IFN-β exposure and ARTS deficiency promote the generation of hyper-efferocytic Ly-6C+ macrophages during the resolution of inflammation

Ariel A[1], Zeituni-Timor O[1], Soboh S[1], Yaseen H[1], Satyanarayanan S[1], Abu Zeid M[1], Silberberg E[1], Schif-Zuck S[1], Larisch S[1]
[1]The department of Human Biology, University of Haifa

During the resolution of inflammation, Ly-6C+F4/80- monocytes differentiate to Ly-6C-F4/80+ macrophages that exert efferocytic properties and consequently convert to IFN-β-producing macrophages. Here, we report that exposure to IFN-β, or TGF-β, or a deficiency in the pro-apoptotic protein ARTS, results in the conversion of mature macrophages to a rejuvenated Ly-6C+F4/80+CCR2+ phenotype. This phenotype appeared only in peritoneal resolution phase macrophages and not in their splenic or bone marrow counterparts or unchallenged peritoneal macrophages. Moreover, IFN-β-triggered rejuvenated macrophages were hyper-efferocytic and expressed higher levels of the efferocytic receptor CD36. Inhibition of CD36 ligation resulted in complete abrogation of efferocytosis ex vivo in both mature and rejuvenated macrophages. Altogether, our findings indicate an unprecedented phenomenon in which IFN-β promotes macrophage rejuvenation and efferocytosis that are limited by ARTS-mediated apoptosis during the resolution of inflammation.

P3-069 Differential innate antiviral signaling in human and bat cells

Mossman K[1], Demian W[1], Jacob R[1], Banerjee A[2]
[1]McMaster University, [2]University of Saskatchewan

Background

Emerging viruses pose substantial threats to health, biodiversity, and economic prosperity. Understanding mechanisms of antiviral signaling in taxa that act as viral reservoirs, such as bats, can identify mechanisms for viral suppression in non-human species. Bats are a diverse order containing over 1,400 species, several of which are implicated as reservoir hosts in virus spillovers. Experimental viral infections demonstrate that bats exhibit effective antiviral responses, including strong interferon responses, and dampening of inflammatory responses that correlate with negative outcomes in humans.

Methods

We performed computational analysis of mammalian interferon regulatory factor (IRF) family members, including multiple species within the two suborders of bats. We subsequently produced in depth sequence alignments and performed functional analyses on residues found to be positively selected in multiple bat IRF species. Additional RNA-Seq analyses were performed in wild-type and IRF3 knockout human and bat cells, under resting or poly(I:C) treated conditions.

Results

Our studies revealed strongly conserved immune modulators, signaling pathways and antiviral responses between humans and bats. However, we also identified positively selected residues in key IRFs and identified unique regulation of signaling pathways, including the regulation of IL-6.

Conclusion

Our studies provide genetic and functional evidence for enhanced IRF-mediated antiviral responses in bats and adds further support to speculations that bats have positively selected for multiple adaptations in their antiviral immune responses. A fulsome understanding of the strategies used by bats to augment innate antiviral immunity while dampening proinflammatory sequela will inform strategic development of novel pan-antiviral therapeutic strategies, such as small molecules and mRNA delivery, to effectively skew the human immune response to a “bat-like” immune response.

P3-070 Type III interferons regulate hyperinflammation and prevent neuroinvasion during genital HSV-2 infection

Feng E[1], Monteiro J[1], Vahedi F, Ashkar A[1], Kotenko S[2]
[1]McMaster Immunology Research Centre, [2]Rutgers New Jersey Medical School

Background:
Proper regulation of inflammatory immune responses during viral infections is critical to promote viral clearance and prevent of tissue immunopathology. Type I and III interferons (IFN) promote antiviral responses to infections and have been used as an antiviral therapy. Unfortunately, type I IFN antiviral therapy clinical trials have failed due to their ability to potentiate hyperinflammatory responses. Type III IFNs are suggested to be a safer alternative therapy due to their inability to induce inflammation. However, their mechanism of action is poorly defined and until recently, believed to be independent of an immunoregulatory function. The aim of this study is to assess the protective and immunoregulatory functions type III IFNs that can promote host survival.

Methods: C57BL/6 mice and Ifnlr-/- mice were infected with HSV-2 intravaginally and administered recombinant type III IFN (rIFN-λ2) or type I IFN (rIFN-β). Mice were assessed for survival, viral titres, and histopathology. Ifnar-/- mice were used to model viral induced immunopathology. We have previously published that Ifnar-/- mice develop severe IL-6 dependent vaginal tissue pathology.

Results: We demonstrate that type III IFNs are superior in protecting against lethal genital HSV-2 infection compared to type I IFNs. Administration of rIFN-λ2 significantly reduced mortality via reduced of neuroinvasion and dissemination to the enteric nervous system, independent of influencing local vaginal viral loads, while rIFN-β showed little effect. Furthermore, rIFN-λ2 treatment attenuated hyperproduction of IL-6 production to baseline levels to suppress the severity of vaginal tissue pathology in Ifnar-/- mice. Similarly, Ifnlr-/- mice deficient in type III IFN signaling display increased neuroinvasion and IL-6 dependent tissue pathology

Conclusion: Overall, we demonstrate that type III IFN can suppress cytokine-driven hyperinflammation and prevents neuroinvasion during mucosal viral infection. These findings contribute to our understanding of the functions of type III interferons and highlight their potential as a therapeutic intervention.

P3-071 Gasdermin B (GSDMB) associates with, and regulates, GSDMD in intestinal-derived goblet cells and is upregulated in inflamed tissues of ulcerative colitis (UC) patients

Williams J[1], Privitera G[1], Li Y[1], Antanaviciute A[3], Huang E[4], Chan R[2], Corridoni D[3], Simmons A[3], De Salvo C[1], Pizarro T[1]
[1]Case Western Reserve University Department of Pathology, [2]Institute for Computational Biology, Case Western Reserve University School of Medicine, [3]MRC Human Immunology Unit, MRC Weatherhall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, [4]Department of Surgery, UT Southwestern Medical Center

Gasdermins (GSDMs) are a family of structurally-related proteins originally described for their role in pyroptosis. Dysregulation of various GSDMs have been observed in several chronic inflammatory disorders, including inflammatory bowel disease (IBD). Our group recently described increased GSDMB in ulcerative colitis (UC) patients, specifically in colonocytes/crypt top colonocytes; however, a significant increase in GSDMB was also observed in goblet cells (GCs). We aimed to confirm the presence and determine the functional consequences of increased GC-derived GSDMB in IBD. GO analysis was performed on GSDMB-expressing GCs from scRNA-Seq-derived intestinal epithelial cells (IEC) from UC patients and healthy controls, and co-expression of other GSDMs evaluated. Based on these results, further analysis was performed on GCs positive for both GSDMB and D. GCs, identified by Alcian blue/PAS staining, and GSDMB and D were immunolocalized in full-thickness colon tissues and colonoids. Using the LS174T GC cell line, regulation of GSDMB and D was determined and co-localization/proximity evaluated using Duolink®. Our results showed GSDMB and D overexpression in GCs from inflamed areas of UC patients, with enriched pathways associated with antigen processing/presentation, protein folding, ER stress, but none with pyroptosis or cell death. GSDMB and D were increased in GCs from inflamed tissues of UC patients, and further elevated as disease became more severe. Co-localization of GSDMB and D was observed in a subpopulation of GCs in UC-derived colonoids and confirmed in LS174T cells. Increased full-length, but not cleaved, GSDMB and D were detected in GCs after IFN-gamma stimulation, with accumulation in the cytoplasm, but with sparse translocation to the plasma membrane. Finally, GSDMB-dependent GSDMD regulation was noted comparing IFN-gamma treated WT vs. GSDMB-/- IECs. Our results indicate GSDMB/GSDMD upregulation and co-operation in GCs derived from active UC, implying an important role in mucin secretion and potential downstream effects in the pathogenesis of IBD.

P3-072 Attenuated CMV infection impairs cytokine production of memory T cells

Dimonte S[1], Gimeno-Brias S[1], Marsden M[1], Chapman L[1], Amratia P[1], Clement M[1], Humphreys I[1]
[1]Systems Immunity University Research Institute/Division of Infection and Immunity, School of Medicine, Cardiff University

The ability of cytomegalovirus (CMV) to trigger the generation and long-term maintenance of functional memory T cells has attracted considerable interest in developing CMV-based vaccine vectors. Despite promising pre-clinical data in models of cancer and infectious diseases, the potential for human CMV to promote immunopathology in immunocompromised individuals has hindered the translational development of these agents. Attenuated CMV vectors, such as replication- or spread-deficient viruses, are viable alternatives to fully replicating vaccine vectors. However, the immunogenicity of these vectors, and their impact on T-cell memory, remains unclear. We, therefore, used a spread-deficient variant of murine CMV (□gL MCMV) to investigate these immunological outcomes in mice following subcutaneous and intranasal immunisation. These routes of administration are common to vaccination. We found that □gL MCMV (2x105 PFU) induced significantly fewer MCMV-specific memory CD8+ T-cells than native MCMV controls, and expanded memory T-cells showed impaired IFNγ production. We further observed a preferential loss of polyfunctional (IFNγ+ TNFα+) T-cells after subcutaneous immunization with □gL MCMV. Increases in the subcutaneous □gL MCMV inoculum (up to 2x106 PFU) did not rescue this loss of cytokine effector function. Interestingly, whilst vector attenuation reduced recruitment of dendritic cells (DCs) to draining lymph nodes in both administration routes, systemic depletion of conventional DCs during acute infection with WT-MCMV reduced T Cell responses in the intranasal model, suggesting that the diminished immunological priming seen with □gL MCMV was attributable to impaired innate DC activation. In summary, these data suggest that CMV replication is required for the generation and functional expansion of antigen-specific memory T cells.

P3-073 Candida tropicalis infection during DSS colitis induces expansion of IL-1-beta and IL-23-producing NOD2-expressing macrophages

Vidmar K[1], Mahabaleshwar G[1], Ghannoum M[1], Pizarro T[1], De Salvo C[1]
[1]Case Western Reserve University, School of Medicine

Inflammatory bowel disease (IBD) is a chronic, relapsing condition of the gastrointestinal tract, resulting from dysregulated gut mucosal immune responses reacting to environmental triggers in genetically-predisposed individuals, and whose etiology is unknown. Previous studies have reported an increased presence of Candida tropicalis (Ct) in IBD patients, mainly in Crohn's disease (CD), and that the pattern recognition receptor (PRR), NOD2, plays a critical role in innate immune responses during CD, but also in the recognition of fungal wall components. How NOD2 regulates mucosal immunity against fungal infection, however, remains unclear. Here, we sought to determine the direct effects of Ct on NOD2- expressing macrophages during DSS-induced colitis. We challenged DSS colitic WT mice with Ct two days before and during DSS administration. Confocal imaging performed on colonic mucosa from WT mice under different experimental conditions (untreated, DSS, Ct, DSS+Ct) showed that Ct induces NOD2-expressing macrophage infiltration into the colonic mucosa of infected DSS colitic mice. To better elucidate the effects of Ct on NOD2-expressing macrophages, we isolated bone marrow-derived macrophages (BMDM) from Nod2-/- and WT mice, and infected them with Ct and chitin, a primary component of fungal cell walls; cell pellets and supernatants were collected and assessed for macrophage-associated cytokines (IL-1β and IL-23) by qPCR and ELISA. Our results showed a decrease in both IL-1ß and IL-23 in Nod2 deficient mice compared to WT, while Il1b was markedly decreased in Nod2-/- BMDMs infected with chitin and Ct. Finally, confocal imaging clearly showed that WT BMDMs were more differentiated, showing distinct patterns of IL-1ß and IL-23 staining, compared with Nod2-/- BMDMs. Taken together, our data suggests that NOD2 contributes to maintain gut mycobiome homeostasis driving innate immune responses via a macrophage-mediated IL-1ß/IL-23 release. Thus, the overgrowth of Ct that may contribute to chronic intestinal inflammation, like in CD, may be prevented.

P3-074 The Temporal Regulations of 14-3-3η in MDA5-Dependent Type-I Interferon Induction

Chan Y[1], Liu N[1], Hsin F[1], Liu H[1]
[1]Graduate Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University

The 14-3-3 family is a group of important chaperone proteins which are ubiquitously expressed in eukaryotic cells to modulate different signaling pathways. Our previous studies have reported that during RNA virus infection, full-length 14-3-3η positively regulates melanoma differentiated-associated protein 5 (MDA5)-mediated type I interferon (IFN) induction. To prevent overactivation, MDA5 activation triggers caspase-3-dependent apoptosis to cleave some signaling molecules such as MAVS and IRF3. However, whether 14-3-3η is also regulated by caspase-3 cleavage remained unclear. Our study showed that coronavirus and/or enterovirus infections caused the production of caspase-3 cleaved 14-3-3η. We also found that inhibition of caspase-3 suppressed the 14-3-3η sub-isoform production, confirming that 14-3-3η was cleaved by caspase-3. The structure of 14-3-3 protein is composed of nine α-helices (αA to αI), and the loops and the C-terminal tail affect the target binding affinity. According to ScreenCap3 database, Asp239 and/or Asp209 are the most likely caspase-3 cleavage sites of 14-3-3η which could remove the C-tail and/or the 9th helix respectively. Our results showed that the truncation mutants of 14-3-3η, 14-3-3ηΔαI (1-209), but not 14-3-3ηΔC (1-239), had a stronger interaction with MDA5 to alter the MDA5 redistribution and dramatically suppress the MDA5-dependent IFN-β induction, indicating that cleaved 14-3-3η is a negative regulator of MDA5-dependent IFN-β induction. Additionally, in coronavirus infected cells, overexpression of 14-3-3ηΔαI inhibited the IFN-β induction and ISG expressions. Our study revealed the temporal control of MDA5 activation/deactivation by caspase-3-dependent 14-3-3η cleavage, which provides a negative feedback mechanism of MDA5-mediated type I IFN induction.

P3-075 MDA5-bound IFN-Stimulated Long Non-Coding RNAs Enhances Type I IFN Induction

Hsin F[1], Liu H[1]
[1]National Taiwan University

The induction and response of type I interferon (IFN-I) are critical for antiviral innate immunity. Cytosolic RNA sensors, including RIG-I and MDA-5, recognize the non-self RNA in cytoplasm and trigger downstream signaling to induce IFN-I expression. Secreted IFN-I signals to the neighbor cells and triggers the production of interferon-stimulated genes (ISGs) to set the cells at the antiviral stage. Previous studies showed that both proteins and RNAs could be induced by IFN-I as ISGs. Certain IFN-stimulated self-RNAs (IS-RNAs) are reported to interact with RIG-I to restrict RIG-I activation, which shows the opposite role of the viral RNA ligands. However, whether specific IS-RNAs could control IFN-I through cytosolic RNA sensors is still unknown. Here we report that IFN-stimulated long non-coding RNAs (IS-lnc-RNAs) may interact with MDA5 to prime MDA5 activation against pathogen invasion. We first observed that transfection of IS-RNAs purified from IFNβ-treated cells induced IFNB1 mRNA expression. However, knock-down of MDA5, but not RIG-I, abolished IFN-I induction by IS-RNAs, suggesting that IS-RNAs may trigger IFN-I induction through MDA5. Independent from ISG protein synthesis, according to an RNA-IP-sequencing analysis, we identified certain IS-lnc-RNAs, including lnc-CCDC122-2:1 and CATIP-AS2, interacted with MDA5. Ectopic expressing these two IS-lnc-RNAs enhanced IFNB1 and IFIT1 mRNA expression levels induced by HMW poly (I:C) transfection, which is known to activate MDA5 dependent signaling. Co-expression of these IS-lnc-RNAs with full-length (FL)-MDA5, but not with N-MDA5, could enhance IFN-I expression, suggesting that RNA binding domain of MDA5 is essential for IS-lnc-RNA recognition. Besides, we observed that both lnc-CCDC122-2:1 and CATIP-AS2 were accumulated in cytoplasm after IFNβ treatment, which made it feasible for them to interact with MDA5. The fluorescence in situ hybridization (FISH) further demonstrated the interaction between MDA5 and IS-lnc-RNAs in cytoplasm directly. All these data indicated that MDA5-bound IS-lnc-RNAs sustain IFN-I induction.

P3-076 The cross-species role of coronavirus nucleocapsid protein in innate immune evasion and its effect on viral replication

Laine L[1], Erdin M[2], Kolehmainen P[3], Julkunen I[3], Kakkola L[3], Sironen T[2], Österlund P[1]
[1]Expert Microbiology Unit, Finnish Institute for Health and Welfare, [2]Department of Virology, Medicum, Faculty of Medicine, University of Helsinki, [3]Infection and Immunity, Institute of Biomedicine, University of Turku

Coronaviruses (CoVs) are a significant source of zoonoses and have potential to cause severe outbreaks and pandemics. During the past 20 years, SARS-CoV, MERS-CoV, and SARS-CoV-2 have been a notable cause of morbidity, mortality and public health crises. In addition to the severe CoVs, there are four circulating seasonal CoVs, which cause mild respiratory infections. As human CoVs originate from bats and crossover to humans via other intermediate animal hosts, research on CoVs from various species will give insight into the mechanisms behind host switching and the development of future zoonoses.

We are studying how human severe and seasonal, bat and rodent CoVs evade the initial host cell innate immune response and how this evasion affects the replication of the virus. Our focus is on the nucleocapsid (N) protein, which potentially interferes with the activation of host innate immune responses. The N proteins from different CoV species are structurally very conserved so we aim to find out whether they also hamper activation of innate immunity in a similar fashion and what implications this has for viral evolution and cross-species spillover events.

Initial studies include structure prediction analyses of novel bat and rodent CoV N proteins to be compared to the known human CoV N structures. We are setting up the system for overexpression of the N proteins in relevant cell lines for each CoV species. With these we will examine whether the N proteins are inhibiting virus induced activation of innate immune signalling molecules and suppress the production of interferons and anti-viral molecules. Depending on these results, subsequent studies will be carried out to dissect further the N protein interactions with host regulatory molecules.

P3-077 IL-38 shapes regulatory T cell activation

Huard A[1,2], Kiprina A[2], Elewa M[2], Raue R[2], Schmidt T[2], Palmer G[1], Brüne B[2], Weigert A[2]
[1]University Of Geneva, Faculty of Medicine, [2]Institute of Biochemistry I, Faculty of Medicine

Interleukin-38 (IL-38) is an IL-1 family member that shares homology with the IL-1 and IL-36 receptor antagonists, and was therefore proposed to act as a negative regulator of inflammation. IL-38 released from apoptotic cells was shown to limit inflammatory macrophage activation and downstream T lymphocyte IL-17 production. IL-38 polymorphisms are further associated with increased susceptibility for inflammatory diseases. However, the mechanisms by which IL-38 regulates inflammation in vivo are still ill defined. The aim of this study was to identify how IL-38 regulates resolution of inflammation.

WT and IL-38 KO mice were injected intraperitoneally with zymosan A (10 mg/kg) to induce zymosan-induced peritonitis (ZIP) and followed for up to 6 days. Cell populations, gene expression and protein secretome in peritoneal lavages were examined respectively by flow cytometry, bulk RNA sequencing and cytokine profiling.

The resolution of ZIP was delayed in IL-38 deficient, as compared to WT mice, as indicated by persistent neutrophilia and lower production of pro-resolving mediators, such as TGF-β, on day 6. This coincided with reduced levels of regulatory T cells (Tregs) in the peritoneal lavage. Unexpectedly, the TGF-β production capacity of macrophages did not influence the induction of Tregs from naïve T cells in vitro, but reduced Treg activity markers, particularly features of the adenosine production machinery. Finally, blocking adenosine production in WT mice by injecting the CD39 inhibitor POM-1 on day 2 and 5 of ZIP mimicked the delayed resolution of inflammation and persistent neutrophilia observed in IL-38 KO mice.

These data suggest a role for IL-38 in the regulation of Treg biology, which may be relevant in the context of autoimmune diseases. The pro-resolving function of IL-38 may be linked to the regulation of TGF-β production by macrophages downstream of efferocytosis, and consequently to the activation and function of Tregs in the peritoneal cavity.

P3-078 Utilising IFN-lambda stimulation and systematic analysis of viral regulation to identify novel mechanisms of innate immunity.

Kite J[1,2], Davies C[1,2], Zerbe C[1,2], Antrobus R[2], McCaughan F[1], Albarnaz J[1,2], Weekes M[1,2]
[1]Department of Medicine, University of Cambridge, [2]Cambridge Institute for Medical Research, University of Cambridge

Type III interferons, or interferon lambdas (IFNλ), are the first-line of defence against viral infections of epithelia. Despite acting via a distinct receptor, initial reports suggested that they functionally resemble type I interferons. Both activate intracellular signalling pathways and antiviral functions, and both are induced by viral infection. Recent data has hinted that there are type III-specific activities, although these have not been studied at the protein level. Furthermore, whether IFNλ subtypes exhibit distinct activities at the level of protein expression is unknown.

We have recently established primary-like human bronchial cells, which retain the ability to differentiate, as a tractable, physiologically relevant model for dissecting the IFNλ response. Using a highly multiplexed, systemic, temporal tandem-mass-tag-based proteomic approach, we have identified common and novel cellular responses to each subset of IFNλ in comparison to IFNα. For example, known modulators of the IFN response, STAT1 and IFITs were induced by all IFNs. Fascinatingly, the response to IFNλ was quantitatively different to type I IFN, as well as quantitatively different between IFNλ subtypes. Furthermore, as viruses often regulate key cellular antiviral proteins, we compared our data to a compendium of our proteomic studies across multiple different viral infections to discover factors that are both stimulated by IFNλs and regulated by viral infection, since these may identify novel innate antiviral factors.

Overall, this systematic approach will advance our understanding of the mechanisms of action of IFNλs and the antiviral immunity they provoke in addition to viral evasion of these critical molecules.

P3-079 Macrophages negatively regulate interferon responses during viral respiratory infection

Hoagland D[1], Rodriguez-Morales P[1], Mann A[1], Lai A[1], Yu S[2], Medzhitov R[2], Franklin R[1,3]
[1]Department of Immunology, Harvard Medical School, [2]Department of Immunobiology, Yale School of Medicine, [3]Department of Stem Cell and Regenerative Biology, Harvard University

Innate immunity must be tightly regulated to ensure host survival during pathogen challenge. Robust antiviral responses are required to clear acute viral infections and initiate the adaptive immune response. In turn, the host immune system must mitigate these responses to avoid deleterious effects on host physiology and preserve maintenance of tissue function. Macrophages are important players in both initiation of innate immune responses and coordinating tissue function during homeostasis. We investigated the role of a cytokine that is readily produced by macrophages in response to various stressors including pathogen-associated molecular patterns (PAMPs): Oncostatin-M (OSM). Given the roles of macrophages and their propensity to rapidly synthesize OSM after stimulus with PAMPs, we hypothesized that macrophage-derived OSM plays a role during the innate immune response to infection. We find that Osm-deficient mice succumb to sublethal influenza challenge. This increase in morbidity and mortality occurs despite no increases in viral burden locally or systemically. However, Osm-deficient mice have higher levels of Type-I Interferon (IFN-I) in the lung. To investigate whether the elevated IFN-I responses in Osm-deficient mice are a direct result of the induction of the innate immune response, we administered poly(I:C) – a viral replication product mimetic and PAMP – intratracheally to mice. Osm-deficient mice succumb to poly(I:C) challenge and display higher levels of IFN-I and interferon-stimulated genes than wild-type mice. We find that poly(I:C) challenge is lethal in vivo with only macrophage-specific depletion of Osm, utilizing a CD64-driven Cre. Critically, this phenotype can be fully rescued by blockade of IFN-I signaling during challenge. We have identified a mechanism by which macrophages negatively regulate interferon-mediated tissue inflammation and damage to promote host survival.

P3-080 IL-27 produced during acute malaria infection regulates the development of unique parasite-specific memory CD4+ T cells

Macalinao M[1], Inoue S[1], Tsogtsaikhan S[1], Matsumoto H[1], Bayarsaikhan G[1], Jian J[1], Kimura K[1], Yasumizu Y[2], Inoue T[1], Yoshida H[3], Hafalla J[4], Kimura D[1], Yui K[1]
[1]Nagasaki University, [2]Osaka University, [3]Saga University, [4]London School of Hygiene and Tropical Medicine

Malaria infection elicits both protective and pathogenic immune responses, and IL-27 is a critical cytokine that regulates T cell immune responses during infection. Here, we identified a critical window of CD4+ T cell responses that is targeted by IL-27. IL-27ra-/- mice display reduced parasite burden during chronic infection and enhanced CD4+ T cell responses following rechallenge infection. In this study, we investigated the role of IL-27 in the development of immunological memory to malaria during chronic malaria infection using MHC-II restricted malaria antigen-specific TCR transgenic mouse, PbT-II.
Mice were transferred with PbT-II cells and were infected with malaria parasite, Plasmodium chabaudi. Neutralization of IL-27 during acute infection expanded high levels of specific CD4+ T cells, which were maintained at high levels thereafter. In the chronic phase, malaria-specific memory CD4+ T cells were maintained at high levels in IL-27-neutralized mice in a manner independent of active infection. These memory PbT-II cells consisted mainly of CD127+KLRG1- and CD127-KLRG1+ unique subpopulations that displayed distinct cytokine production and proliferative capacity. Single cell RNA-seq analysis revealed that both PbT-II cell subpopulations formed independent clusters that express unique Th1-type genes. These IL-27-neutralized mice exhibited enhanced cellular and humoral immune responses and protection against challenge infection. These findings demonstrate that IL-27, which is produced during the acute phase of malaria infection, inhibits the development of unique Th1-type memory precursor CD4+ T cells, suggesting potential implications for the development of vaccines and other strategic interventions.

P3-081 Age-dependent expression and antiviral activity of IFNε in respiratory airway epithelium.

Mccabe M[1], E. Groves H[1], Lopez Campos G[1], Getty E[1], G.G. Bamford C[2], F. Power U[1]
[1]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, [2]School of Biological Sciences & Institute for Global Food Security, Queen's University Belfast

Respiratory syncytial virus (RSV), a single-stranded negative-sense RNA virus, is the commonest viral cause of severe lower respiratory tract infection in infants. RNA-seq analysis of RSV-infected well-differentiated primary nasal epithelial cell cultures (WD-PNECs) derived from infants sampled at birth and one-year-old revealed that interferon epsilon (IFNε) was significantly increased following RSV infection versus uninfected controls. Furthermore, IFNε expression was higher in 1 year- versus newborn-derived WD-PNECs, both at baseline and following RSV infection, suggesting a more robust role in antiviral activities at 1 year compared to newborns.

To further investigate the potential antiviral role of IFNε we characterised IFNε expression in airway epithelial cell lines (HEp-2, BEAS-2B, and A549) infected with a recombinant RSV reporter virus expressing a far-red fluorescent protein (RSV-A2/mKate2) or mock-infected. Constitutive IFNε expression was detected in all cell lines, irrespective of infection status. Interestingly, the induction of interferon-stimulated genes following IFNε treatment of airway epithelial cells was different to other epithelium-expressed IFNs, including IFNβ1 or IFNλ1 (100 ng/mL), with IFNβ1 inducing a more potent enduring response than IFNε or IFNλ1. To determine the relative antiviral activities of IFNε compared to IFNβ1 and IFNλ1, cell lines pre-treated with recombinant IFNε, IFNβ1 or IFNλ1 (100 ng/mL) were infected with RSV-A2/mKate2. IFNε pre-treatment resulted in a dose-dependent reduction in RSV infection in BEAS-2B and HEp-2 cells, although the reduction was less than that for IFNβ1. A similar dose-dependent antiviral activity of IFNε was evident against other respiratory viruses (Sendai virus, Influenza virus and SARS-CoV-2).

Therefore, we hypothesise that constitutive production of IFNε within airway epithelium mitigates viral infection and reduced IFNε expression at birth may increase susceptibility to severe virus-induced disease. Future work will explore the consequences of IFNε gene knockdown and IFNε pre-treatment of the more physiologically relevant WD-PNECs on viral replication and induction of antiviral responses.

P3-082 TRIM14 Regulates Mitochondria Homeostasis During Mycobacterium tuberculosis Infection

Mabry C[1], Coleman A[1], Weindel C[1], Patrick K[1], Watson R[1]
[1]Texas A&M University School of Medicine

Mycobacterium tuberculosis (Mtb) has evolved a variety of mechanisms to suppress macrophage apoptosis and form a favorable niche for survival. Because cell death is a key determinant of Mtb infection, we sought to identify host factors critical for regulating cell death. One family of factors, TRIM (tripartite motif-containing) proteins, have emerged as key regulators of innate immunity and cell death. Our previous work has shown that TRIM14 acts as a scaffold between the kinase TBK1 and STAT3 to increase STAT3 activity through phosphorylation at Ser727 whereas without TRIM14 STAT3 is preferentially phosphorylated at Ser754 inhibiting its activity. STAT3 is a classical transcription factor, but recent studies have revealed STAT3 regulates mitochondria-associated metabolism, cell death, and opening of the mitochondria permeability transition pore (MPTP). We hypothesized that TRIM14 may regulate mitochondria homeostasis through modulating STAT3 activity. Here, we show that in the absence of TRIM14, STAT3 is preferentially phosphorylated at Ser754 and is not properly targeted to mitochondria. The lack of mito-STAT3 in Trim14-/- primary macrophages is associated with significantly increased apoptotic cell death in response to Bcl2 inhibitors and Mtb infection. This cell death is STAT3-dependent, where STAT3 inhibition in wild-type cells phenocopied Trim14-/- macrophages. Consistent with this idea, we found that MPTP pore inhibition significantly reduced cell death in Trim14-/- macrophages, suggesting TRIM14 may regulate STAT3 activity at the level of the MPTP to reduce apoptosis. Remarkably, overexpression of TRIM14 blocks cell death in response to the same stimuli, suggesting TRIM14 controls cell death. Lastly, we also found that Trim14-/- macrophages exhibit altered mitochondrial metabolic profiles during Mtb infection. Collectively our data supports a model whereby TRIM14 reduces apoptosis and maintains mitochondria homoeostasis in a STAT3-dependent manner. We are currently working to link TRIM14's regulation of macrophage mitochondria homeostasis and a mouse model of tuberculosis disease.

P3-083 Deficiency of NAD(P)H:quinone oxidoreductase 1 Alleviates Inflammation through Regulation of TLR Signaling Pathway

Kim T[1], Chathuranga K[1], Lee J[1]
[1]College of Veterinary Medicine, Chungnam National University

IκB kinase (IKK) is an enzyme complex involved in the cellular signal transduction of inflammatory response. IKK phosphorylates IκBα that inhibits NF-κB, which results in the dissociation of IκBα from nuclear factor κB (NF-κB). Here we report that NAD(P)H:quinone oxidoreductase 1 (NQO1) regulates inflammation by stabilizing IKKα, a component of IKK complex. NQO1 Knockout mice showed higher resistance to LPS-induced septic shock than wildtype mice. Moreover, NQO1 knockout BMDMs exhibits low level of cytokines in response to TLR2 and TLR4 stimulation compared with wildtype BMDMs. We also demonstrated that the regulation of inflammation is mediated by interaction of NQO1 with IKKα via kinase domain upon TLR2 and TLR4 stimulation. Moreover, we showed that NQO1 is responsible for stabilization of IKKα but not IKKβ and IKKγ. Taken together, our study shows the mechanism by which NQO1 regulates inflammatory responses by interacting and stabilizing IKKα upon TLR stimulation.

P3-084 Investigate the role of IRF5 in macrophages and myeloid cells in anti-tumor immunity.

WANG M[1], Lim K[1], Dotse E[1], TIU C[1], LI Y[1], CHOW K[1]
[1]City University of Hong Kong

IRF5 is a crucial transcription factor that regulates the inflammatory immune response, which is a key driver of anti-tumor immunity. However, the pathways and factors that regulate IRF5 are not well-defined, especially in myeloid cells. IRF5 plays a key role in M1 vs. M2 macrophage polarization, and tumor-associated macrophages (TAMs) that display M2 phenotype are often associated with bad prognosis. Thus, we hypothesize that IRF5 activation within macrophages (Mϕ) and myeloid cells is a key regulator of anti-tumor immunity. To reveal the role of IRF5 in anti-tumor immunity, we established the B16F10 melanoma tumor model in mice with a myeloid-specific deficiency of Irf5 (Irf5-MKO) and wild-type (WT) mice. Our results indicated that as compared to WT, mice with myeloid-specific Irf5 deficiency showed suppressed tumor growth. Tumors harvested from Irf5-MKO mice displayed increased leukocytes and M1 Mϕ infiltration. These results suggest that Irf5 activation in myeloid cells favors tumor progression in the melanoma mouse model. We therefore investigated the molecular pathways that regulate IRF5 activity and downstream inflammatory cytokines expression in myeloid cells and Mϕ. We found that contrary to plasmacytoid dendritic cells (pDCs), TLR7 and 9 stimulations failed to activate IRF5 in myeloid cells and Mϕ. We hypothesize that a negative regulator is preventing the activation of IRF5 downstream of endosomal TLR stimulation. Moreover, IRF5 is marginally activated by high concentration of TLR8 agonist in monocytes but not Mϕ, while knockout of IRAK2 interferes with the activation of IRF5. We are currently further characterizing the molecular basis of IRF5 activation in myeloid cells and investigating its role in immune modulation for the design of cancer immunotherapy.

P3-085 Structural and functional insights into the role of Themis1 in T cell receptor signalling

Clancy D[1], Sanz Sanjuan A[1], Bloch Y[1], Furones A[1], Tougaard P[1], Vandenabeele P[1], Savvides S[1]
[1]Vib-UGent Inflammation Research Centre

Themis1, a recently discovered T cell-specific protein, is a key regulator of thymocyte development. Maturation from double positive thymocytes to effector T cells is blocked in Themis1-deficient mice, resulting in a marked reduction in peripheral T cells.

Themis1 contains tandem copies of a novel structural domain, dubbed the cysteine-containing all beta in Themis (CABIT) domain, crucial for Themis activity and signalling. CABIT domain-containing proteins have been identified in a wide variety of species, pointing to Themis1 as the archetypal member of a new metazoan protein family. Themis1 acts as a molecular scaffold in TCR signalling and binds to the cytosolic adapter protein Grb2, which facilitates recruitment of Themis1 to the TCR after activation.

In addition to its crucial role in thymocyte development, accumulating evidence also links Themis to inflammatory diseases, such as rheumatoid arthritis, yet the structural and mechanistic basis of these pleiotropic functions remain poorly understood. Themis1 has been shown to act as a positive regulator of TCR signalling in mature T cells, with reduced IL-2 production and in vitro effector functions in Themis1-deficient CD4+ T cells. Moreover, Themis1 is required for IL-2 and IL-15-induced T cell proliferation via Jak-Stat and mTOR.

We have combined an integrative structural biology approach with cellular and in vivo experiments to elucidate the structure-function landscape of Themis1 and to investigate the unexplored roles of Themis1 in inflammatory signalling and disease models of rheumatoid arthritis. Here, we present a cryo-EM structure of the Themis1-Grb2 complex associated with an engineered Themis-specific single-domain VHH antibody fragment. We characterize the kinetics and affinity dictating the Themis1-Grb2 interaction and identify key residues on Grb2 mediating complex formation in binding studies and cellular assays. Structure-function analysis of Themis1-Grb2 complexes provide timely insights into the function of CABIT domain-containing proteins as signalling scaffolds in T cell signalling and inflammatory pathways.

P3-086 Systematic characterisation of IFN stimulated genes and pathways in normal physiological and pathological states

Newsham I[1,2], Torra I Benach M[1], Jungkurth E[1], Huang J[1], Gong Z[1], Samarajiwa S[1,3]
[1]MRC Cancer Unit, University of Cambridge, [2]MRC Biostatistics Unit, University Of Cambridge, [3]Systems Medicine Division, Imperial College London

Background: The interferons (IFNs) are a family of pleiotropic cytokines, which mediate host defence, anticancer and inflammatory responses. Three classes of IFN have been identified, and they regulate the transcription of many genes, termed Interferon Stimulated Genes (ISGs). While a few thousand ISGs have been identified, only a subset are produced in any given cell type in response to a specific stimulus.

Objectives: We aim to uncover ISG expression signatures and pathways in a variety of cell types, under different stimuli (type I, II and III IFN treatment and in pathogenic states such as viral infections, various cancer types and inflammatory diseases).

Methods: 77 IFN treated human RNA-Seq studies were processed with the same pipeline and integrated to form our reference dataset. A number of disease studies where IFNs play a role were also processed and analysed similarly. These included ~60 studies representing viral infections (Zika, SARS-CoV-2, Influenza), SLE, Alzheimer's disease and Cancer. Pathway modelling was used to build ISG pathways. An algorithm was produced to identify upstream regulators of IFN-signatures and a web application was created using graph database technology and a javascript visualization library.

Results: Using our IFN reference RNA-seq datasets, we identified 5284 ISGs. Computational approaches were applied to functionally characterise these. Pathway modelling methods were utilsed to extract IFN-stimulated pathways active in either normal physiology or pathological states from ISG signatures. We curated ~37 novel IFN-regulated pathways, including those involved in immune checkpoints and epigenetic regulation. These pathways were integrated into a web application, enabling users to analyse their own dataset. The ISGs, their functional properties, literature evidence, interactions and upstream regulator information were integrated into a Knowledge-Graph. This will form the foundation for modelling ISG pathways and networks systematically with leading edge artificial intelligence methods, namely graph neural networks and large language models.

P3-087 ‘Take 1’ on TAOK2: structural insights into TAOK2 and its involvement in antiviral cytokine signaling

Thierens L[1,2], Savvides S[1,2]
[1]Department of Biochemistry and Microbiology, Ghent University, [2]Unit for Structural Biology, VIB-UGent Center for Inflammation Research

Thousand-and-one-amino-acid kinase 2 (TAOK2) is a member of the MAP3K family that plays critical roles in various physiological and pathophysiological cellular responses, such as regulation of microtubule dynamics and antiviral immunity. The literature links sensing of viral dsRNA and the interaction of TAOK2 with ubiquitin ligase protein TRIM4 to the activation of innate immunity by type-I interferon signaling. However, the structural and mechanistic basis of these pleiotropic functions remains poorly understood, thereby presenting the field with a progress bottleneck.

TAOK2 is the largest of three TAOK family members and features a conserved kinase domain (KD) coupled to a much larger segment predicted to adopt a coiled-coil region and an additional leucine-rich region. These scaffolding parts of TAOK2 are thought to mediate interactions with TRIM4, dsRNA, and cellular membranes. At the same time, the most readily studied function of TAOK2 as a kinase via its conserved KD has not yet been linked to its much more substantial non-kinase segment.

We have embarked on an integrative structural biology approach to elucidate the structure-function landscape of TAOK2 and its complexes with TRIM4 and viral dsRNA. Our studies comprise TAOK2 protein production in mammalian expression systems to enable the study of candidate interactors of TAOK2 (dsRNA and TRIM4) via biophysical methods and structural studies by single-particle cryo-electron microscopy. Such knowledge will provide insights into the modular function and mechanistic synergies comprised in the enigmatic structure of TAOK2 and will facilitate further interrogation of TAOK proteins in physiology and disease, including, its role in cytokine induction in response to viral infections.

P3-088 TLR9 IFNa responses are highly variable and are associated with clearance of acute hepatitis C virus infection.

Sugrue J[1], Bondet V[1], Albert M[1], Rahal F[1], Bourke N[2], O'Farrelly C[2], Duffy D[1]
[1]Institut Pasteur, [2]Trinity College Dublin

Owing to differences in age, sex, genetics and environmental exposure, human immune responses are highly variable. This variation in immunity can predispose to, or protect against disease and infection. To investigate natural variation in anti-viral immunity we quantified IFNβ and all IFNα subtypes after whole blood stimulation with the viral agonists polyIC (TLR3), R848 (TLR7/8), GARD (TLR8), ODN (TLR9), and two live viruses: influenza A and Sendai, in 1,000 healthy donors of the Milieu Interieur Cohort. We observed age, sex and environmental factors to be associated with specific IFN responses. Current analysis is focused on integrating genetics, cellular, and epigenetic datasets to identify mechanisms underlying this immune variability. Interestingly, the ODN TLR9 response was the most variable of all agonists tested (coefficient of variation for ODN = 52%, polyIC = 13%, R848 = 12%, GARD = 14%, IAV = 19%). 18.3% (181/987) of donors responded to ODN with less than 10fg/ml of IFNα. To investigate the variable TLR9 response in a disease context we leveraged a separate cohort of women exposed to the hepatitis C virus (HCV) through a contaminated blood product. Based on records post exposure, 3 groups were identified: resistors, acute resolvers and those who developed chronic HCV infection and required therapeutic intervention. In response to whole blood stimulation with ODN we found that women who had acute HCV infection have reduced responses characterised by reduced interferon regulated gene upregulation, decreased IFNα signalling and reduced IFNα production. Associated with this reduced IFNa response to ODN we found enrichment for mTOR and mitochondrial respiration related genes in acute resolvers compared with resistors and those who were chronically infected, in which genes for fatty acid oxidation are enriched. Future work will assess whether the factors identified to explain variability in healthy IFN responses may underly protective immunity to HCV infection.

P3-089 High-dimensional immunomonitoring of human immune cell subsets by spectral flow-cytometry

Ziegler A[1], Sadeghi M[1], Mulenge F[1], Hennemann A[2], Kalinke U[1]
[1]TWINCORE, Centre for Experimental and Clinical Infection Research, [2]Hannover Medical School, Clinic for Neurology

Background
Human immunology is challenged by the limited availability of tissue specimens and blood samples. Thus, to understand human immune responses, the aim is to get as many information as possible from the scarce samples. Flow cytometry is a powerful method to analyze the distribution and activation status of specific immune cell subsets in a mixed cell suspension. For many years, high-parameter analysis was only possible by mass spectrometry, which is elaborative and costly. The development of spectral flow-cytometry facilitated an in-depth analysis of human immune cell subsets and promoted human immunology research.

Methods
To monitor human immune responses, peripheral-blood mononuclear cells (PBMCs) were isolated from blood samples and phenotypically analyzed by spectral flow-cytometry (ID7000, Sony Biotechnology). Three different panels of up to 30 fluorochrome-conjugated antibodies were established to investigate cell subsets and maturation-stages of B cells, T cells, and myeloid cells. The antibodies were carefully titrated for optimal labeling efficiency and specificity at prolonged overnight incubation times. An R-based pipeline for dimensionality reduction and FlowSOM-based clustering was used to compute the high-dimensional data in an unbiased manner.

Results
The established flow-cytometry panels allowed for a detailed analysis of immune cell subsets among PBMCs and whole blood samples. Applying the methodology to samples from post-COVID patients confirmed that disease-induced alterations in the distribution and the activation status of blood-derived immune cells can be detected with the described methodology.
With the extended incubation time of the cells with the fluorochrome-conjugated antibodies, the amount of antibodies needed for identification of different cell-subsets could be reduced by a factor three to ten.

Conclusion
We established an efficient flow-cytometry-based immunomonitoring pipeline that allows for in-depth analysis of human immune cell subsets. The thorough titration of the fluorochrome-conjugated antibodies and the increased incubation time dramatically decreased the reagent costs associated with the flow cytometric analysis.

P3-090 DDX3X suppresses RIG-I dependent type I Interferon production and promotes Mammarenavirus growth

Chiale C[1], Thelen F[1], Zuniga E[1]
[1]University of California San Diego

Type I interferons (IFN-I) are crucial antivirals and immune-regulators, but many viruses, such as Mammarenaviruses (MA), have evolved means to suppress them. We have previously identified that the host factor DEAD-box ATP-dependent-RNA-helicase 3 (DDX3X) suppresses IFN-I production and promotes MA replication in an immortalized cell line. To assess the in vivo role of DDX3X, we herein generated mice (DDX3XDCko) with selective deletion of DDX3X in Dendritic Cells (DCs), which are critical immune players targeted by MA, and infected them with lymphocytic choriomeningitis virus (LCMV). DDX3XDCko mice exhibited dramatically reduced viral loads in both plasmacytoid (p) and conventional (c) DC subsets, accompanied by decreased levels of antigen-specific CD8 T cells, particularly within the effector-like subset. We next generated DDX3X-deficient human primary fibroblasts and again detected reduced viral replication, and in this context, also observed increased IFN-I production upon LCMV infection. Importantly, follow-up studies using different Retinoic acid-inducible gene I (RIG-I) agonists revealed that the increased IFN-I production caused by DDX3X deficiency was independent of MA infection. Finally, we analyzed primary fibroblasts from patients with DDX3X Neurodevelopmental Disorder, a disease caused by de-novo DDX3X mutations and characterized by intellectual disabilities, and found that a mutation in residue 417 (Q417P) caused enhanced IFN-I signature after MA infection. Notably, this DDX3X mutation did not affect LCMV replication, demonstrating that DDX3X pro-viral and IFN-I-antagonistic activities can be biochemically dissociated. Together, our results demonstrate that DDX3X suppresses MA replication both in vivo and in primary human cells, and revealed that the DDX3X capacity to inhibit IFN-I production is independent of MA infection and may have broad implications for multiple RIG-I-mediated IFN-I responses. Our findings further uncover that a DDX3X disease-associated mutation causes enhanced IFN-I signature, which raise the possibility that a subset of patients with DDX3X Neurodevelopmental Disorders could be classified and perhaps treated as interferonopathies.

P3-091 Cell line adaptation of fowl adenovirus-4 induces genetic modification and generates protective immunity

Lee R[1], Yeo J[1], Sung H[1], Park J[1]
[1]Kangwon National University

(Background)
Fowl adenovuris-4 (FAdV-4) is a major avian virus that causes acute and lethal hepatitis. It gives a huge economic loss in poultry industry. Among structural proteins of FAdV-4, hexon and fiber2 are associated with immunopathogenesis.
(Methods and Results)
A virulent FAdV-4 strain was adapted in Leghorn male hepatoma (LMH) cell line by serial passages and we found the frameshift of fiber2 amino acid sequences. Then, we treated the attenuated virus (80 times passaged) before the virulent FAdV-4 challenge and examined pathogenesis. The treatment protected hosts from the infection and cleared the invading virus. In protected animals, activated CD4+ and CD8+ T cell populations were bigger. Myeloid cells, however, did not expand in the presence of the immunization. The functional genes for immune modulation were partially related with immune cell changes in the spleen but showed the deeper association in the liver.
(Conclusion)
The results imply that the genetic modification by cellular adaptation generated an attenuated virus and this can be a candidate for FAdV-4 vaccine strain.

P3-092 Fruits extract from Astilbe chinensis promotes anti-mycobacterial activity in macrophages

Son D[1,2], Lee J[1,2,3], Son S[1,2], Nguyen T[1,2], Kim J[1,2], Song C[1,2,3], Choi J[1,2,3]
[1]Department of Medical Science, College of Medicine, Chungnam National University, [2]Department of Microbiology, College of Medicine, Chungnam National University, [3]Translational Immunology Institute, Chungnam National University

Background: Astilbe chinensis is a perennial herb and one of the Saxifragaceae family that usually grows in moist fields. A.chinensis has been known as a traditional medicine to treat chronic bronchitis, arthralgia, cough, inflammation, pain, and headache. However, the effect of A.chinensis on anti-bacterial activity remains unclear.
Methods: We evaluated the effectiveness of fruits extract (FE) from Astilbe chinensis in Mycobacterium tuberculosis (Mtb)-infected bone marrow derived macrophages (BMDMs). We measured the activation of nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs) and autophagy pathway in Mtb-infected macrophages after FE stimulation using by western blot analysis. We also measured the levels of pro-inflammatory cytokines using by CBA analysis and colony-forming unit counts following treatment of Mtb with FE.
Results: To investigate the antimycobacterial activity of FE, we determined whether FE activates NF-κB and MAPKs pathways in BMDMs. We found FE stimulation enhanced the phosphorylation of Iκbα and JNK during Mtb infection. FE induced reactive oxygen species (ROS) production and inflammatory cytokines such as TNF-α and MCP-1 in Mtb infected BMDMs. Next, we assessed the expression of autophagy-related molecules in FE-treated BMDMs after Mtb infection. As expected, FE treatment increased the level of LC3-II and decreased the level of P62 during Mtb infection. Remarkably, the total amount of Mtb H37Rv was significantly reduced in FE-stimulated BMDMs.
Conclusion: Administration of FE in Mtb-infected macrophages resulted in the activation of MAPK, induction of ROS and inflammatory cytokine synthesis. The accumulated ROS induced autophagy reaction, leading to suppression of Mtb in macrophages. Our findings might facilitate the development of new therapies for TB patients using herbal extracts such as FE.

P3-093 Identification of neuronal interferon-dependent antiviral signaling upon viral infection

Negatu S[1], Vazquez C[1], Bannerman C[1], Saldaña X[1], Han B[1], Jurado K[1]
[1]University of Pennsylvania, Perelman School of Medicine

Neurotropic viruses are the most common cause of infectious encephalitis. Published studies have increased our understanding of how resident glial cells and infiltrating leukocytes contribute to neuroimmune responses during pathogen invasion. Despite neurons being readily infected by many neurotropic viruses, neuron-intrinsic immune responses remain poorly described. This leads to our central question of how do neurons orchestrate effective antiviral immunity during CNS infection? Using La Crosse Virus (LACV), an emerging RNA bunyavirus, we observed widespread infection and viral growth in both murine cortical neurons and human stem cell-derived cerebral organoids. Transcriptomics approaches revealed infection of cortical neurons induced expression of interferon-stimulated genes (ISGs). In cerebral organoids, we found that bystander uninfected neurons have higher ISG expression than neighboring infected neurons and expression of the ISGs were IFNβ- and Jak- dependent. Future directions aim to characterize how infected neurons communicate with uninfected bystander neurons during infection. To address this in murine cortical neurons, I have optimized a ProbeSeq pipeline where I infect cortical neurons and characterize immune activation in bystander uninfected and highly infected populations using fluorescence in situ hybridization and downstream bulk RNA-sequencing. To address this in cerebral organoids, I will be infecting organoids with LACV and conducting spatial transcriptomics on organoid sections. Together, these techniques will define neuron specific protective anti-viral gene signatures.

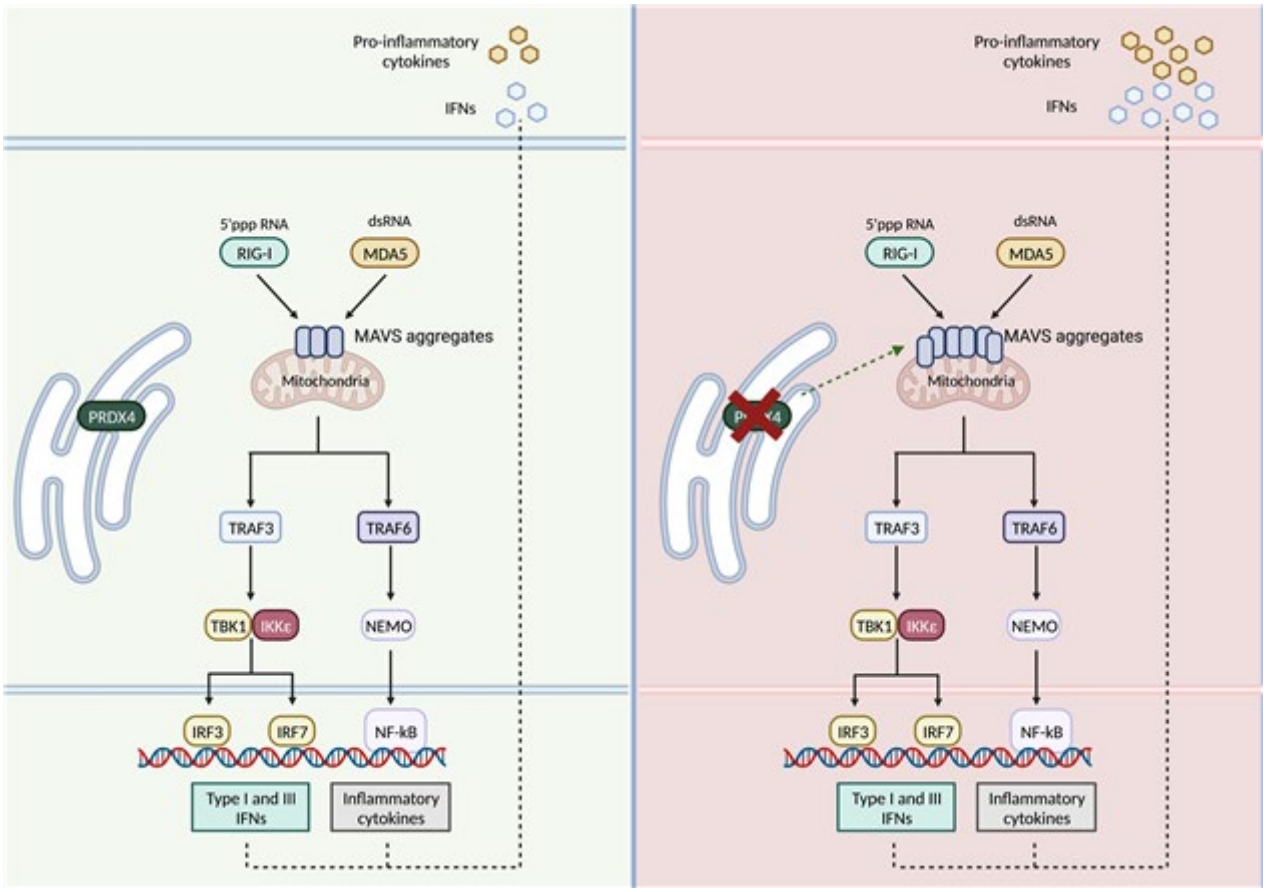
P3-094 Peroxiredoxin 4 links redox signaling to antiviral innate immunity in hepatocytes

Sularea V[1], Sugrue J[1], Alexandridi M[2], Palermo E[2], Hiscott J[2], O'Farrelly C[1,3]
[1]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, [2]Pasteur Laboratories, Istituto Pasteur Italia – Fondazione Cenci Bolognetti, [3]School of Medicine, Trinity College Dublin

Background:
Infection of hepatocytes can lead to cellular oxidative stress through excessive production of reactive oxygen species (ROS). ROS have a physiological role acting as secondary messengers in cellular signaling, and are fundamental in activation of several immune activities, including innate antiviral response. ROS homeostasis, regulated by ROS production and antioxidant enzymes, is crucial in modulating cell signaling and avoiding cell damage during viral infection. However, the molecular mechanisms through which antioxidant enzymes are involved in the innate antiviral response are not fully understood.

Results:
By analysing publicly available RNA-sequencing and proteomic datasets from in vivo hepatotropic infection with LCMV we found differential expression of several antioxidant proteins, including downregulation of peroxiredoxin 4 (PRDX4). To assess the effect of PRDX4 on hepatic antiviral activity we silenced PRDX4 in the Huh7s hepatocyte cell line. Silencing of PRDX4 resulted in increased type I and III IFN, as well as pro-inflammatory cytokine expression following stimulation with dsRNA (transfected polyI:C). These results were confirmed in the context of an in vitro infection with Dengue virus. To test whether this phenotype was also present in other cell types we used HEK293T fibroblasts. Silencing of PRDX4 increased their antiviral activity upon transfection with polyI:C, suggesting that this mechanism may be conserved in different cell types. Lastly, as transfected polyI:C signals via MDA5, we sought to determine whether PRDX4 silencing increases activation of the MDA5 pathway. To this end, we quantified the aggregation or mitochondrial antiviral protein (MAVS), a key protein located downstream of MDA5, and found that PRDX4 silencing increases MAVS aggregation.

Conclusion:
Here, we identified a novel role for the antioxidant enzyme PRDX4, which restrains cytosolic PRR-dependent antiviral activity by limiting MAVS activation. Future work will focus on determine the molecular mechanism through which PRDX4 deficiency increases MAVS-dependent antiviral activity.


Pro-inflammatory cytokines
IFNs
5'ppp RNA
dsRNA
RIG-I
MDA5
MAVS aggregates
Mitochondria
PRDX4
TRAF3
TRAF6
TBK1
IKKε
NEMO
IRF3
IRF7
NF-kB
Type I and III IFNs
Inflammatory cytokines
Pro-inflammatory cytokines
IFNs
5'ppp RNA
dsRNA
RIG-I
MDA5
MAVS aggregates
Mitochondria
TRAF3
TRAF6
TBK1
IKKε
NEMO
IRF3
IRF7
NF-kB
Type I and III IFNs
Inflammatory cytokines

P3-095 IL-1 and chemokine responses are modulated by long-term in vivo vitamin D3 supplementation in the bovine model

Reid C[1], Flores-Villava S[2], Remot A[3], Kennedy E[4], O'Farrelly C[1], Meade K[2]
[1]School of Biochemistry and Immunology, Trinity College Dublin, [2]School of Agriculture and Food Science, University College Dublin, [3]INRAE, Université de Tours, [4]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark

Background: In vitro studies have found vitamin D induces an increased anti-inflammatory and decreased pro-inflammatory innate cytokine profile in an array of immune cell populations. Therefore, it is thought vitamin D supplementation may protect against inflammatory disease through modulating cytokine responses. However, current evidence remains controversial, partially due to a lack of controlled long-term vitamin D supplementation trials in vivo and reliable approaches for reproducibility assessing immune function.
Methods: A standardized whole blood immunophenotyping assay was used to compare innate immune responses, including gene expression of an array of innate cytokines and IL-1β, IL-6 and IL-8 protein production, to infection relevant TLR ligands (LPS, Pam3CSK4 and R848). Blood cells from Holstein-Friesian calves supplemented with vitamin D (n=12) from birth until 7 months of age were compared with cells from control calves (n=10) raised on an industry standard diet.
Results: Transcriptomic analysis in unstimulated whole blood cells revealed increased expression of type I interferons and chemokines in vitamin D supplemented calves. In contrast, IL-1 and inflammasome gene expression was decreased. In response to LPS stimulation, vitamin D supplementation increased expression of CASP1, CX3CR1, CAT, whereas STAT1 was decreased. Stimulation with Pam3CSK4 revealed increased expression of IL1A, IL1B and CAT genes; and decreased C5AR1 expression in response to vitamin D. In response to the viral ligand R848, STAT1 and S100A8 expression was significantly decreased. Vitamin D supplementation also increased IL-1 and inflammasome gene expression signature in response to LPS and Pam3CSK4, with ELISA confirming increased IL-1β protein production. In contrast, vitamin D supplementation decreased chemokine gene expression signature in response to R848, with decreased IL-8 protein expression exhibited in response to all TLR ligands also found.
Conclusion: These results demonstrated expression of several cytokine, chemokine and inflammasome genes were impacted by vitamin D supplementation which could have important implications for disease susceptibility.

P3-096 Hyper-induction of IL-6 in response to TLR1/2 ligand stimulation in calves with bovine respiratory disease

Reid C[1], Donlon J[2], Remot A[3], Kennedy E[4], O'Farrelly C[1], McAloon C[2], Meade K[5]
[1]School of Biochemistry and Immunology, Trinity College Dublin, [2]School of Veterinary Medicine, University College Dublin, [3]INRAE, Université de Tours, [4]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, [5]School of Agriculture and Food Science, University College Dublin

Background: Variability in how viral infection primes inflammatory responses has consequences for disease progression. Use of standardized assays found variation in ex-vivo stimulated type I interferon and pro-inflammatory cytokine responses in SARS-CoV-2 infected individuals associated with disease severity. Bovine respiratory disease (BRD) is a polymicrobial disease where viral infection weakens the immune system resulting in pneumonia. Despite a leading cause of mortality in young calves, the induced cytokine responses during BRD infection have not been explored in a reproducible manner.
Methods: A standardized whole blood immunophenotyping assay was used to compare innate immune responses, including gene expression of an array of innate cytokines and IL-1β, IL-6 and IL-8 protein production, to infection relevant TLR ligands (LPS, Pam3CSK4 and R848). Blood cells from calves diagnosed with BRD by lung ultrasonography were compared with cells from uninfected controls from the same farms.
Results: Hyper-induction of IL-6 protein expression in response to all TLR ligands was found in calves with BRD, most significantly to TLR1/2 ligand Pam3CSK4. An optimal IL-6 threshold of >1780pg/ml had a 71% true positive rate and 5% false positive rate in response to Pam3CSK4 in diagnosing BRD. Gene expression analysis in response to Pam3CSK4 revealed BRD infection significantly increased expression of IL-6 receptor gene IL6R, IFN-γ receptor gene IFNGR1, and vitamin D pathway transcription factor gene RXRA. Conversely, BRD infection significantly decreased expression of IL-1 family genes IL1A and IL1RN, and the gene encoding a vitamin D pathway enzyme, CYP27B1. IL-1 and inflammasome pathway and chemokine gene expression signatures were found decreased in response to Pam3CSK4.
Conclusion: These results show BRD infection altered immune responsiveness upon TLR1/2 activation via hyper-induction of IL-6 and impaired chemokine and inflammasome gene expression signatures. This study highlights cytokine responses with potential diagnostic and prognostic value to help reduce disease burden and antimicrobial usage.

P3-097 Convergent evolution of SARS-CoV-2 variants of concern to enhance innate immune suppression

Reuschl A[1], Thorne L[1], Bouhaddou M[2], Whelan M[1], Furnon W[3], De Lorenzo G[3], Cowton V[3], Patel A[3], Palmarini M[3], Zuliani-Alvarez L[2], Krogan N[2], Towers G[1], Jolly C[1]
[1]University College London, [2]Quantitative Biosciences Institute , [3]MRC-University of Glasgow Centre for Virus Research

Airway epithelial cells are a critical first line of defence against SARS-CoV-2, mounting potent interferon (IFN) and inflammatory responses to inhibit infection and to instruct and determine local and systemic immune responses to the virus. SARS-CoV-2 adaption to humans is driven by selective pressure from host immunity, resulting in increasingly transmissible variants of concern (VOCs). These include Alpha, Beta, Gamma, Delta, and Omicron, all of which arose independently. Here, we combined global proteomic and genomic approaches with molecular virology to compare VOC replication and host responses to understand the selective forces driving SARS-CoV-2 evolution. We discovered VOCs evolved convergent strategies to remodel the host response by modulating viral RNA and protein levels, altering viral and host protein phosphorylation levels, and rewiring virus-host protein-protein interactions. Specifically, suppression of IFN and interferon stimulated gene (ISG) responses correlated with the expression of viral innate immune antagonist proteins, including Orf6, N and Orf9b. We validated the contribution of these key innate antagonists to the antiviral response during VOC infection using reverse-genetics mutants.
Although Alpha, Beta, Gamma, and Delta ultimately converged in the suppression of IFN and ISGs relative to earliest circulating SARS-CoV-2 viruses, Omicron sublineage BA.1 and BA.2 did not. Strikingly, later Omicron subvariants BA.4 and BA.5 did more potently suppress innate immunity than early subvariants, which again correlated with Orf6 protein levels, akin to our observations for VOCs Alpha to Delta. In conclusion, we have shown that evolution of enhanced innate antagonism is a convergent feature of SARS-CoV-2 VOCs likely contributing to improved transmission and decreased immune protection from severe disease. Importantly, these findings highlight the need for genetic monitoring of non-spike regions in the SARS-CoV-2 genome.

P3-098 Innate Lymphoid Cells Reside in the Human Female Reproductive Tract and Display Antiviral Properties

Werner A[1], Moreno de Lara L[2], Parthasarathy S[1], Carrillo-Salinas F[2], Rodriguez-Garcia M[2]
[1]Immunology Program, Tufts University Graduate School of Biomedical Sciences, [2]Department of Immunology, Tufts University School of Medicine

Sexual contact is the main HIV-acquisition route in women. While low transmission rates per sexual act indicate effective local protection against HIV infection in the female reproductive tract (FRT), these mechanisms remain largely unknown. Identification of the innate mechanisms that protect against infection in the FRT will enable strategies to prevent HIV acquisition in women. Innate lymphoid cells (ILCs) are tissue-resident cells specialized in cytokine secretion and important for mucosal protection against infections in mice. However, human mucosal ILCs remain poorly characterized and their potential role in HIV prevention is completely unknown. Here, we characterize ILCs in the human FRT and determine their ability to respond to HIV.

Human hysterectomy samples (n=30) were enzymatically digested to generate mixed-cell suspensions from distinct anatomical regions (endometrium, endocervix, and ectocervix). Phenotypical and functional ILC characterization was performed using high-dimensional flow cytometry (23-parameters). To define anti-HIV responses, mixed-cell suspensions were stimulated with HIV for 30 minutes prior to cytokine analysis by flow cytometry.

FRT ILCs represented <5% of mononuclear cells. ILC subset characterization revealed anatomical compartmentalization, with ILC3 predominance in the endometrium (63% of ILCs), while ILC1s were predominant in the ectocervix (48%). ILCs expressed intraepithelial residency (CD69, CD103), cytotoxicity markers (CD161, CD49d), and HIV receptors (CD4, CXCR4, CCR5), with CD4 expression restricted to ILC1s. ILC1s constitutively produced the antiviral cytokine IFN-γ (14%). ILC3s constitutively produced IL-22 (46%), important for barrier maintenance, with production enhanced in CCR6+ CD103+ ILC3s. In vitro HIV stimulation induced rapid ILC degranulation, and changes in intracellular IL-22 content, indicating antiviral responses in FRT ILCs.

Our study represents the first deep phenotypical and functional characterization of ILCs throughout the human FRT. Our findings that genital ILCs express receptors to recognize HIV, and are constitutively armed with antiviral cytokines, suggest ILCs could represent a novel mechanism for protection against HIV acquisition.

P3-099 Gasdermin D modulates the protective cGAS/STING/ type I Interferon pathway in schistosomiasis.

Kalantari P[1]
[1]Penn State University

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

P3-100 Noroviruses encode a MLKL-like pore forming protein that initiates cell death to induce viral egress

Wang G[1], Orchard R[1], Hancks D[1], Reese T[1]
[1]University Of Texas Southwestern Medical Center

Non-enveloped viruses require cell lysis to release new virions from infected cells, suggesting that these viruses require mechanisms to induce cell death. Noroviruses are one such group of viruses, but a mechanism of norovirus-infection triggered cell death and lysis are unknown. Here we have identified a molecular mechanism of norovirus-induced cell death. We found that the norovirus-encoded NS3/NTPase contains a N-terminal four helix bundle domain homologous to the membrane disruption domain of the pseudokinase Mixed Lineage Kinase Domain-Like (MLKL). Norovirus NS3 acquired a mitochondrial localization signal, thereby inducing cell death by targeting mitochondria. NS3 full length (NS3-FL) and N-terminal fragment (NS3-NT) bound mitochondrial membrane lipid cardiolipin, permeabilized mitochondrial membrane and induced mitochondrial dysfunction. Both the N-terminal region and the mitochondrial localization motif of NS3 were essential for cell death, virus egress from cells and virus replication in mice. These findings suggest that noroviruses stole a MLKL-like pore forming domain and co-opted it to facilitate viral egress by inducing mitochondrial dysfunction.

P3-101 IFNAR signaling plays a role regulating redox responses of brain and peripheral myeloid cells.

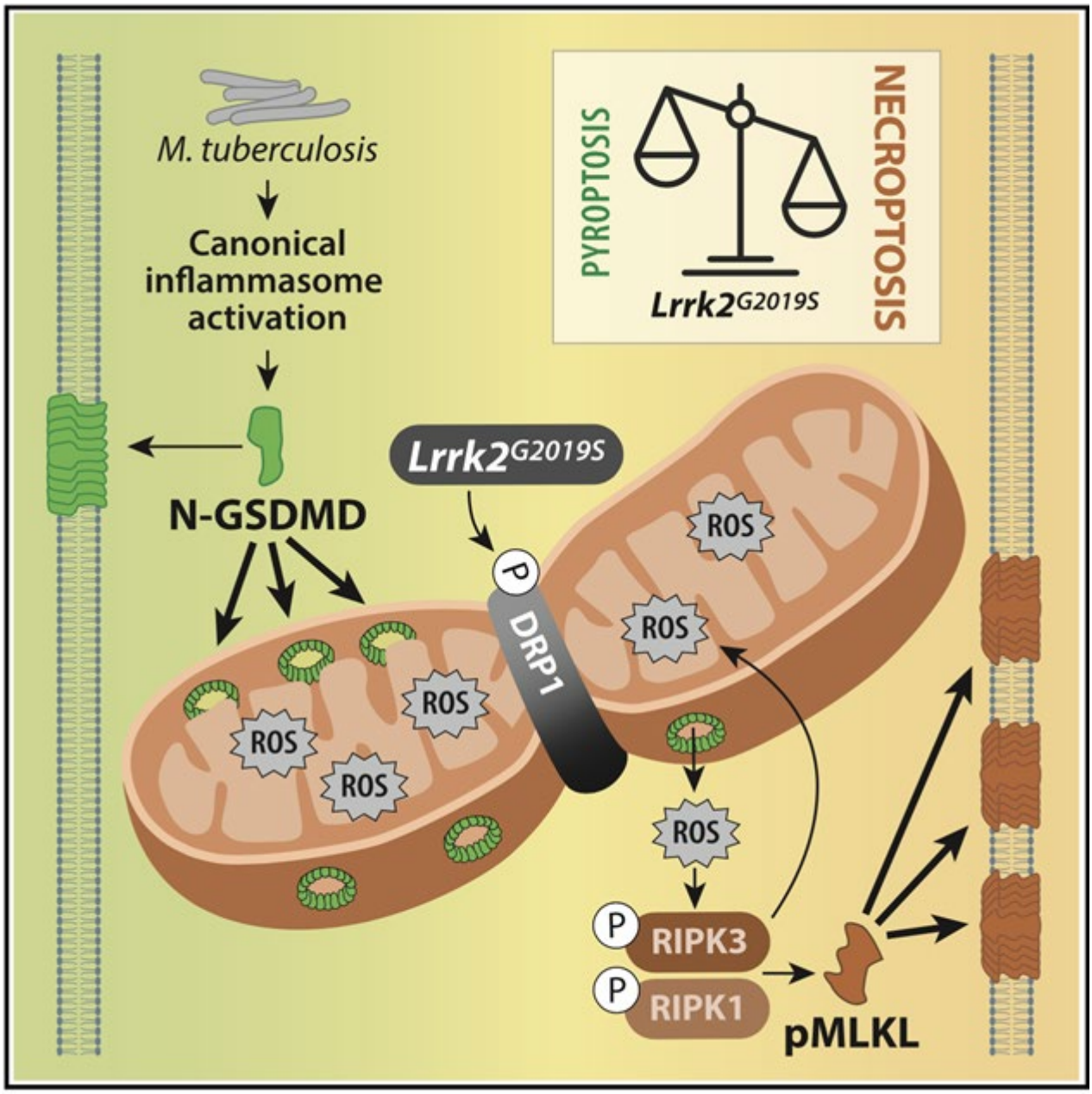
Weindel C[1], Ellzey L[1], Huntington T[1], Bell S[1], Vail K[1], Kang T[2], Welsh J[2], Srinvasan R[1], Patrick K[1], Watson R[1]
[1]Texas A&M School Of Medicine, [2]Texas A&M School of Veterinary Medicine

Chronic inflammation in the periphery can precede the classical symptoms of Parkinson's disease (PD), suggesting that peripheral inflammation and bidirectional crosstalk between the brain and periphery might contribute to PD disease progression. The "two-hit hypothesis" purports that genetic predisposition, combined with environmental insults like bacterial infection, leads to the development of PD. We set out to test the two-hit hypothesis by investigating how genetic disruption of PD-associated genes impacts the ability of peripheral and brain-resident immune cells (macrophages and microglia, respectively) to sense and respond to infection and stress. We discovered that loss of leucine-rich repeat kinase 2 (Lrrk2), induces mitochondrial stress in peripheral macrophages resulting in an elevated basal type I interferon (IFN) response due to mitochondrial stress and subsequent mitochondrial DNA engagement with the cGAS DNA sensing pathway. Interestingly, we identified that loss of LRRK2 results in a paradoxical response in microglial cells of the brain. Specifically, we found that basal IFN responses are reduced, and mitochondria are protected from stress, tempering proinflammatory cytokine expression in response to IFN. Through these LRRK2-dependent phenotypes in distinct macrophage populations we uncovered a role for IFNAR signaling in the regulation of the major redox transcription factor NRF2. This new function of IFNAR helps explain why Lrrk2 KO microglia and macrophages differ in their responses to mitochondrial stress. Consistent with an altered inflammatory phenotype in vitro, loss of LRRK2 impacts activation of microglial cells in PD-relevant regions of the brain in vivo during peripheral infection with the important human lung pathogen, Mycobacterium tuberculosis. These results illustrate that LRRK2 can play dichotomous roles within different immune cell populations and supports a two-hit hypothesis model where peripheral infection coupled with LRRK2 genetic variants creates a brain-peripheral immune repertoire that may contribute to PD.

P3-102 Mitochondrial ROS promotes susceptibilityto Mycobacterial tuberculosis infection via gasdermin D-mediated necroptosis

Watson R[1], Weindel C[1], Martinez E[1], Mabry C[1], Coleman A[1], Patrick K[1]
[1]Texas A&m University, School Of Medicine

Although mutations in mitochondrial-associated genes are linked to inflammation and susceptibility to infection, their mechanistic contributions to immune outcomes remain ill-defined. We discovered that the disease-associated gain-of-function allele Lrrk2G2019S (leucine-rich repeat kinase 2) perturbs mitochondrial homeostasis and reprograms cell death pathways in macrophages. When the inflammasome is activated in Lrrk2G2019S macrophages, elevated mitochondrial ROS (mtROS) directs association of the pore-forming protein gasdermin D (GSDMD) to mitochondrial membranes. Mitochondrial GSDMD pore formation then releases mtROS, promoting a switch to RIPK1/RIPK3/MLKL-dependent necroptosis. Consistent with enhanced necroptosis, infection of Lrrk2G2019S mice with Mycobacterium tuberculosis elicits hyperinflammation and severe immunopathology. Our findings suggest a pivotal role for GSDMD as an executer of multiple cell death pathways and demonstrate that mitochondrial dysfunction can direct immune outcomes via cell death modality switching. To better understand the consequences of this cell death “switch” in vivo, we performed single-cell RNA sequencing of CD45+ cells and identified significant differences in the both the number and transcriptional signature of neutrophils in the lungs of Mtb-infected WT and Lrrk2G2019S mice. Specifically, we found that neutrophil recruitment to the lung is enhanced in Mtb infected Lrrk2G2019S mice and a large portion of these neutrophils are immature. In response to treatment with the LRRK2 inhibitor DN9713 (Denali Therapeutics), Lrrk2G2019S mice still experience elevated lung neutrophil infiltration, but these neutrophils are mature/terminally differentiated. Consequently, Lrrk2G2019S mice treated with DN9713 have significantly reduced bacterial loads in the lung and spleen. These data suggest that although there is an increase in neutrophils and “inflammation” early during infection in Lrrk2G2019S mice, this inflammation ultimately leads to an immunosuppressive environment that supports Mtb replication. Together, our work provides insights into how LRRK2 mutations manifest or exacerbate human diseases and identifies GSDMD-dependent necroptosis—and potentially LRRK2 itself—as targets to limit immunopathology during Mtb infection.


M. tuberculosis
Canonical inflammasome activation
N-GSDMD
PYROPTOSIS
NECROPTOSIS
Lrrk2G2019S
Lrrk2G2019S
P
DRP1
ROS
ROS
ROS
ROS
ROS
ROS
P
RIPK3
P
RIPK1
pMLKL

P3-103 Interleukin 27 (IL-27) signaling at the maternal-fetal interface contributes to innate antiviral immunity during congenital infection

Merlino M[1], Clements R[1], Barksdale B[1], Lopez A[1], Hunter C[2], Jurado K[1]
[1]University of Pennsylvania, Department of Microbiology, [2]University of Pennsylvania, Department of Pathobiology

Cytokines maintain homeostasis throughout pregnancy and have diverse responses to pathogens at the maternal-fetal interface. Interleukin 27 (IL-27) is a cytokine that is highly expressed in the placenta, however its functional role during pregnancy is unknown. In other contexts, IL-27 is a potent regulator of inflammation and mediates antiviral immunity in the skin through antiviral gene expression. Therefore, our objective was to determine how IL-27 contributes to immune responses at the maternal-fetal interface during congenital viral infection. To begin, we utilized an immunocompetent mouse model for Zika virus (ZIKV) infection to assess pregnancy and infection outcomes in the presence and absence of IL-27 signaling. IL-27-deficient mice were obtained by delivering IL-27 neutralizing antibody to pregnant dams via intraperitoneal injection at regular timepoints prior to and during congenital ZIKV infection, or by genetic deletion of the IL-27 receptor (IL27RA). At gestational day 13.5, mice were sacrificed and gross morphologies, weights, and ZIKV burdens of fetal and placental tissues were assessed. I observed significantly higher levels of fetal pathology (resorption) in infected, IL-27-deficient dams relative to control dams, despite similar fetal viral burdens. Interestingly, the placental tissues of infected, IL-27-deficient dams had significantly higher ZIKV burdens relative to IL-27-competent dams. These data indicate that IL-27 is antiviral in the placenta. To further define the antiviral activity of IL-27 in the placenta, I will utilize a primary human trophoblast organoid model, which I have shown recapitulates placental IL-27 expression and signaling in vitro. In future studies, I will assess IL27RA+ immune cell responses at the maternal-fetal interface during congenital infection as a possible mechanism of fetal pathology. Collectively, our studies will enhance our understanding of the immunological crosstalk between mother and fetus during healthy pregnancies and could ultimately improve fetal and neonatal outcomes during congenital infections.

IP3-104 L-27 signaling supports regulatory T cells at steady state and during infection

Lanzar Z[1], Aldridge D[1], Perry J[1], Phan A[1], Hunter C[1]
[1]University Of Pennsylvania

Interleukin-27 is a heterodimeric cytokine that mediates pro- and anti-inflammatory activities. While IL-27 levels are low at homeostasis, antigen presenting cells rapidly produce IL-27 upon receiving stimulation via toll-like receptors, interferons, or infection. IL-27 signaling on regulatory T (Treg) cells promotes localization and suppressive action in models of autoimmune disease and infections, such as Toxoplasma gondii. Previous studies utilizing Treg-specific IL27 signaling deficient (Foxp3 YFP-Cre+ IL27Rflox/flox) mice have described minimal impact on the Treg compartment in the absence of IL-27 signaling at steady state. Utilizing male Foxp3-YFP-Cre+ IL27Rflox/flox and female Foxp3-YFP-Cre+/- IL27Rflox/flox mice, we show that constitutive loss of IL-27 signaling at steady state does not alter the total number of Tregs, but IL-27 signaling does confer an advantage in a competitive setting at steady state. The use of high parameter flow cytometry on the Treg compartment shows that loss of IL-27 signaling led to a reduction in the frequency of effector Treg (eTreg) cells. Complimentary studies utilizing IL27R-/-:WT (50:50 ratio) mixed bone-marrow chimera mice demonstrate that the proportion of Tregs deficient in IL-27 signaling declines at steady state, similar to the transgenic female mice. Induction of selective pressure on the IL27R-/- and WT compartments with Toxoplasma gondii induces a greater decline in the proportion of IL27R-/- Tregs than at steady state. While IL-27 signaling is dispensable for determining the size of the Treg pool, our data implicates IL-27 signaling as an important factor in the overall fitness of the Treg population.

P3-105 IL-27 regulates monocyte development and function during acute Toxoplasma gondii infection

Aldridge D[1], Lanzar Z[1], Phan A[1], Christian D[1], Hunter C[1]
[1]University Of Pennsylvania, Department of Pathobiology

IL-27 is an immuno-modulatory cytokine composed of the subunits p28 and EBi3 and can directly impact hematopoietic stem and progenitor cells (HSPCs). During infection with the protozoan parasite Toxoplasma gondii, mice that lack IL-27 show dramatic alterations in their responses to infection, culminating in lethal immunopathology. While this pathology is characterized by enhanced inflammatory monocyte responses, it is unclear whether these responses are driven by aberrant CD4+ T cell responses or direct interactions between myeloid cells and IL-27. HSPCs show the highest expression of the IL-27R in the bone marrow and activate STAT1 in response to treatment with IL-27 in vitro. During toxoplasmosis, there was an increase in monocyte progenitors in IL-27 deficient mice, peaking at 5 dpi and consistent with the kinetics of IL-27 production in the bone marrow. This enhanced monopoiesis resulted in increased peripheral monocyte numbers, with enhanced inflammatory cytokine production. As HSPCs are the only cells in the monocyte lineage that express high levels of the IL-27R, these results suggest that IL-27 can regulate both monocyte development and function through direct interaction with HSPCs in the bone marrow. To test the IL-27 intrinsic role of these effects, mixed bone marrow chimeras were generated with bone marrow from WT and IL-27R -/- mice. After reconstitution, these mice were infected and at 5 dpi had an enhanced proportion of inflammatory, CCR2hi monocytes from the IL-27R -/- lineage. Taken together, these data suggest a novel role for IL-27 in biasing cell fate and restraining the induction of a monocytic response during infection, implicating IL-27 as a novel target for the regulation of aberrant myeloid responses ranging from infection to malignancies.

P3-106 Deep Characterization of Viral- and Self-Peptide Induced Cellular Immune Responses

Nickle R[1], Zemlickova E[1], Tam M[1], Wiethe C[1]
[1]BioLegend

Biological processes are complex, and to understand them in a holistic manner, researchers need to use multiple tools and innovative approaches to capture that complexity. As our understanding of cell physiology and pathological conditions advances, the need for more sophisticated technology and applications continues to push the development of integrated solutions, including quantification of cytokines and other secreted factors as they are at the core of the cellular processes that regulate immune responses. Here, we describe and compare the activation and expansion of specific T cell responses against peptide pools (15-mers with 11-aa overlap) containing SARS-CoV-2 WT or Delta Variant Spike Protein, Myelin Basic Protein (MBP, Self/Multiple Sclerosis), or Melan-A/MART-1 (Self/Neoantigen). Cellular phenotype was characterized by flow cytometry and single cell multiomics, and cytokine profiling was performed with LEGENDplex™ Panels which can measure up to 14 soluble factors simultaneously. Assessing a total of 26 cytokines, we were able to correlate cellular functional state with functional cytokine and chemokine secretion. Our results indicate that SARS-CoV-2 peptide responses strongly polarize towards CD4 inflammatory cytokine responses, self-peptide MBP also polarizes towards CD4 responses but less potently and in a more regulatory manner than SARS-CoV-2, whereas self-peptide/neoantigen stimulation (Melan-A) polarizes toward CD8 inflammatory cytokine responses. By characterizing the cell surface and intracellular protein expression along with cytokine secretion profile, we can better correlate the cells' phenotype with their functional state. Taken together, our data demonstrate that Regulatory T cell and Effector T cell populations expand differently and possess unique cellular and molecular signatures in response to viral vs. self-peptide stimulation, with a specific cytokine expression profile associated with distinct T cell subsets.

P3-107 Antagonism of Innate Immune is not a Mechanism of Hantavirus Persistence in Reservoir

Klimaj S[1], Kell A[1]
[1]University Of New Mexico

Hantaviruses are pathogenic agents endemic to rodent reservoirs throughout the world. Seoul orthohantavirus (SEOV) is a unique member of this family as the only orthohantavirus species found worldwide in its reservoir, the common Norway rat (Rattus norvegicus). Zoonosis from rats to humans can result in hemorrhagic fever with renal syndrome. This disease is characterized by acute infection of endothelial cells, resulting in strong inflammatory responses, vascular leakage leading to systemic edema, and organ failure. The underlying mechanisms for hantavirus disease are thought to be immune-mediated. Yet, in SEOV's reservoir, infection is persistent and asymptomatic. Here we demonstrate that, contrary to the robust innate immune activation elicited by SEOV infection in human endothelial cells, SEOV infection in rat endothelial cells does not induce interferon-stimulated gene (ISG) expression. Importantly, we report that rat endothelial cells do respond to virus challenges from other hantaviruses and rodent-borne viruses, Sendai and encephalomyocarditis viruses. We hypothesized that SEOV has evolved to antagonize reservoir type I interferon (IFN) signaling pathways, thus inhibiting innate immune activation. We tested the ability of SEOV to inhibit innate immune signaling through the RIG-I-like receptors, RIG-I and MDA5, and the type I IFN receptor. We infected primary rat endothelial cells with SEOV and subsequently treated the cells with agonists for RIG-I and MDA5 (ssRNA and dsRNAs) or recombinant type I IFN. We then measured ISG expression by RT-PCR and western blotting compared to mock-infected agonist-treated controls. Surprisingly, we observed no inhibition of ISG expression in SEOV-infected cells, suggesting that SEOV does not directly antagonize these signaling pathways. These findings emphasize substantial differences in innate immune responses to SEOV infection between the reservoir and human. Ongoing studies will define how SEOV modulates host innate immune responses and how sensing of SEOV infection by human endothelial cells is linked to vascular dysfunction.

P3-108 The role of CD200 in the development and function of regulatory T cells induced by B cells

Liu Y[1], Chu K[1], Chiang B[1]
[1]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University

Background
The ways to suppress inflammation could be divided into two parts, including downregulate inflammatory cells directly and upregulate tolerance induction. Our Previous studies have identified a particular subset of regulatory T cells induced by B cells, termed Treg-of-B cells. CD200 has been demonstrated to plays the role in regulating inflammation. CD200 transduces anti-inflammatory signal through engaging with its receptor, CD200R. Moreover, CD200 could induce Foxp3 expression in regulatory T (Treg) cells with direct or indirect pathway. In this study, we aimed to clarify the role of CD200 in the development and functions of Treg-of-B cells.

Methods
To further identify the functional surface molecules on Treg-of-B cells, we assayed the expression of CD200 level on these T cells. In addition, anti-CD200 and anti-IL20 RB antibodies were used to assess the development and functions of Treg-of-B cells. Particularly, the cytokine profile and suppressive activities of these Treg-of-B cells after CD 200 inhibition were also assayed in the study.

Results
Here we demonstrated that CD200 plays the important role in Peyer's patch B cell inducing Treg (so-called Treg-of-B(P)) cells. Peyer's patch B cells provide the CD200 as the first signal to help T cells expressing elevated CD200 and CD200R. with the phosphorylation of STAT6, T cells express CD39 to increase the expression of LAG3 and IL-10, which regulate the suppression function of Treg-of-B(P) cells.

Conclusion
In this study, we reported a particular subset of regulatory T cells induced by B cells. We further found that CD200 was very important for the induction and function of these Treg-of-B cells. Further elucidation on the functional molecules on these Treg-of-B cells might shed light on future development of novel therapeutic approaches for immune regulation.

P3-109 Zika virus infection induces IL-1β-mediated inflammatory responses by macrophages in the brain of an adult mouse model

Kim D[1,2], Jeong G[1,3], Lee S[1], Yoon G[1], Kwon Y[1,2]
[1]Korea Research Institute Of Chemical Technology, [2]University of Sciences and Technology (UST), [3]Harvard Medical School

Background: During the 2015/16 Zika virus (ZIKV) epidemic, ZIKV-associated neurological diseases were reported in adults, including microcephaly, Guillain-Barre syndrome, myelitis, meningoencephalitis, and fatal encephalitis. However, the mechanisms underlying the neuropathogenesis of ZIKV infection are not yet fully understood.
Methods: In this study, we used an adult ZIKV-infection mouse model (Ifnar1−/−) to investigate the mechanisms underlying neuroinflammation and neuropathogenesis.
Results: ZIKV infection induced the expression of proinflammatory cytokines, including IL-1β, IL-6, IFN-γ, and TNF-α, in the brains of Ifnar1−/− mice. RNA-seq analysis of the infected mouse brain also revealed that genes involved in innate immune responses and cytokine-mediated signaling pathways were significantly upregulated at six days post infection. Furthermore, ZIKV infection induced macrophage infiltration and activation, and augmented IL-1β expression, whereas microgliosis was not observed in the brain. Using human monocyte THP-1 cells, we confirmed that ZIKV infection promotes inflammatory cell death and increases IL-1β secretion. In addition, expression of the complement component C3, which is associated with neurodegenerative diseases and known to be upregulated by proinflammatory cytokines, was induced by ZIKV infection through the IL-1β-mediated pathway. An increase in C5a produced by complement activation in the brains of ZIKV-infected mice was also verified.
Conclusion : Taken together, our results suggest that ZIKV infection in the brain of this animal model augments IL-1β expression in infiltrating macrophages and elicits IL-1β-mediated inflammation, which can lead to the destructive consequences of neuroinflammation.

P3-110 Humoral Immunity against SARS-CoV-2 in Patients with Solid Cancer

Lee K[1], Lee E[1], Oh J[1]
[1]Graduate School of Medical Science and Engineering, KAIST

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic has resulted in severe infections and fatalities, particularly among the elderly and immunocompromised individuals. Cancer patients, in particular, are known to be highly susceptible to infections and exhibit reduced vaccine effectiveness due to dysregulated systemic immunity resulting from cancer itself and various cancer treatments. Therefore, the development of effective SARS-CoV-2 vaccine strategies tailored specifically for cancer patients has become of utmost importance for oncologists. However, the majority of studies on SARS-CoV-2 humoral immunity have primarily focused on healthy individuals, resulting in extremely limited data regarding cancer patients. In this study, we evaluated the antibody and memory B-cell response to SARS-CoV-2 in a cohort of gynecological cancer patients within the context of vaccination and breakthrough infections. To assess the vaccine response of cancer patients, blood samples were collected from healthcare workers (n=6) and cancer patients (n=7) before and after the administration of the second and third vaccine doses. Additionally, the response to breakthrough infections was examined using blood samples from healthcare workers (n=15) and cancer patients (n=17). Antibody titers and potency against variants of concern (VOCs) were measured using enzyme-linked immunosorbent assay (ELISA) and pseudovirus neutralization assay. Furthermore, the proportion and heterogeneity of SARS-CoV-2 receptor binding domain (RBD)-specific memory B-cells were analyzed using multicolor flow cytometry in conjunction with the B-cell tetramer assay. Our findings demonstrated significantly lower antibody responses to the Omicron variant and its subvariant in cancer patients compared to healthy controls. Cancer patients exhibited an imprinted antibody response, although the extent of imprinting varied among antibody isotypes. Moreover, the proportion of SARS-CoV-2 RBD-specific memory B-cells was substantially lower in cancer patients, and their subset distribution was significantly altered. Our research offers valuable insights for the development of vaccine strategies tailored to cancer patients against SARS-CoV-2 and potential future viral outbreaks.

P3-111 Loss of methyl-CpG binding protein 2 (MeCP2) disrupts IFN responses to influenza A virus infection

Forero A[1], McFadden M[1], Kenney A[1], Speaks S[1], Schott E[1], Sanders C[1], Gong Q[1], Hemann E[1], Yount J[1]
[1]The Ohio State University

Mutations in Methyl-CpG-Binding Protein 2 (MeCP2), an epigenetic regulator of gene expression, are predominant in individuals with Rett syndrome (RTT). Although, RTT is a neurocognitive regression disease, lower respiratory tract infections (LRTI) are amongst the leading causes of death in this patient population. MeCP2 deficiency has been linked to increased neuroinflammation, but whether MeCP2 can also regulate host responses to infections and overall lung inflammation remains to be addressed. Influenza A virus (IAV) is a highly prevalent respiratory pathogen that can cause LRTI. Here, we demonstrate that mice lacking Mecp2 display increased morbidity and mortality following infection with IAV (A/Puerto Rico/8/1934 (H1N1)). Histopathology analysis revealed increased lung pathology concomitant with increased CD45+ cellular infiltration that enhanced peribronchial cuffing in Mecp2-deficient mice. However, genome-wide transcriptional profiling of IAV-infected lungs revealed that Mecp2-null mice had delayed lung interferon (IFN) and IFN-stimulated gene (ISG) expression relative to wild-type (WT) littermate controls. The loss of MeCP2 also associated with dampened proinflammatory cytokine and chemokine gene expression and the muted expression of genes associated with T cell activation and differentiation after infection. These data indicate potentially disrupted immune cell dynamics in Mecp2-null infected mice relative to WT infected animals. Consequently, Mepc2-deficient mice had greater extrapulmonary dissemination of IAV and detection of IAV in the heart, a hallmark of severe IAV disease. Taken together our findings implicate MeCP2 as a novel positive regulator of antiviral responses that tunes immune cell recruitment and function during respiratory infection. Our study suggests that the increased susceptibility to LRTI in patients could stem from a decrease in host inflammatory responses to respiratory pathogens and reveals pleiotropic immunological vulnerabilities across tissue types in MeCP2 deficiencies.

Index of Authors

A

Abdel-Hakeem, M
Abedi, M P3-002
Abhiraman, G P2-003
Abida, O P1-025
Abraham, P P3-037
Abrol, R P1-091, P2-020
Abt, M
Abu Zeid, M P3-068
Acevedo Woods, M
Achuthan, A P1-055, P1-085
Acosta, L P2-098
Adamczyk, A P3-045
Adamo, J P2-039
Addison, M P2-007
Adler, C P3-034
Afshar-Sterle, S P2-077
Agareva, M P2-058, P2-070
Aguiar, J P3-017
Ah Kioon, M
Ahlborg, N P1-018
Ahmed, R P2-009, P2-010, P3-062
Ahn, E P2-010, P3-027
Aicher, S P3-017
Ailal, F
Akalu, Y P1-083
Alameh, M P3-011
Albarnaz, J P3-078
Albert, M P1-004, P1-005, P3-088
Albrich, W
Alcaide, M P2-098
Alcorn, J
Aldridge, D P1-097, P3-104, P3-105
Aldrighetti, L P2-057
Alekseev, A
Alexandre, Y
Alexandridi, M P3-094
Alexopoulos, L P1-026
Ali, M P2-023
Ali Akbari, S P2-091
Altman, J
Alvarado, A P2-095
Alvarez-Martinez, M P1-070
Amariliz, R
Amratia, P P3-072
An, H P2-007
Anastasiadou, D P1-002, P2-001
Anderson, J
Anderson, M
Anderson, M P2-015, P2-061
Andreakos, E P2-076, P2-078 P3-060
Andrews, R
Andries, J P1-088
Ang, E P3-016
Annamneedi, V P2-059
Ansel, K P1-098
Antanaviciute, A P3-071
Antonchuk, J P3-016
Antos, D
Antrobus, R P3-078
Argyropoulou, O
Ariel, A P3-068
Arlen, P
Arranz, J P2-104
Arranz Herrero, J P3-056
Arreguin, M P1-104
Arslanow, A P2-092
Arzani, H P1-021
Asano, S
Aschenbrenner, I P2-066
Ashkar, A P3-070
Ashuo, A P2-047
Asimakopoulos, A P3-058
Asrat, S P1-056, P2-042
Asurappulige, H P1-107
Atar-Karo, D P2-068
Athar, M P3-008
Atiq, M
Atwal, G P3-034

Alipour Symakani, R	P1-007
Allam, A	
Alpar, A	P1-013
Alphonse, N	
Alrehaili, A	
Alterauge, D	
Au Yeung, E	P3-034
Aufiero, M	
Aunins, E	P3-011
Avila, S	
Ayuso, A	P2-104

B

Bach, L	
Bachu, M	
Bae, H	P2-019
Bae, J	P2-059
Baek, H	P1-069
Baek, S	P3-020
Baek, S	P2-101
Baez Vazquez, A	
Bagavant, H	P1-057
Baid, K	P3-017
Baillif, V	P2-078
Baines, S	P3-062
Bajetto, G	P2-004
Baker, D	P3-002
Baker, P	P3-052
Balasubrahmanyam, P	P2-022, P2-023
Banchereau, J	P1-001
Banday, A	P2-094
Banerjee, A	P3-017, P3-069
Bannerman, C	P3-054, P3-093
Baral, I	P2-063
Baran, M	P1-006
Barbeta, G	P3-046
Barkhymer, A	P3-049
Barksdale, B	P3-103
Barrat, F	
Barretto, A	P2-093
Barron, A	
Bartley, O	
Basting, C	P2-098, P3-031
Batlle, E	P2-057
Baudino-Garcia, C	P1-012
Bauer, M	P2-022, P2-023
Baumjohann, D	
Bayarsaikhan, G	P3-080
Bazan, F	P1-068
Becher, B	
Becker, J	P3-051
Bianchi, N	P1-082
Biasizzo, M	P1-023
Bilbao, J	
Bilusic, M	
Biondo, M	P1-055
BIRCK, C	P1-068
Biswas, B	P1-058
Biswas, P	P1-084
Blackhall, F	
Blish, C	P2-003
Bloch, Y	P1-007, P3-085
Blum, H	
Boddaert, N	
Bodykevich, G	P3-016
Boehmer, D	
Bogunovic, D	P1-083, P1-086, P1-096
Bohlen, J	P3-061
Boisson-Dupuis, S	P3-061
Boki, K	
Bolado-Carrancio, A	
Bondet, V	P3-088
Boneparth, A	P1-086
Bongiovanni, A	P3-054
Böning, H	P1-039
Borgonia, D	P2-010
Borthwick, L	
Bošnjak, B	P3-005
Botelho, F	P2-071
Botrugno, O	P2-057
Bouhaddou, M	P3-097
Bound, K	
Bouras, E	P3-058
Bourdin, B	P1-108
Bourgey, M	
Bourke, N	P2-062, P3-088
Bousfiha, A	
Bousso, P	P2-037
Bowie, A	P1-006

Becker, J
Becker, J P3-049
Becker, M
Begitt, A P1-008
Behnke, J P3-050
Behren, A
Behrendt, C
Behrmann, I P2-092
Belkaid, Y
Bell, S P3-101
Belman, J
Beltra, J P1-108,
Belz, G
Ben, L P1-056, P2-042
Ben amar, M P1-025
Ben Jashar, N
Bender, M
Bengeochea, J P1-006
Benhsaien, I
Benitez, A P3-034
Bennett, A P2-015
Bennett, N P3-046
Bennett, T P1-065
Berger, S
Bergerson, J
Bergmann, C P1-071
Berkhout, B
Bertoni, A
Berzins, S
Best, S
Beyaz, S
Beyer, M
Beyer, S P1-039
Béziat, V
Bhattacharyya, A P1-031
Bialucha, C
Bowman, A P3-036
Bracke, K P1-030
Braiman, A P2-083
Brauner, C
Brenchley, J
Brendolan, A P2-057
Briceño, O
Briken, V P3-052
Brod, S P1-099
Brodsky, I P3-063
Broedlow, C P2-098, P3-031
Broggi, A
Brooks II, J
Brownell, I P2-061
Brüne, B P3-077
Buadze, T P3-007
Bubier, J P3-010
Budde, H P1-057
Buenconsejo, G P2-096
Buffone, C P2-022, P2-023,
Bugda Gwilt, K
Bugno, J P1-013
Bujotzek, A P2-030
Bunk, P
Burgener, S P2-020
Burn, T
Burnett, S P1-022
Büssow, K P2-006
Bustamante, J P3-061
Bustamente, J P1-096
Buta, S P1-083, P1-086, P1-096
Butler, K P2-094
Buttiglione, V P2-057
Byerly, J P2-096
Bykov, Y P2-104

C

Calà, O P1-077
Calderon-Gonzalez, R P1-006
Callery, J P1-018
Campbell, A P2-007
Campillo-Poveda, M
Campos, J P1-081
Campos Duran, J
Chin, H
Chinnaswamy, S P1-051
Chiu, C P2-040
Chiu, T P2-067
Chiu, Y P2-053
Cho, M P1-049,
Cho, M P1-037, P1-076, P2-073

Canar, J P2-095, P2-100
Caneparo, V P2-004, P2-005
Canna, S
Cantaluppi, V P2-005
Capellini, T P3-017
Carbone, F
Card, J
Cardus Figueras, A
Carpenter, S P1-064, P1-094
Carrillo-Salinas, F P3-098
Carriqui, B P1-027
Carroll, K
Caruso, A P1-075
Caruso, F P2-057
Casanova, J P1-096, P3-061
Casella, V
Casey, A
Casper, M P2-092
Castano, D
Castellanos Rubio, A
Castellucci, C
Castiglioni, F P2-005
Caston, J P1-098
Catteeuw, D P1-068
Cavazza, A
Caveney, N
Chae, J P3-012, P3-013
Chakraborty, A P2-094
Chambers, J P1-106
Champelovier, D P2-037
Chan, A P3-024
Chan, C P2-052, P2-053
Chan, L P1-044
Chan, R P3-071
Chan, Y
Chan, Y P3-074
Chandra, V P1-067
Chandran, E
Chang, J P2-101
Chang, W P3-030
Chang, Y
Chang, Y P1-010
Chapman, L P3-072
Cho, M P3-038
Cho, O P2-087
Cho, S P2-061
Cho, S P3-038
Cho, S P2-030
Cho, Y P2-069, P3-027 P3-053
Choi, A P2-026
Choi, B P2-035
Choi, D P2-010, P2-101
Choi, H P1-069
Choi, J P1-080, P3-038
Choi, J P3-092
Choi, J P2-036
Choi, S P1-046, P1-062 P3-028
Choi, Y P2-101
Choteau, M P2-075
Chou, S P2-007
CHOW, K P2-084, P2-085, P3-084
Chowdhury, K P1-050
Choy, E
Christensen, S
Christian, D P3-105
Christo, S ,
Chu, K P3-108
Chu, P P2-028
Chung, C
Chung, I P3-040
Chung, K P2-051
Chung, S P1-072
Chuong, E
Cicin-Sain, L P3-051
Cipriani, F P2-057
Clancy, D P1-088, P3-085
Clatch, A
Clement, M P3-072
Clements, R P2-007, P3-103
Clissi, B P2-057
Clouston, A P2-020
Cohn, I P2-096
Coirada, F P1-073
Coleman, A P1-079, P3-082, P3-102
Coleman, S
Collins, N

Chapoval, S P2-034
Chathuranga, K P3-083
Chaturvedi, D P2-022
Chavaki, S
Chavakis, T
Cheang, L P1-092
Chen, C P2-006
Chen, J
Chen, J P2-047
Chen, J P3-058
Chen, M P1-002
Chen, M P1-047, P1-060
Chen, Q P1-102
Chen, S P2-028
Chen, T P2-090
Chen, T P2-053
Chen, W P3-066
Chen, W
Chen, W P1-053
Chen, X
Chen, Y P1-053
Cheng, I P2-050
Cheng, J
Cheng, P P3-039
Cheou, P P2-075
Cherenack, E P2-098
Chesi, G
Cheuk Ying, T P2-085
Chiale, C P3-090
Chiang, B P1-053, P1-054, P2-040, P2-043, P2-049, P2-050, P2-090, P3-039, P3-108
Chiang, C P2-032
Chieppa, M
Chigvinadze, N P3-007
Chin, C P3-039

D

D, N P1-074
Dagher, M P2-105
Daher, W
Dahl, S P3-048
Dahlmann, J P1-027
Dahlström, F
Dalai, S P1-016, P1-017
Deswarte, C
DeVeaux, M
Devlin, J P3-034
Dey, I P1-084
Dhaliwal, H P3-037
Dho, S P1-037, P1-076, P2-073
Dhulst, L P2-074
Coloma, R P1-005
Colpitts, C P3-017
Cominelli, F
CONCORD-19 Consortium
Conde Garcia, E P1-056
Condeelis, J P1-002
Conrey, P P1-081
Cooper, M P1-081
Corà, D P2-005
Cordes, L
Cornelius, J
Corridoni, D P3-071
Costa, A
Costa, B P3-051
Courtain, C P1-077
Cousin, A P1-014
Covarrubias, S
Covolan, L P1-073
Coward Smith, M
Cowton, V P3-097
Cristofaro, M
Croci, S
Cromarty, R
Crosby, D
Crow, Y
Crowe, D
Crowley, T
Cruz-Morales, E P3-011
Cuadrado, S P2-104
Cui, A
Cummings, F P3-046
Cunningham, K P1-101
Curson, J P1-091, P2-020

Dalalo, M
Damm, D P3-059
Dang, V
Daniel, B
Dansercoer, A P1-068
Das, A
Das, J
Das Gupta, K P2-020
Davidson, S P3-067
Davies, C P3-078
Davis, M
Dawodu, G P2-104
Dawson, R P2-072
Day, E P1-009
D'Cruz, A
De Andrea, M P2-004, P2-005
De Backer, R P1-007
De Bleecker, J P1-030
De Lorenzo, G P3-097
De Los Santos, G
De Paepe, B P1-030
De Salvo, C P2-107, P3-071, P3-073
De Santis, S
De Waal Malefyt, R P2-022, P2-023,
DeAntoneo, C P2-012
Decaluwe, H P1-108,
Dedoussis, G
Dehghan, A P3-058
Del Pinto, R P2-107
Del Rio, P P1-073
Delgi-Esposti, M P1-065
Demian, W P3-069
Dende, C
Denny, J
Denz, P P3-036

Depledge, D P1-039
Derrac Soria, A
Desai, J
Desamero, M P1-106
Deshane, J P3-008
Deshmukh, U P1-057
Deswaerte, V P2-072

de Steiger, R P1-055

E

Di Cristofano, A P2-001
Di Santo, J
Diabangouaya, P P2-008
Dickenson, R
Diefenbach, A P2-002
Dijkstra, C
Dimonte, S , P3-072
Dimopoulos, M
Dinu, A P3-026
Dittel, B P1-099
Dittel, L P1-099
Dittmer, U P3-044, P3-045
Dittrich, A P2-024
Dive, C
Djerdjouri, B P3-043
Djokic Lišanin, M P1-032
Doglioni, C P2-057
Doisne, J
Dolinar, K P1-023
Domingo, C
Donahue, R
Donlon, J P3-096

Dotse, E P2-085, P3-084
Douglas, J P1-011

Dov, A P1-003
Dower, K
Doxey, A P3-017
Drew, P P1-011
Drosten, C P3-017
Drummond, G
Du, Y
Dubourdeau, M P2-078
Duffy, D P2-062, P3-088
Dumigan, A P1-006
Dumoutier, L P1-077, P1-078, P2-074, P2-075

Dunbar, C
Duprey, H
Duran, C P1-002
Duran, V
Durum, S
Dvorkin-Gheva, A P2-071
Džafo, E P1-082

E. Groves, H P3-081
Eaves, A P3-016
Eberhard, J P2-054
Eberl, G P2-008
Eddy, A P3-036
Eddy, R P1-002
Egli, N
Einsenlohr, L P2-007
Eisenlohr, L
Eivazi, M P1-028
Ekalle-Soppo, C P2-057
El- Hachem, N P1-108
El Hamidie, A
Eleminiadou, E P3-060
Elewa, M P3-077
Elewski, B P3-008
Elgaher, W P1-027
Ellisdon, A P2-030
Ellzey, L P3-101
Elmets, C P3-008
Elmore, U P2-057
Elmzzahi, T

F

F. McGettrick, A P1-009
F. Power, U P3-081
Fabre, T
Fagioli, C P2-057
Faixová, Z P2-027
Fajardo, E P1-090, P1-093
Fakhfakh, R P1-025
Falcón, J P1-004
Falduto, G P2-063
Falqui, M P1-005
Fan, J P1-054
Fanaroff, R P2-034
Fantini, D
Farmer, J P1-081
Fassett, M P1-098
Fattinger, S P2-013
Faught, E P2-037
Faure, P P3-017
Favero, F P2-005
Favier, A P1-014
Feige, M P1-071, P2-066, P3-050
Emmerich, J P2-022
Endres, N P3-017
Engelhart, E P2-039
Entenberg, D P1-002
Epple, M P3-059
Erdin, M P3-076
Ergin, I
Erhard, F P3-051
Ernst, M P2-077
Eryilmaz, E
Escalona, D P1-064
Escandon, K
Escher-Toller, J P1-009
Espinsoa, V
Esposito, A P2-057
Esser, S P3-044
Esteban, M P1-005
Evangelou, K
Evrard, M
Ewen, C P3-016
Exposit, M P3-002
Ferreira Espinoza, L P2-057
Ferrero, R P2-072
Fielding, C
Figg, W
Fikarová, N P2-079, P2-081
Fish, E
Fisher, A
Fitzgerald, K P1-006
Flores-Villava, S P3-095
Fonseca, D P3-025
Fonseca Nogueira, N P2-098
Foong Ngiow, S
Forbes, L
Forero, A P3-111
Förster, I P1-055, P2-054,
Förster, R P3-005
Fortin, L P2-097
Fotis, C P1-026
Fraenkel, E
France, M P2-098
Frankel, W P2-086

Feijoo, C P2-008
Fein, L P2-098
Feki, S P1-025
Felber, B P2-103,
Felix, J P1-007, P1-068
Felwick, R P3-046
Feng, E P3-070
Fenton-May, A P3-046
Feriotti, C P1-006
Fernandez, P P3-056
Ferrando-Martinez, S P2-009, P2-010, P2-015, P2-061
Ferrara, B P2-057

G

G.G. Bamford, C P3-081
Gabay, C P1-075
Gaffen, S P1-084
Gaidt, M P3-037
Gaigneaux, A P2-092
Gaiozishvili, M P3-007
GALANI, I P2-076, P2-078, P3-060
Galatík, F P2-081
Gale, Jr., M
Galindo, F P1-052
Galland, F
Gamero, A P2-095, P2-100
Gancarčíková, S P2-027
Gandolfo, L
Gangopadhyay, A P2-023,
Gao, H P2-034
Garbers, C P1-063, P3-048
Garcia, K P1-097, , P2-003,
Garcia baudino, C P2-008
Garcia-Sastre, A P2-104, P3-025
Gariglio, M P2-004, P2-005
Gattorno, M
Gauchat, J P1-090, P1-093
Gavrilova, A P2-070
Gentile, M P2-068
Gern, O P3-051
Gerold, G P1-027
Getty, E P3-081
Ghannoum, M P3-073
Ghilas, S P2-077
Gong, Q
Gong, Q P3-111
Gong, Z P3-086
Gonzalez Paredes, P P1-057
González-Motos, V P1-039
Goodman, W P2-107
Goodnight, D P2-039
Gordon, C
Gorgoulis, V
Gorman, J P1-057
Goswami, R P1-058
Gotthardt, D P1-021
Gottschalk, M P1-055
Goules, A
Gowda, P P2-088
Gralinski, L P3-010
Grassi, S P2-057
Greb, H P2-022,
Green, C
Green, D P3-063
Greene, J
Gregorieff, A P2-068
Gros, G P1-012, P2-008
Gros, P
Groß, O P3-063
Gruber, C P1-096
Grutzendler, J P2-041
Gu, S P3-006
Gualdrini, F P1-082
Guendel, F P2-008
Frank-kamenetskii, A
Franklin, L
Franklin, R P3-079
Freeman, A
Frezza, C
Frizzell, N
Froc, C P2-037
Fujita, S P2-082
Fülle, L P2-054
Furnon, W P3-097
Furones, A P3-085

Ghita, L
Ghosh, S
Giangrazi, F
Giavedoni, L P1-018
Giles, J
Gill, D P3-058
Gilliam, B P3-062
Gimeno-Brias, S P3-072
Girard-Guyonvarc'h, C P1-075
Gjonbalaj, M
Glover, L
Gnafakis, S P2-002
Gnasso, C P2-057
Godin, S
Gokhale, N
Gómez, C P1-005
Gower, t

H

Ha, H P3-023
Habel, J
Hachicha, H P1-025
Hacohen, N
Haddad, E P1-108
Hafalla, J P3-080
Hafer, M P1-068
Hafler, D P2-041
Halasz, H
Haljasmägi, L P1-059
Halle, S
Hamelin Morrissette, J P2-097
Hamilton, J P1-055, P1-085
Hamza, Z P1-025
Han, B P3-054, P3-093
Han, S
Han, S P1-038
Han, Y P3-033
Hancks, D P3-100
Hankin, A
Hansen, J P3-026
Hao, M P3-030
Haque, A
Hara, I P3-018
Harari, D P1-003
Hargreaves, D P3-004
Guerin, T P2-103
Guérin, A
Guerra, S P1-004, P1-005
Guerrero, C P3-031,
Guha Roy, D P1-051
Guidotti, L P2-057
Guillaume, S
Guleria, R P1-061
Gulley, J
Gunther, K P1-091, P2-020
Guo, K P3-045
Guo, Y
GUPTA, R P3-003
Gutiérrez-Melo, N
Guzman, W
Hinck, A P1-101
Hirota, K P3-022
Hisaeda, H P2-065
Hiscott, J P3-094
Ho, T P1-109
Hoagland, D P3-079
Hodara, V P1-018
Hohl, T
Holíček, P P2-081
Holland, S
Holloway, K P1-011
Holm, J P2-098
Holy, X P1-014
Holzmann, B P3-050
Homad, L P2-039
Hong, J P3-020
Hong, S P1-040, P1-072, P2-106
Hood, S
Hooftman, A P1-009
Hooper, L
Hopkins, J
Horan, M
Horlbeck, M
Hoser, G P3-047
Hou, C
Hsiao, S

Hart, K
Harwig, A
Hasenauer, J
Hasiuk, M
Haskins, B P2-096
Hausler, M
Haxhinasto, S P3-034
Hay, C
Haystead, T P1-048
He, K
Heath, N P3-019
Heink, S
Heissmeyer, V
Held, J P3-050
Hemann, E P3-111
Henderson, N
Hendricks, M P3-026
Hendrickx, E P2-075
Hennemann, A P3-089
Henrickson, S P1-081,
Heo, T P1-087, P2-087
Herich, R P2-027
Hernandez, P P2-008
Hernandez-Cerda, P P1-012
Herzig, K P3-058
Heynen, S
Hixon, j
Hsieh, C P2-032
Hsin, F P3-074, P3-075
Hsu, L P1-010
Hsu, T P2-093
Hua, T P2-047
Huang, A
Huang, E P3-071
Huang, H
Huang, J P3-086
Huang, P P2-032
Huang, T
Huang, Y
Huard, A P3-077
Hubbell, J P1-013
Huber, C
Hudec, E P1-033
Humphreys, I P3-072
Hunter, C P1-097, P2-096, P3-011, P3-103, P3-104, P3-105
Huntington, N P2-077
Huntington, T P3-101
Huo, L
Hur, G P3-020
Hur, J P1-040
Hwang, E P3-020
Hwang, Y P3-023
Hynkova, M P2-079

I

Iannucci, A P2-004, P2-005
Ilangumaran, S P2-091
Iliev, I P3-001
Im, S P3-035
Im, S P2-101
Imamichi, T P1-102, P3-030
Inchausti, I P3-056
Inguanti, C
Inoue, S P3-080
Inoue, T P3-080
Ioannides, A P3-058
Ireton, R
IRF1-Consortium
Irvine, K P2-020
Ishida, Y P1-045, P2-025, P3-018
Ishigami, A P1-045
Ishihara, J
Ishimori, M
Ivashkiv, L
Iwahashi, Y P3-018
Iwakura, Y P1-106
Izquierdo, A P3-058
Izquierdo, H

J

Jabbari, A P2-086
Jiang, Z P1-006

Jacob, R	P3-069
Jain, R	P1-061
Jalkanen, S	P3-058
Jane, S	P3-002
Jang, A	P3-053
Jang, Y	P1-040
Jankovic, D	P2-011
Jarvelin, M	P3-058
Jayaraman, B	P2-022, P2-023
Jefferies, C	
Jeker, L	
Jena, K	
Jenkins, B	P2-072
Jenkins, K	
Jenull, S	P1-021
Jeon, E	P2-099
Jeon, H	P1-049,
Jeon, M	P2-099
Jeong, C	P2-101
Jeong, G	P3-109
Jeong, H	P2-106
Jeong, H	P3-038
Jeong, Y	P2-087
Jeong, Y	P2-101
Jeznach, A	P3-047
Jian, J	P3-080
Jiang, M	P3-029
Jin, L	P3-008
Jin, W	P3-066
Johansson, C	
Johnson, M	
Johnson, P	
Jokhadze, T	P3-007
Jolly, C	P3-097
Jones, A	P1-091
Jones, G	
Jones, S	P1-065, P1-092
Jones, S	
Joo, I	P2-064
Jost, P	
Jouvenet, N	P3-017
Joyner, O	P3-065
Julkunen, I	P3-029, P3-076
Jun, C	P3-040
Jung, D	P2-069, P3-027, P3-053
Jung, H	P1-020
Jung, H	P3-013
Jung, K	P2-056
Jung, W	, P3-032
JUNG, Y	P2-026
Jungkurth, E	P3-086
Jurado, K	P1-104, P2-007, P3-054, P3-093, P3-103
Jürgens, C	P1-039
Jurisic, V	P1-032

K

Kaempfer, R	P3-057,
Kakkola, L	P3-076
Kakuta, S	P1-106
Kalafati, N	
Kalantari, P	P3-099
Kalinke, U	P3-005, P3-051, P3-089
Kallies, A	
Kaltsas, G	
Kalveram, B	P3-010
Kambas, K	
Kandane-Rathnayake, R	P1-065
Kane, C	P1-011
Kang, B	P1-020
Kim, M	P1-105, P3-013
Kim, M	P1-029
Kim, M	P2-021
Kim, N	P3-040
Kim, S	
Kim, S	P2-106
Kim, S	
Kim, S	P2-036
Kim, S	P2-019
Kim, T	P3-083
Kim, T	P3-023
Kim, T	P2-064
Kim, W	P2-069, P3-027, P3-053

Kang, H	P1-029, P2-021
Kang, I	P1-019
Kang, T	P3-101
Kang, T	P2-059
Kang, Y	P1-046, P1-062, P3-028
Kang, Y	P2-101
Kang, Y	P2-064
Kannourakis, G	
Kany, A	P2-006
Kapetanovic, R	P1-091
Karaffová, V	P1-033, P2-027
Karagiannis, G	P1-002, P2-001
Karaköse, Z	P3-044
Karaliota, S	P2-103
Karatza, E	
Karhunen, V	P3-058
Karsten, C	P3-044
Karunakaran, D	P2-020
Karzai, F	
Kashyap, M	P3-008
Kastelein, R	P2-022, P2-023,
Katopodis, A	
Kaur, A	P1-057
Kaur, N	P1-056,
Kawaguchi, M	P1-045, P2-025
Ke, J	P3-024
Keegan, A	P2-034
Kell, A	P3-107
Kelly, M	P3-046
Kennedy, E	P1-104
Kennedy, E	P3-095, P3-096
Kenney, A	P3-036, P3-111
Kenyon, A	P1-082
Kerle, A	P2-066
Keskin, D	
Kespohl, B	P1-063
Khalilova, J	P3-048
Khalmuratova, R	P2-031
Khandhar, A	
Khatooni, Z	P3-017
Khatun, O	P3-041
Khelil, A	P1-093
Kilgour, E	
Kilty, I	P3-046
Kim, B	P1-041
Kim, Y	P2-069, P3-027, P3-053
Kim, Y	P3-013
Kim, Y	P2-031
Kim, Y	P2-055
Kim, Y	P1-037
Kim, Y	P3-032
Kim, Y	P2-101
Kim, Y	P3-020
KIM, H	P1-029, P2-021
KIM, S	P1-040, P1-041
Kimura, A	P1-045, P2-025, P3-018
Kimura, D	P3-080
Kimura, K	P3-080
Kimura, O	P2-082
Kinchington, P	P1-039
King, I	P2-068
King, J	P1-018
Kingwell, B	P1-055
Kiprina, A	P3-077
Kirchmeyer, M	P2-092
Kirti, M	P2-088, P2-089
Kisand, K	P1-059
Kissick, H	P2-010
Kiššová, Z	P1-033
Kitas, C	
Kite, J	P3-078
Klapholz, M	
Klatt, N	P2-098, P3-031,
Klimaj, S	P3-107
Knight, M	
Knoll, M	
Ko, S	P2-051
Kofoed Branzk, M	P2-002
Köhler, N	P2-024
Kokaji, A	P3-016
Kolehmainen, P	P3-076
Koliesnik, I	P2-022, P2-023
Kollias, G	P1-015
Kondo, T	P1-045, P2-025, P3-018
Kondoh, G	P3-022
Kong, H	P2-101
Koo, B	P3-020
Kopitar-Jerala, N	P1-023
Korn, T	
Koronaiou, E	,

Kim, B	P2-035
Kim, C	P1-019
Kim, C	P3-040
Kim, D	P1-049,
Kim, D	P2-064
Kim, D	P2-069, P3-027, P3-053
Kim, D	P3-109
Kim, G	P1-080
Kim, G	P3-020
Kim, H	P1-087
Kim, H	P1-046, P1-062, P3-028
Kim, H	P1-019, P1-020
Kim, H	P3-040
Kim, H	
Kim, H	P2-064
Kim, H	P1-019
Kim, H	P1-040, P1-041
Kim, J	P3-092
Kim, J	P3-023
Kim, J	P3-023
Kim, J	P2-035
Kim, J	P3-032
Kim, J	P2-101
Kim, J	P2-021
Kim, J	P3-040
Kim, K	P1-020
Kim, K	P1-015
Kim, L	P1-037, P1-076, P2-036, P2-073
Kim, L	P1-087
Kim, M	P1-015
Kim, M	P1-072
Kim, M	P3-013
Kim, M	P1-069
Khvichia, N	P3-007
Koščová, J	P2-027
Kotenko, S	P3-070
Kotov, D	P2-013
Koukaki, E	P2-078
Koutsoukou, A	P2-078
Kozlov, S	P2-103
Kravarik, K	
Krawczyk, M	P2-092
Krebs, S	
Krey, T	P1-039
Krishnakumar, A	P3-002
Krishnamohan, M	P2-083
Krogan, N	P3-097
Kropp, K	P1-039
Krulova, M	P2-079
Krulová, M	P2-080, P2-081
Ku, K	P1-020
Kuchel, G	P1-001
Kuchler, K	P1-021
Kugler, D	P2-011
Kumar, B	P2-072
Kumari, A	
Kumari, R	P1-061
Kuninaka, Y	P1-045, P2-025, P3-018
Kurachi, M	
KURMASHEVA, N	P1-027
Kwarteng, E	P1-108
Kwok, S	P1-049,
Kwon, D	P2-101
Kwon, E	P2-019
Kwon, H	P1-069
Kwon, Y	P3-109
Kyuwa, S	P1-106

L

Lacarbonara, D	P2-004, P2-005
Lachmann, N	P1-039
Lacour, A	P1-027
Lage, S	P1-052
Lai, A	P3-079
Lai, M	P2-049
Lee, S	P1-062, P3-028
Lee, S	P3-109
Lee, S	P3-040
Lee, T	P2-069, P3-027, P3-053
Lee, W	P1-046, P1-062, P3-028
Lee, Y	P1-072

Laidlaw, E	P1-052
Laine, L	P3-029, P3-076
Lakhani, J	
Lambert, E	P1-007
Lambrecht, B	
Lambropoulou, V	P2-078
Lambrou, E	P1-018
Lammert, F	P2-092
Lampasona, V	P2-057
Landolfo, S	P2-005
Landy, E	
Lang, K	P3-045
Langat, R	P3-031
Langlais, D	
Langlois, R	P3-042, P3-049
Lanoce, E	P3-054
Lanzar, Z	P1-097, P3-104, P3-105
Laplante, V	P1-090, P1-093
Larisch, S	P3-068
Lariviere, L	P2-030
Larsen, P	
Laschinger, M	P3-050
Latorre, D	P3-061
Lauterbach, A	P1-013
Lavallée, V	P1-108
Laverdure, S	P1-102
Lazarevsky, S	P1-098
Le Fevre, T	P3-016
Le Floc'h, A	P2-042
Le Pen, J	
Le Voyer, T	
LeBlanc, E	P3-017
Lech, M	
Lee, A	P1-096
Lee, A	P3-037
Lee, B	P2-009, P2-010, P2-015, P2-061
Lee, C	P2-101
Lee, C	
Lee, D	P2-021
Lee, E	P1-069
Lee, E	P3-110
Lee, H	P1-019, P1-020
Lee, H	P2-029
Lee, Y	P1-037
Lee, Y	P3-013
Lee, Y	P3-022
Lee, Y	P2-069, P3-027, P3-053
Legarda, M	
Lehtimäki, T	P3-058
Leiding, J	P1-081,
Leonard, W	P1-067
Lesage, S	P1-090, P1-093
Leu, C	P1-047
Levesque, M	
Levkut, M	P2-027
Levraud, J	P2-008, P2-037
Levy, M	P3-057
Lewis, M	
Lezhava, T	P3-007
Li, P	P1-067
Li, S	
Li, Y	P2-047
Li, Y	P1-084
Li, Y	P3-071
Li, Y	P2-047
Li, Z	
LI, Y	P3-084
Liao, C	P2-040
Liao, Y	
Liebl, K	P2-066
Lim, G	P2-064
Lim, K	P1-085
Lim, K	P2-084, P2-085, P3-084
Lim, W	P2-042, P3-034
Lim, Y	P2-028
Lim, Y	P3-066
Lim Kan Sian, T	
Limnander, A	P1-056,
Lin, B	P1-109, P2-052, P2-053, P2-067, P2-093
Lin, Y	P1-010
Lin, Y	P1-002, P3-039
Lindsay, A	
Lionakis, M	
Liong, S	
Lisco, A	P1-052, P2-015, P2-061
Liu, H	P3-074, P3-075

Lee, H	P2-044, P2-045
Lee, H	P3-023
Lee, H	
Lee, H	P1-069
Lee, I	P3-024
Lee, J	
Lee, J	P2-036
Lee, J	P1-037
Lee, J	P3-083
Lee, J	P3-032
Lee, J	P2-087
Lee, J	P2-009, P2-010
Lee, J	P3-035
Lee, J	P3-092
Lee, K	P1-055, P1-085
Lee, K	P3-110
Lee, K	P2-101
Lee, K	P1-037
Lee, K	P3-023
Lee, K	P3-035
Lee, K	P3-040
Lee, M	P2-003
Lee, O	P3-037
Lee, P	P2-048
Lee, R	P3-091
Lee, S	P3-006
Lee, S	P1-015
Lee, S	P2-014, P3-012, P3-013
Lee, S	P2-064
Lee, S	P2-035
Lee, S	P2-101
Li, W	
Liu, J	P2-086
Liu, L	P1-091
Liu, N	P3-074
Liu, Q	P3-017
Liu, S	
Liu, X	P2-015, P2-061
Liu, Y	P2-086
Liu, Y	P3-108
Liu, Z	
Liu, Z	
Livis, T	P2-072
Lkhagva-Yondon, E	P2-099
Lo Cigno, I	P2-005
Locci, M	
Lohan, A	
Lokau, J	P1-063, P3-048
Lone, B	P2-094
Long, M	
Lopez, A	P3-103
Lopez, R	P2-039
Lopez Campos, G	P3-081
Lösch, J	P2-054
Louis, S	P3-016
Low, J	P1-082
Lowe, A	
Luc, J	P3-017
Lucas, R	P1-091
Lueder, Y	
Luo, L	P1-091
Lupancu, T	P1-055, P1-085
Lupardus, P	P1-097, P2-022, P2-023,

M

M, N	P1-074
Ma, A	
Mabry, C	P3-082, P3-102
Macalinao, M	P3-080
MacDonald, W	
Macfarland, S	P1-081
Machado, Y	P3-017
Mackay, L	
Macoritto, M	
Madan, R	
Madden, J	P3-046
Meisalu, S	P1-059
Meisel, M	
Meissner, F	
Mele, A	P1-102
Mele, F	P3-061
Melgar, S	
Menachery, V	P3-010, P3-017
Mendoza, L	
Menshikov, M	P2-058
Merceron, R	P1-007
Merlino, M	P3-103

Mahabaleshwar, G	P3-073
Maher, T	
Mahfooz, N	
Majithia, M	P1-073
Majlesain, Y	
Makower, B	P1-018
Makrecka-Kuka, M	P3-046
Malekos, E	P1-094, P3-064
Malheiros, J	P1-073
Malik, S	P1-055
Malkova, N	
Malle, L	
Maloney, E	P2-062
Mambu, J	
Mandias, H	P3-004
Manion, M	P1-052
Manioudaki, M	P2-078
Mann, A	P3-079
Manne, S	
Manry, J	
Maples, R	
Mar, D	P1-098
Marcos, L	P1-005
Mariadason, J	
Markovic, I	P1-068, P1-089
Marsden, M	P3-072
Marte, J	
Martin Fernandez, M	
Martinello, G	P2-004
Martinez, E	P3-102
Martin-Fernandez, M	P1-086, P1-096
Martinon, F	
Marvisi, C	
Marzinotto, I	P2-057
Maslanka, J	
Masmoudi, H	P1-025
Matchett, W	P3-042
Mathew, D	
Matsumoto, H	P3-080
Matta, B	P1-042
Matzelle, M	
Mauracher, A	P1-081,
Merolle, M	P2-096
Meta, I	
Meyer, A	P2-048
Meyer, T	
Meyts, I	P1-083
Miceli, I	
Michurina, S	P2-058, P2-070
Mickelson, C	P3-042
Mielke, L	P2-077
Miggiano, R	P2-004
Mikdache, A	P2-008
Miles, M	
Milieu Interieur Consortium	P2-062
Miller, G	P2-020
Miller, M	P3-017
Miller-Jensen, K	P3-055
Millet, V	
Millrine, D	
Mills, K	
Milner, J	P1-083, P1-086
Min, H	P1-034, P1-035
Min, J	P2-101
Minch, K	P2-039
Ming, G	P3-054
Miorin, L	P3-025
Miranda-Bedate, A	P3-056
Mishra, B	P3-008
Mitra, D	P1-061
Mitsialis, V	
Mo, J	P2-038
Mohammed Salih, M	P1-094
Monack, D	P3-063
Monaco, F	
Monestiroli, A	P2-057
Montano, E	
Monteiro, J	P3-070
Monticelli, S	P1-082
Moon, J	P2-029
Morales, R	P1-012
Morand, E	, P1-065
Moreno de Lara, L	P3-098
Moresco, P	

Maxwell, M
Mayer-Barber, K P3-052
McAloon, C P3-096
McArthur, A P3-017
Mccabe, M P3-081
McCaughan, F P3-078
McCauley, S
McCune, B
McFadden, M P3-111
McGettrick, A
McGraill, J P1-005
McGuire Jr, D P2-009
McLaughlin, M
McMahon, J
McMillan, L P3-046
McPherson, A
McRae, B
McRae, H P3-004
Mdluli, N
Meade, K P3-095, P3-096
Mears, K
Medena-Yanez, I P1-012
Medzhitov, R P3-079
Meijer, A P2-037
Meininger, L

N

Nagashima, K P2-042
Nahm, M P1-001
Nakayama, H P1-106
Nakayama, J P1-106
Nally, K
Namer, L
Nandi, B P2-018
Naquet, P
Narayan, R P3-041
Natoli, G P1-082
Neal, T P3-042
Neehus, A
Negatu, S P3-054, P3-093
Neubert, P
Neumann, C
Newsham, I P3-086
Nguyen, H P3-017
Moriya, K
Morrissette, J
Morse, R P1-107
Mossman, K P3-017, P3-069
Mubareka, S P3-017
Mudroňová, D P1-033
Muglia Amancio, A
Mukaida, N P1-045, P2-025, P3-018
Mukhtar, S P3-008
Mukoyama, H P3-022
Mukundan, A P1-101
Mulenge, F P3-005, P3-051, P3-089
Müller, M P3-017
Mun, S P2-099
Munoz-Sandoval, P P1-098
Munzar, J P2-105
Muratore, F
Murer, P
Muroyama, Y
Murphy, A P1-056, P2-042
Murphy, M
Murray, K P2-041
Murray, K P3-031
Muusse, T P1-091
Myers, B
Nguyen, T P3-092
Nguyen Truong, D P3-016
Ni Cheallaigh, C P2-062
Niavi, C P2-009, P2-010
Nicholson, b
Nickle, R P3-106
Nieto-Patlan, A
Nikitopoulou, E
Nikolouli, E P1-039
Nikovics, K P1-014
Nio-Kobayashi, J P1-106
Nistal-Villan, E P2-104, P3-056
Nogueira, F P1-021
Noh, J P1-038
Nold, C P2-030
Nold, M P2-030
Nosaka, M P1-045, P2-025, P3-018

Nguyen, J
Nguyen, M P2-041

O

O'Farrelly, C P2-062, P3-088, P3-094, P3-095, P3-096
O'Mahony, L
O'Neill, L P1-009
Obeng, R P2-010
Obers, A
Obradovic, J P1-032
O'carroll, S P1-009
Ochando, J P3-056
O'Dea, K P2-096
Odendall, C
Oft, M P2-022, P2-023,
O'Garra, A P1-070
Ogawa, S P1-106
Ogishi, M
Ogunsulire, I P3-048
Oh, E P2-014
Oh, J P3-110
Oh, T P3-020
Oh, Y P2-101
Ohara, D P3-022
Ohnmacht, C
Ohteki, T P3-022
Okamura, Y P3-026
Oktay, M P1-002
Olagnier, D P1-027
Oland, S P2-011
Olazagoitia Garmendia, A
Olmer, R P1-027
Omelia, D
O'Neil, J
O'Neill, L
Ongo, G P2-105
Oram, R
Orchard, R P3-100
Orcutt-Jahns, B P2-048
Orengo, J P1-056, P2-042, P3-034
O'shea, J P2-011
Osman, M
Ostrouska, S
O'Toole, C
Overall, C P3-017

Ö

Österlund, P P3-029, P3-076

P

Pal, B
Palacín, I P2-104
Palamidas, D
Palermo, E P3-094
Palm, N P2-041
Palmarini, M P3-097
Palmer, G P3-077
Pandey, S
Pantsulaia, I P3-007
Papadaki, M P2-078
Papelis, D P2-078
Parathan, P
Pardy, R P2-096
Parfyonova, Y P2-058, P2-070
Parisi, B P2-013
Pellegrini, S
Pendse, M
Peng, G P2-068
Peng, X P1-083
Penninger, P P1-021
Perdiguero, B P1-005
Pereira Ribeiro, S P1-073
Perez, J
Pérez-Díez, A P2-015, P2-061
Perry, J P1-097, P3-104
Pervin, M
Pessler, F P1-027, P2-006
Petersen, L
Pfeiffer, J
Phan, A P3-011, P3-104, P3-105
Nurieva, R

Park, J	P3-020
Park, J	P2-044, P2-045
Park, J	P1-019, P1-020
Park, J	P3-091
Park, J	P3-040
Park, J	P2-069, P3-027, P3-053
Park, J	P2-064
Park, J	P1-029, P2-021
Park, J	P2-035
Park, J	P2-036
Park, J	P2-059
Park, S	P1-037
Park, S	P3-033
Park, S	
Park, S	P2-014, P3-006, P3-013
Park, S	P3-032
Park, S	P2-101
Park, S	P2-064
Park, Y	P3-023
Park, Y	P1-049,
Parthasarathy, S	P3-098
Pasquesi, G	
Pasquin, S	P1-090
PASXALIDIS, N	P2-078
Patel, A	P3-097
Patel, D	P1-016, P1-017
Patel, R	P1-022
Patil, S	P1-074
Patino-Molano, L	P3-066
Patrick, K	P1-079, P3-082, P3-101, P3-102
Patrick, S	P2-088
Pavlakis, G	P2-103,
Pavlos, E	P2-078
Pavlou, A	P3-005, P3-051
Peace, C	P1-009
Peauroi, E	
Pederzoli-Ribeil, M	
Pedica, F	P2-057
Phelps, C	
Philippot, Q	P3-061
Picton, L	P2-003
Piehler, J	P1-068
Piemonti, L	P2-057
Pietropaoli, D	
Pinelli, E	P3-056
Piperakis, A	P2-076
Pirkmajer, S	P1-023
Pisanelli, G	P3-025
Pitzalis, C	
Pizarro, T	P2-107, P3-071, P3-073
Pizarrro, T	
Pliaka, V	P1-026
Plückebaum, N	P1-039
Poholek, A	
Polissidis, A	
Polletti, S	P1-082
Pollmann, C	P1-068
Polyzou, A	
Porubska, B	P2-079
Porubská, B	P2-080
Postler, T	
Poulakou, G	P2-078
Prag, H	
Prasad, V	P1-055
Previato de Almeida, L	P1-057
Privitera, G	P3-071
Pross, L	P3-042
Protzer, U	P2-066
Provost, M	P1-007
Pryimych, N	P2-095
Puca, P	
Puchan, J	P3-061
Puel, A	P3-061
Puigdevall, L	P2-074, P2-075
Puri, P	P3-015

Q

Qiang, X	P3-062
Quinn, L	
Quiroz, R	

R

R Melo-Silva, C	P3-019
Rabahi, S	P1-012, P2-008
Raccamarich, P	P2-098
Radhakrishnan, G	P2-018
Rafferty, T	P1-011
Raghu, D	
Rahal, F	P3-088
Rahmberg, A	
Raitakari, O	P3-058
Raj, T	
Ramadass, M	P2-023,
Ramage, H	P3-019
Raman, C	P3-008
Ramanathan, S	P2-091
Ramesh, V	P3-015
Ramnath, D	P2-020
Ramon, A	
Rao, H	
Rao, K	P1-081
Rao, W	P2-105
Rappe, J	P3-067
Rapti, V	P2-078
Rastogi, S	P3-052
Ratti, N	P2-022, P2-023
Rau, A	
Raue, R	P3-077
Ravel, J	P2-098
Raven, K	
Ravi, S	P1-001
Raviola, S	P2-004, P2-005
Ray, A	
Reed, S	
Reese, T	P3-100
Reichel, J	
Reich-Zeliger, S	P1-003
Reid, C	P3-095, P3-096
Remot, A	P3-095, P3-096
Ren, J	P2-003
Reschke, S	
Reuschl, A	P3-097
Revajová, V	P2-027
Reyes-Moreno, C	P2-097
Ribas, A	P2-003
Riccobono, D	P1-014
Rice, C	
Rice, C	
Richmond, N	P3-046
Richter, K	
Rico, R	
Ricordi, C	
Riener, R	
Rigas, Y	P2-060
Ring, A	
Ritter, B	P1-039
Rius, S	P2-104, P3-056
Robertson, S	
Robichaud, N	P2-105
Robinson, E	P2-046
Robinson, R	
Rocco, J	P1-052
Rodin, A	P2-048
Rodrigues, G	
Rodriguez, E	P1-075
Rodriguez, G	P2-003
Rodriguez-Garcia, M	P3-098
Rodriguez-Morales, P	P3-079
Rojas Márquez, H	
Rojas-Sánchez, L	P3-059
Rokkam, D	P2-022, P2-023,
Romano Bruno, F	P1-073
Romberg, N	
Romera Hernandez, M	P3-016
Romero, H	P1-108
Rontogianni, M	P3-058
Rosadini, C	
Rosain, J	
Rosati, R	P2-057
Rose-John, S	P3-048
Rosen, D	P2-022
Ross, C	P1-018
Rotkopf, R	P1-003
Roupe, J	P2-023
Rousseau, M	P1-090, P1-093
Rovina, N	P2-078
Roynard, M	
Rubino, R	
Rudloff, I	P2-030
Ruffner, M	P1-081
Ruisseaux, C	P1-108
Rupert, A	P1-052
Ruscher, R	
Russo, L	P2-107

Rich, H
Richard, D P3-017
Richards, C P2-071
Richardson, A P1-083

R

Rosati, m

S

Saad, M
Saco, J P2-003
Sadeghi, M P3-089
Sadhukhan, R
Said, E P1-100
Saini, C P3-015
Salagianni, M P2-078,
Salazar, A P2-098
Salazar, U
Saldaña, X P3-093
Salgado, G
Saller, B P3-063
Salloum, Y P1-012, P2-008
Sallusto, F P1-082, P3-061
Salmi, M P3-058
Salvador Rocha, E P3-055
Salvarani, C
Samarajiwa, S P3-086
Samelis, V P3-067
Sanchez, L P1-002
Sanchez-Elsner, T P3-046
Sander, C
Sanders, C P3-111
Santiago, M P3-045
Sanz Sanjuan, A P3-085
Sa-Pessoa, J P1-006
Saporita, A P3-062
Sapra, L P3-015
Saqib, U
Sarić, A P1-032
Sato, K P2-102
Sato, S P2-102
Satyanarayanan, S P3-068
Savan, R P3-026
Savvides, S P1-007, P1-068, P1-088, P1-089, P3-085, P3-087
Sawden, M
Ryan, D
Ryan, T
Ryu, G P2-038
Ryu, J P3-021
Shin, H P2-044, P2-045, P3-021
Shin, H P2-031, P2-038
Shin, S P1-037
Shin, S P2-019
Shin, S P3-027
Shirasaki, T
Shourian, M P1-108
Sidor, K P3-047
Sigal, L P3-019
Sigua, T P3-007
Silberberg, E P3-068
Sim, Y P3-023
Simmons, A P3-071
Simmons, J P1-018
Simpson, K
Singh, S P1-101
Sinha, R P3-008
Siouti, E P2-078
Sironen, T P3-076
Sitia, G P2-057
Siva Venkatesh, I P1-024
Siwak, K P3-017
Skirecki, T P3-047
Skön, M P3-029
Skowyra, A
Slawinski, H P1-070
Sleeman, M P1-056, , P2-042, P3-034
Smieszek, S
Smith, E P2-096
Smith, E
Smith, K
Smith, K
Smith, M P1-079
Smith, M
Snapper, S
Soboh, S P3-068

Saxton, R	P2-003,
Sayed, S	P1-081,
Sazeides, C	P1-086
Scarneo, S	P1-048
Schacker, T	
Schaper, F	P2-024
Scheffold, A	P3-048
Schifanella, L	P3-031
Schif-Zuck, S	P3-068
Schiller, L	P2-066
Schlerman, F	
Schlom, J	
Schmid, K	
Schmidt, T	P3-077
Schmitt, E	
Schott, E	P3-111
Schreiber, G	P1-003, P2-083
Schroder, K	P2-020
Schroeder, J	
Schroeder, T	P3-031,
Schumacher, F	P2-030
Schumertl, T	P1-063
Schuster, I	P1-065
Schwartz, D	P2-063
Schwartz Wertman, R	P3-063
Schwerdtfeger, M	P3-044, P3-045
Scott, G	P2-042
Scott, H	P1-079
Scott, W	
Scotti, G	P2-057
Sebastian-Delacruz, M	
Selemidis, S	
Semana, M	P2-023
Sen, E	P2-088, P2-089
Seo, D	P2-064
Seo, E	P2-035
Seo, I	P2-069, P3-027, P3-053
Seo, M	P1-069
Seo, M	P2-099
Seo, Y	P3-013
Sereti, I	P1-052, P2-015, P2-061
Servais, F	P2-092
Soh, W	P3-040
Solorzano, C	P1-005
Solstad, A	
Somova, V	P2-079, P2-080, P2-081
Son, C	P2-106
Son, D	P3-092
Son, S	P3-092
Song, C	P3-092
Song, D	P2-007
Song, E	P3-053
Song, H	P3-032
Song, M	P2-101
Song, W	P1-046, P3-028
Sonnenburg, E	P2-046
Sonnenburg, J	P2-046
Southern, P	P3-031
Sowrirajan, B	P1-102
Speaks, S	P3-036, P3-111
Speed, T	
Spel, L	
SPINELLA, M	P1-082
Spinelli, L	
Spolski, R	P1-067
Sposito, B	
Srinvasan, R	P3-101
Srivastava, R	P1-043, P3-015
Ssebyatika, G	P1-039
St. Leger, A	P2-060
Stacey, H	P3-017
Stacey, K	P1-091
Stafeev, I	P2-058, P2-070
Stahl, N	
Stamou, P	
Stangel, M	
Stanijevic Pirkovic, M	P1-032
Stellas, D	P2-103
Stermer, K	P2-061
Sterneck, E	P1-084
Stewardson, C	P1-078, P2-074, P2-075
Stock, A	P1-057
Stocker, C	
Stow, J	P1-091

Sexl, V	P1-021
Shah, V	
Shane, J	P2-060
Shanks, R	P2-060
Shao, S	
Shapira, J	
Sharma, A	P3-015
Sharma, M	P3-041
Sharma, P	P1-016, P1-017
Sharma, S	
Sharpe, H	
Sher, A	P2-011
Sherlock, R	
Shestakova, E	P2-070
Shestakova, M	P2-070
Shi, W	
Shih, H	P1-095
Shim, K	P3-020
Shimada, E	P1-045
Shimokawa, C	P2-065
Shin, D	P1-066
Shin, E	P1-066
Shin, H	P1-055

Š

Ševčíková, Z	P2-027

S

srivatsan, s	P2-042

T

Tabatabaei-Zavareh, N	P3-016
Taft, J	P1-083
Tahuil, N	
Takahashi, J	
Takaoka, A	P2-102
Takeuchi, Y	P3-022
TALY, A	P1-074
Tam, M	P3-106
Tamvakopoulos, C	P2-078
Tang, L	P3-019
Tang, T	P1-065
Tangye, S	
Tarique, M	P3-015
Tasker, C	
Tauriello, D	P2-057
Tavernier, J	P1-068
Stravokefalou, V	P2-103
Strecker, B	P1-057
Strickland, M	P3-046
Striepen, B	P2-096
Strugnell, R	
Su, H	P1-081,
Su, L	P2-003
Sudek, L	
Sugrue, J	P2-062, P3-088, P3-094
Suh, I	P3-012
Sui, H	P1-102, P3-030
Sularea, V	P3-094
Sulka, K	
Sullivan, M	P2-020
Sun, E	P2-072
Sung, H	P3-091
Suri, V	P2-088, P2-089
Sutter, K	P3-044, P3-045
Swanson, R	P2-039
Sweet, M	P1-091, P2-020
Swiecki, N	P3-034
Szabóová, R	P2-027
Szomolay, B	

Štofilová, J	P1-033

Toftdal Dynesen, L	
Toller-Kawahisa, J	
Tonon, G	P2-057
Torra I Benach, M	P3-086
Torres-Juarez, F	P3-052
Torres-Ruiz, F	
Totagrande, M	P2-022, P2-023,
Tougaard, P	P3-085
Tounsi, N	P3-043
Towers, G	P3-097
Tran, K	
Tran, K	P2-022, P2-023
Trapani, J	P3-067
Treat, B	P2-060
Tree, T	
Tregoning, J	

Taylor, J
Teahulos, I P2-105
Temchura, V P3-059
Ten Caten, F P1-073
Tennakoon, M P3-021
Terpos, E
Thanopoulou, K
Thanos, D
Thelen, F P3-090
Thelin, M
Theobald, H P3-059
Theodoropoulou, K
Theoharides, T P3-014
Thiagarajah, J
Thiem, M P1-055,
Thierens, L P1-089, P3-087
Thomas, A P1-107
Thorne, L P3-097
Tian, Y P1-106
Tiefenthaler, G P2-030
Timonen, M P3-058
Ting, O P3-024
TIU, C P3-084
Tlapakova, T P2-079
Tobin, M P3-058
Triantafyllia, V P2-078, P3-060
Tripathi, S P3-041
Trivic, T P1-103
Trollope, G
Trstenjak-Prebanda, M P1-023
Tsai, Y
Tsilidis, K P3-058
Tsilioni, E P3-014
Tsirigotaki, A P1-068
Tsogtsaikhan, S P3-080
Tsolakos, N P1-026
Tsui, C
Tsymala, I P1-021
Tummers, B P3-063
Tur Planells, V P2-104
Turbow, B P1-018
Turcotte, C
Turel, O
Turgeman, O P3-057
Turk, B P1-023
Turner, N
Tur-Planells, V P3-056
Tutau, C
Tzioufas, A
Tzoulaki, I P3-058

T

Ten Dijke, P P1-101

Ü

Überla, K P3-059

U

Ucar, D P1-001, P2-016, P2-017
Uchida, K P1-106
Umdor, S P2-088, P2-089
Uno, K P2-082
Utz, P
Utzschneider, D

V

Vahedi, F P3-070
Vail, K P3-101
Vajjhala, P P1-091
Valanparambil, R P2-009, P2-010
Vallee, A P1-108
Van Dis, E P1-036
Van Gelder, R
Van Putte, W P1-068
Vance, R P2-013, P3-037
Velmurugan, J
Venuturupalli, S
VEROUTIS, D
Verrou, K P1-026
Verschueren, K P1-068, P1-089
Verstraete, K P1-068
Vidmar, K P2-107, P3-073
Viejo-Borbolla, A P1-039
Vijayanand, P P1-067

Vandenabeele, P P3-085
Vanker, M P1-059
Varghese, J
Vasek, D P2-079
Vašek, D P2-080, P2-081
Vasilogianni, M P3-060
Vaughn, L P1-022
Vazquez, C P3-054, P3-093
Vázquez, J P1-004
Veikko, S P3-058
Velez, A P3-031,

V

van Dinther, M P1-101
van Uelft, M

W

Wack, A P3-067
Wadsworth, M
Wagner, A P1-079
Wallace, D
Walter, J P1-081
Waltl, I P3-005
Wan, Y P2-020
Wanford, J
Wang, A P2-041
Wang, C
Wang, C P1-060
Wang, G P3-100
Wang, H
Wang, J
Wang, S
Wang, Y P2-020
Wang, Z
WANG, M P2-085, P3-084
Waqas, F P1-027, P2-006
Wargo, H P2-107
Watanabe, H P3-022
Wataru, F P1-106
Watson, R P1-079, P3-082, P3-101, P3-102
Weekes, M P3-078
Weerasooriya, M P3-024
Weichselbaum, R P1-013
Weigert, A P3-077
Weighardt, H P1-055,
Weinberg, S
Villablanca, E P1-012
Villadangos, J
Villarino, A P2-011
Vivona, S P2-022, P2-023,
Vogel, T
Volchinski, V P1-003
Volpi, S
Von Kriegsheim, A
Voronov, E P2-083
Vu, S
Vuillier, F
von Pein, J
Weng, T
Werner, A P3-098
West, A P2-072
Westendorf, A P3-045
Westfall, S P2-068
Whalen, B
Whelan, M P3-097
Wherry, J
Whisstock, J P2-030
White, A
Whitmore, L
Wieking, G
Wielscher, M P3-058
Wiethe, C P3-106
Wiggett, H P2-037
Wilcox, J
Wilkinson, E
Williams, D
Williams, J P2-107, P3-071
Williams, N
Williams, N
Wilson, H P3-017
Wilson, T
Winer, H
Wingfield, M
Witt, K P3-037
Wolfarth, A P2-009, P2-010, P2-015
Woo, W P1-037, P1-076, P2-073
Woo, Y P3-020

Weindel, C P3-082, P3-101, P3-102
Weissman, D P3-011
Weissman, J
Wells, A
Welsh, J P3-101
Wen, L
Weng, C P2-043
Woznicki, J
Wray-McCann, G P2-072
Wu, C
Wu, C
Wu, P P1-053
Wu, X P1-070
Wynn, T

X

Xu, Z
Xue, H

Y

Yakou, M P2-077
Yamamoto, H P1-045
Yancopoulos, G P2-042
Yang, C
Yang, J P2-064
Yang, J P1-102
Yang, M
Yang, O P3-031
Yang, R
Yarmolinsky, J P3-058
Yaseen, H P3-068
Yasumizu, Y P3-080
Ye, J P2-047
Ye, P P2-061
Yen, M P2-003
Yeo, J P3-091
Yeo, J P1-069
Yerov, O
Yesudhas, D P2-094
Yin, M
Yongliang, Z P1-044, P2-033, P3-024
Yoo, J P2-019
Yoon, G P3-109
Yoon, H P1-040
Yoon, J P3-038
Yoshida, H P3-080
Yoshida, T P2-041
Yoshikawa, T P2-082
You, G P2-101
YOUNG, H
Yount, J P3-036, P3-111
Youssef, M P1-086
Yu, J P3-032
Yu, S P3-079
Yu, S P1-037
Yuan, R
Yuan, Z P2-047
Yui, K P3-080

Z

Zacharias, M P1-071
Zaffalon, L
Zaid, A
Zannino, L P2-057
Zanoni, I
Zapatero, F P1-027
Zecchini, V
Zeiträg, J
Zeituni-Timor, O P3-068
Zemlickova, E P3-106
Zerbe, C P3-078
Zhang, D P3-066
Zhang, Q
Zhang, S
Zhang, Z
Zhao, M P2-006
Zheng, P P2-086
Zhou, C P1-098
Ziegler, A P3-089
Zordan, P P2-057
Zotta, A P1-009
Zubkova, E P2-058
Zuliani-Alvarez, L P3-097
Zuniga, E P3-090
Zuniga, L P2-022

Ž

Žurmanová, J P2-081