Presenter's Name: Al Jawhri, MohdWessam

Additional Author(s): Cameron L

Abstract Title: The effects of Glucocorticoids on Th2 cell phenotype and function

Abstract:

Introduction: Asthma affects more than 339 million people globally, with high morbidity and strain on the healthcare system. It is not a homogeneous disease which increases the complexity of finding appropriate treatments, with the most common form of the disease being the Type 2 high asthma. T helper (Th) 2 cells are an important cell type involved in Type 2 high asthma and their actions are mediated by the type 2 cytokines. Glucocorticoids (GCs) are the primary treatment for Type 2 high asthma as they reduce type 2 cytokines; however, the effects of their prolonged use on these Th2 cells are unknown. Despite taking high dose GC, severe asthmatics have persistent symptoms that are considered to be related to having more Th2/Th17 dual positive cells. We hypothesize that exposure to glucocorticoid shifts Th2 cells towards having Th2/Th17 properties and/or to changes in Th2 cell expression that make them more pathogenic.

Methods: Primary human Th2 cells from donor peripheral blood mononuclear cells (PBMCs) will be used as the model of my study. Here we examined expression changes under Dexamethasone (Dex) treatment by assessing markers of the Th17 phenotype using qRT-PCR and/or Flow cytometry. Treated cells were assessed for the expression of genes part of the Th17 gene signature, which includes IRF4, CD161, and IL-7 receptor (R). Dex with and without IL-7 treatments were used to assess the influence of IL-7 on the expression of the Th17 genes as well as the expression of integrins and adhesion molecules.

Results: Dex treatment resulted in an increased level of IRF4 and CD161 mRNA as well as IL-7R protein. Cells treated with both Dex and IL-7 showed that IL-7 decreased some Th17 genes (CD161, CCR6) but also resulted in a trend towards higher levels of ITGA4 than with Dex or IL-7 alone. ITGA3 was induced by Dex only. These findings suggest Dex may support the differentiation of the Th17 phenotype from Th2 cells and/or drive changes in function that make Th2 cells more pathogenic.

Discussion: Dex upregulating CD161 and IRF4, both Th17 genes, suggests GC may prime Th2 cells to transition into the dual Th2/Th17 cells associated with asthma severity. Alternatively, with IL-7R induction by GC, these Th2 cells could be more sensitive to local IL-7 levels and the ability of this cytokine to influence expression of integrins associated with tissue residency and asthma severity.

POSTER PRESENTATIONS 1
1C: INFECTION, IMMUNITY & INFLAMMATION

Presenter's Name: Gholami, Hasti

Additional Author(s): Jawhri MA, Hong MM, Morin A, Cameron L, Castellani CA

Abstract Title: The Effect of Asthma and Cardiovascular Drug Exposures on Mitochondrial Function

Abstract:

Introduction: The mitochondrion is a membrane-bound organelle that plays a crucial role in adenosine triphosphate (ATP) production for cellular energy. Oxidative capacity changes in mitochondria can lead to mitochondrial dysfunction and cause insufficient cellular energy production, decreasing ATP production and increasing reactive oxygen species (ROS) production. Mitochondrial DNA copy number (mtDNA-CN) is a biomarker for mitochondrial function and decreases in mtDNA-CN have been associated with chronic inflammatory diseases, such as asthma and cardiovascular disease. Modifications to mitochondrial function can be mediated by chemical therapeutics, though research on this topic is limited. My study aims to examine the mechanisms mediating the effect of asthma and cardiovascular disease drugs on mitochondrial function.

Methods: This project utilizes CCRF-CEM and THP1 cell lines to assess the effects of asthma and cardiovascular drugs in vitro, respectively. The study assesses the effect of the drugs dexamethasone and formoterol for asthma, and simvastatin and ezetimibe for cardiovascular disease. DNA was extracted from each treatment assay and mtDNA-CN was quantified using qPCR. Citrate synthase (CS) and lactate dehydrogenase (LDH) assays were used to assess mitochondrial function.

Results: A decrease in mtDNA-CN and mitochondrial function was detected in the CRM-CEM cell line upon exposure to increasing dexamethasone concentrations (0.1-0.9μM). An increase in mtDNA-CN was detected in the CRM-CEM cell line upon exposure to increasing formoterol concentrations (0.01-0.05Mm). The CS and LDH assays are expected to show an increase in mitochondrial function in formoterol treated CCRM-CEM cells. An increase in mtDNA-CN and mitochondrial function following simvastatin, and ezetimibe treatment on THP1cells is expected in standalone. A net increase in mtDNA-CN and mitochondrial function is expected when both dexamethasone and formoterol, and simvastatin and ezetimibe are given in combination.

Discussion: The results of this study reveal the effects of asthma and cardiovascular disease drugs on mitochondrial function and will contribute to refining treatment plans for patients to optimize patient health. Future studies should look at the mechanistic sex differences in mitochondrial function within chronic inflammatory settings by examining the effect of estrogen in addition to these asthma and cardiovascular drugs on mitochondrial function.

Presenter's Name: Hong, Megan

Additional Author(s): Maleki Vareki, S

Abstract Title: Deciphering the underlying immune mechanisms of anti-CTLA-4 efficacy against neuroblastoma tumors with induced DNA mismatch repair deficiency

Abstract:

Introduction: Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) highly expressed on regulatory T-cells (Tregs) inhibit the activation of pro-inflammatory T-cells responsible for eliminating cancer cells. Anti-CTLA-4 can enhance T-cell activation by increasing CD28 co-stimulatory signalling or depleting Tregs through Fc-dependent effector mechanisms. Strategies to improve its therapeutic efficacy are needed as patient response rates to anti-CTLA-4 are low. Defects in the DNA repair pathways can increase tumor mutation burden (TMB) and the production of neoantigens that promote anti-tumor immunity. Patient response rates to anti-CTLA-4 have been positively correlated with TMB in several cancers. Our lab has demonstrated that induced DNA mismatch repair (MMR) deficiency in an immunologically-cold and low TMB tumor model, neuroblastoma, can enhance the anti-tumor response induced by anti-CTLA-4. Here we investigate the underlying mechanism(s) to which MMR deficiency in tumors can enhance the therapeutic effect of anti-CTLA-4. We hypothesize that induced MMR deficiency in neuroblastoma tumors enables anti-CTLA-4 to inhibit/deplete intratumoral Tregs and thereby increase the infiltration and activation of tumor-specific effector T-cells.

Methods: To determine the effects of anti-CTLA-4 and MMR deficiency in tumors, flow cytometry will be used to quantify peripheral and intratumoral Tregs. Mixed-lymphocyte reaction assays will be used to examine the effect of anti-CTLA-4 on expanding tumor-specific T-cells co-cultured with MMR-deficient (dMMR) or -proficient (pMMR) neuro-2a cells in vitro. To determine if Fc-dependent effector mechanisms are involved in the therapeutic efficacy of anti-CTLA-4, anti-FcR antibodies will be co-administered with anti-CTLA-4 in vivo. The presence of intratumoral macrophages that facilitate Fc-dependent Treg depletion will also be examined to assess how MMR deficiency alters the tumor microenvironment to enable anti-CTLA-4 to deplete intratumoral Tregs.

Results: Preliminary results suggest that anti-CTLA-4 increases the intratumoral CD4+ effector: Treg ratio by depleting Tregs and increasing CD4+ICOS+ effector T-cells in dMMR neuroblastoma tumors.
Implications: By understanding the underlying mechanism(s) of anti-CTLA-4 in dMMR tumors, it may justify targeting the MMR pathway to improve the response to ICIs in patients with immunologically cold and/or low TMB tumors.

POSTER PRESENTATIONS 1
1C: INFECTION, IMMUNITY & INFLAMMATION

Presenter's Name: Jackson, Matthew

Additional Author(s): Menassa R, Kiser P

Abstract Title: Use of a VHH-IgA Fc fusion protein targeting surface protein Intimin to block E. coli O157:H7 colonization in GI tract of mice

Abstract:

Shiga toxin-producing Escherichia coli (Enterohemorrhagic E. coli (EHEC); O157:H7 and related serotypes) is a human food-borne bacterial pathogen that can inhabit the gastrointestinal (GI) tract of common livestock, such as cows and pigs. Human infection by consuming EHEC-contaminated animal products lead to over 90,000 illnesses, 3,200 hospitalizations and almost 100 deaths annually in the United States alone annually. Conventional antibiotics exacerbate Shiga toxin-mediated cytotoxicity leaving no effective treatments for EHEC infection aside from supportive care. In the absence of effective treatments for EHEC, prevention and control of the pathogen prior to zoonotic transmission are critical for public health management of this disease.

This study is designed to test the safety and efficacy of a plant-derived antibody, VHH-IgA Fc fusion protein, that targets the E. coli O157:H7 surface protein, intimin. This fusion protein has been shown to effectively block E. coli O157:H7 from binding to human cells in vitro. These antibodies are expressed in Nicotiana benthamiana and the antibody enriched plant leaves are processed whole to produce a dried plant biomass (DNB). DNB is developed to provide a high yield, cost-effective food additive that prevents colonization of E. coli O157:H7 in livestock GI tracts, thereby reducing the pathogen's contamination of the food chain. This study will begin with preliminary testing for safety and efficacy of oral administration of DNB in a low pathogenicity mouse model for E. coli O157:H7 infection. For this initial study, each experimental condition will include male and female mice. Safety of DNB administration will be assessed using clinical signs (i.e. ill thrift, weight loss) with post-mortem gross and histologic assessment for abnormalities relative to untreated mice. Efficacy will involve E. coli O157:H7 challenge at two timepoints: bacterial inoculation before or after DNB supplementation. The measure of DNB efficacy will involve quantification of GI tract colonization and fecal shedding of the bacteria as well as gross and histologic assessment of the mice following infection and/or treatment. This study is the first step in determining whether this plant-produced food additive can be used on livestock to help reduce or eventually eliminate E. coli O157:H7 in a passive, inexpensive manner, as successful results from this study can lead to further testing on livestock animal models.

Presenter's Name: Quadri, Ahmed

Additional Author(s): McClennan A, Hoffman LA

Abstract Title: Regional Differences in the Morphology of the Gastrocnemius Muscle of Duchenne Muscular Dystrophic Mice

Abstract:

Introduction: Duchenne Muscular Dystrophy (DMD) is a X-linked recessive disorder that is characterized by progressive muscle weakness and degeneration. It occurs in individuals that have a mutation in the dystrophin gene which results in a non-functional dystrophin protein being expressed in myocytes. While researchers are focused on the development of dystrophin-gene replacement therapies to treat DMD, few studies aim at correcting the damaged microenvironment of DMD-affected muscles. To find novel therapies that reconstruct the microenvironment of DMD-affected muscles and fosters muscle repair and regeneration, a better understanding of the microenvironment of myocytes was needed. In this study, we attempted to determine whether regional differences in the morphology of the upper, middle, and lower regions of the gastrocnemius muscle of mdx/utrn+/- mice exists.

Methods: Sections of the upper, middle, and lower regions of the gastrocnemius muscle of female mdx/utrn+/- mice and healthy controls were stained using Hematoxylin and Eosin (H&E). The entire upper, middle, and lower regions of the gastrocnemius muscle of mdx/utrn+/- mice and healthy controls was then imaged. ImageJ was used to map out and determine the amount of necrotic, regenerative, and healthy muscle tissue present in different regions of the gastrocnemius muscle. Necrotic, regenerative, and healthy muscle regions were found by looking at the position of the nuclei of each myocyte. A two-tailed T-test was then performed to investigate whether regional differences in the morphology of the gastrocnemius muscle between the mdx/utrn+/- mice and healthy controls existed.

Results: Currently, no data has been collected. Issues with H&E staining and sectioning of tissues have contributed to the delays in the study. However, these issues have since been resolved and the use of the whole-imaging scanner to image the slides have streamlined the imaging stage of this study. Right now, we are in the data-collection stage.

Discussion: By investigating the regional differences in the morphology of the gastrocnemius of DMD mice and healthy controls, it has given us a better view of the morphological changes that occurs to the entire muscle of DMD patients, as well as a broader understanding of the defects found in the microenvironment of the myocytes. The will help in finding novel strategies that correct for these defects in future studies.

POSTER PRESENTATIONS 1
1C: INFECTION, IMMUNITY & INFLAMMATION

Presenter's Name: Safdar, Aisha

Additional Author(s): Sidahmed, A

Abstract Title: Immune Response to SARS-CoV-2 Infection in Immunocompromised Patients

Abstract:

Introduction: COVID-19 is an infectious respiratory disease caused by SARS-CoV-2, a new coronavirus strain, that was discovered in November 2019. COVID-19 can manifest itself in various ways and in severe cases, it can cause individuals to have respiratory failure, cardiac injury, or death. Research shows the immune response in individuals who get severe COVID-19 resembles a cytokine storm, characterized by an increase in pro-inflammatory cytokines such as IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, IL-12, IL-18, IFN-γ, TNF-α, GCSF and CXCL-10. In this study, we evaluated if immunocompromised patients with severe covid present with a cytokine storm, as compared to non-immunocompromised covid patients. We hypothesize that immunocompromised patients are protected from SARS-CoV-2 related cytokine storm due to their immune dysfunction.

Methods: To test this hypothesis, a cohort of 25 immunocompromised patients who were hospital-admitted for COVID had 2-3 blood samples collected during their time at the hospital. Chemokine and cytokine bead array kits were used to analyze cytokine levels in patients' serum samples. The patients were matched with controls, based on age and gender. The cytokine data was analyzed using GraphPad to conduct t-tests and assess if there was a significant ($p<0.01$) difference in cytokine levels of the study subject and their control. The data was assessed at a group level of patient vs control in R using a linear regression and the underlying data was visualized as boxplots. Subsequent analysis comparing cytokine levels will be performed in R.

Results: Our initial results show there is little difference in the cytokine response of immunocompromised patients and covid patients when compared at a group-level. At an individual matching level, there are some cytokines that are significantly lower ($p<0.01$) in immunocompromised patients, suggesting these patients may be protected from an increase in some cytokines that are associated with severe covid.

Discussion: These findings show that the varying nature of COVID-19 and the heterogeneity of each patient make it difficult to use single factors such as being immunocompromised to predict an individual's pathological response to COVID. Given the major implications associated with the potential immunomodulatory treatments needed for immunocompromised patients with severe COVID, further research should be conducted on smaller subsets of covid patients and their pathological responses.

Presenter's Name: Teplitsky, Jack

Additional Author(s): Vinokurtseva A, Liu H, Hutnik CML

Abstract Title: Modulating wound healing in glaucoma surgery: ALK5 inhibition to counteract subconjunctival scarring

Abstract:

Excessive ocular scarring is a common postoperative complication of glaucoma surgery. Currently, the chemotherapeutic agent mitomycin C (MMC) is the gold standard used to manage resulting fibrosis despite its cytotoxicity and unreliable efficacy. Our lab has investigated the drug SB-431542 (SB) as a safer alternative to MMC for reducing ocular fibrosis. Previously, we demonstrated SB's ability to reduce collagen contraction in 3D in vitro tissue model of human Tenon's capsule fibroblasts (HTCFs). To further understand the mechanism underlying anti-fibrotic effects of SB, in the current study, we investigate SB's effects on cell metabolism, cytotoxicity and expression of pro-fibrotic proteins MMP9 and ACTA2 in myofibroblasts. We hypothesize SB will exhibit significantly lower cytotoxicity in HTCFs than MMC while displaying comparable anti-fibrotic efficacy.

HTCFs derived from patients undergoing trabeculectomy were pre-treated with SB (20 nM) for 5, 10 or 20 min or MMC (0.2 mg/mL) for 1, 2 or 4 min followed by incubation with TGFβ (2ng/ml) for 48 hrs. Myofibroblasts underwent MTT and LDH assays to characterize cell metabolic rate and cytotoxicity. Total protein was extracted from treated myofibroblasts and protein expression of ACTA2 and MMP9 was measured using immunoblotting.

TGFβ treatment alone induced an increase in ACTA2 and MMP9 protein expression compared to the vehicle control in HTCFs. Treatment of TGFβ-treated myofibroblasts with SB resulted in a downward trend of ACTA2 and MMP9 expression. Similarly, the MMC treatment resulted in reduced ACTA2 and MMP9 expression relative to positive control. 5-minute SB treatment and 4-minute MMC treatment demonstrated a statistically significant reduction of MMP9 and ACTA2 expression relative to the positive control. SB treatment was associated with mildly elevated cell metabolic activity and reduced LDH release compared to MMC treatment.

SB has shown comparable efficacy to MMC in reducing expression of pro-fibrotic proteins ACTA2 and MMP9, while being less cytotoxic in TGFβ-induced myofibroblasts. In the context of SB being previously shown effective to counteract fibroblast-mediated collagen contraction in 3D in vitro tissue-mimetic, these findings suggest that SB could be a promising new alternative to MMC for management of postoperative ocular fibrosis.

POSTER PRESENTATIONS 1
1C: INFECTION, IMMUNITY & INFLAMMATION

Presenter's Name: Wang, Tan Ze

Additional Author(s): Yu F, Warsi A, Rong K, Mele T, Zheng X

Abstract Title: Characterization of differentially expressed circular RNAs in septic peripheral blood mononuclear cells

Abstract:

Introduction: Sepsis, the leading cause of death in intensive care units (ICU), is associated with alterations in genomic expression in leukocytes. Understanding of molecular mechanisms and current treatment for sepsis remain to be improved. Circular RNAs (circRNAs) generated by back-splicing may be associated with sepsis pathogenesis, which needs to be further investigated. We hypothesize that circRNAs in peripheral blood mononuclear cells (PBMCs) of sepsis patients would show differential expression between ICU admission and discharge, and this change in circRNA landscape would contribute to sepsis pathogenesis.

Materials & Methods: PBMCs were isolated from three sepsis patients before and after intensive care, and RNAseq was conducted with total RNA. CircRNAs were identified using four different annotation pipelines and their expression was determined and compared. Expression of selected circRNAs were validated through quantitative PCR in a larger sample size.

Results: Overall circRNA expression in septic PBMCs were found to be more abundant and less diverse at ICU admission compared with ICU discharge. We identified 939 circRNA species through MapSplice2, the most accurate aligner used. DESeq2 analysis revealed 34 differentially expressed circRNAs between ICU admission and discharge. Among these altered circRNAs, an isoform of circASPH was found to be highly expressed at ICU admission.

Conclusion: This study characterized differential expression of circRNA in septic PBMCs between ICU admission and discharge, and explored their potential role as novel markers or targets for sepsis. This study also contributes to the growing field of circRNA bioinformatics, comparing the efficacy of various circRNA identification pipelines.