

expression and neurons to show signs of impaired development. **DISCUSSION/SIGNIFICANCE OF IMPACT:** We expect that anemia has a differential impact on retinal vascular anatomy and neuronal function. While retinopathy severity may be improved, overall retinal function will be dampened. These findings and the mechanistic work of this study will generate targets for intervention to preserve improved retinopathy outcomes but rescue retinal function.

467

### **Gut-brain mechanisms of COVID-19 in wild type mice**

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**OBJECTIVES/GOALS:** SARS-CoV-2 infection has been shown to impact multiple organ systems, including the brain, and is associated with increased cognitive decline in vulnerable populations. The gut microbiome may play a significant role in modulating these effects, as shifts in microbiota composition have been linked to inflammation and systemic disease processes. **METHODS/STUDY POPULATION:** To explore these interactions, we conducted an acute COVID-19 study using 12-week-old C57 mice intranasally inoculated with  $1 \times 10^4$  PFU of the Mouse Adapted 10 (MA-10) strain of SARS-CoV-2. On day 5 postinfection, we performed 16S ribosomal RNA sequencing on fecal samples, analyzed using QIIME2 and DeSeq2, to assess microbiome alterations and identify changes in metabolic pathways associated with inflammatory responses. **RESULTS/ANTICIPATED RESULTS:** Analysis revealed notable shifts in the gut microbiome during the acute phase of COVID-19 infection, with significant alterations in metabolic pathways related to inflammation and immune regulation. These changes suggest that early gut microbiome disruptions may contribute to the host response in acute COVID-19. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our findings indicate that acute SARS-CoV-2 infection leads to rapid alterations in the gut microbiome, which could underlie inflammatory and immune pathways impacting disease progression. These insights highlight the potential for microbiome-targeted therapies to mitigate acute COVID-19 morbidity and warrant further investigation.

468

### **Effects of butyrate supplementation in modulation of gut microbiome and its metabolites in new-onset rheumatoid arthritis**

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**OBJECTIVES/GOALS:** The gut microbiome and its metabolites, such as short-chain fatty acids (SCFA), are dysregulated in rheumatoid arthritis (RA); however, the significance of this observation and

its implications in pathogenesis and therapeutics is unclear. Here, we explore the role of the SCFA, butyrate, in treatment efficacy in new-onset rheumatoid arthritis. **METHODS/STUDY POPULATION:** We designed a proof-of-principle study to determine the effects of butyrate supplementation in new-onset RA (NORA) patients that fulfilled 2010 ACR/EULAR RA criteria. We evaluated the effects of methotrexate (MTX) plus butyrate in NORA (n = 17; 1 gm butyrate, 3 times daily) compared to MTX alone (n = 19) over 4 months. MTX responders were defined by a change in disease activity score (DAS)-28 ESR of > 1.8 at 4 months. Fecal samples were collected at baseline and followed up for 16S rRNA sequencing and metabolite quantification by 1H NMR spectroscopy. Unpaired-t, paired-t, Wilcoxon and Fisher's exact test were performed as appropriate. **RESULTS/ANTICIPATED RESULTS:** MTX responders in the MTX-only group had a higher concentration of fecal butyrate than nonresponders at baseline (p = 0.045). Fecal butyrate concentration decreased over time in treatment responders in MTX group (p = 0.05), whereas butyrate concentration remained similar in MTX/butyrate group. Prior to treatment, both MTX and MTX/butyrate groups demonstrated similar levels of gut bacterial alpha diversity (Shannon index), yet only the MTX/butyrate group demonstrated a significant increase in alpha diversity by 4 months (p = 0.022). LeSe analysis demonstrated increased abundances of *Bacteroides*, *Clostridium*, and *Phascolarctobacterium* in responders in the MTX/butyrate group by 4 months. Ten (52.6%) patients in MTX and 11 (64.7%) in MTX/butyrate group were considered MTX responders by 4 months (p = 0.516). **DISCUSSION/SIGNIFICANCE OF IMPACT:** Butyrate supplementation increased gut microbial diversity in patients and led to increased abundance of *Bacteroides*, which has been implicated in efficacy of methotrexate, a first line medication in rheumatoid arthritis. Butyrate may have implications for the maximization of therapeutic effectiveness in rheumatoid arthritis.

469

### **Identification of gut barrier protective fruits and vegetables for Latin American infants**

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**OBJECTIVES/GOALS:** Early childhood obesity is a major concern for Latin American children in the U.S., with gut barrier dysfunction as a key risk factor. Diet plays a role in gut development, but few studies have focused on Latin American infants. Our objective is to identify culturally relevant introductory foods that promote in vitro gut barrier development and function. **METHODS/STUDY POPULATION:** Pooled human milk (2.5 mL) from 6-month postpartum Hispanic mothers was combined with fruit and vegetable baby food products (2.5 g) and subjected to a 3-phase in vitro digestion system that simulates oral, gastric, and intestinal digestion. Digested products were then anaerobically fermented for 24-hours

using human stool inoculum, centrifuged, and filter sterilized. Intestinal epithelial cells (Caco-2, ATCC) were grown to confluence on 0.4  $\mu$ m polystyrene transwell inserts using a DMEM + 10% FBS medium and allowed to differentiate for 21-days. Highly differentiated monolayers were treated with a 1:4 dilution of fermenta with medium in triplicate. The cell experiment was conducted twice. Cell layer integrity was measured using transepithelial electrical resistance (TEER) 24- and 48-hours after treatment. RESULTS/ANTICIPATED RESULTS: Dietary intake data from the What We Eat in America database indicated that the top 3 fruit and vegetable exposures for infants with Mexican or Hispanic ethnicity were banana, apple, and carrot. Commercial baby food purees of these fruits and vegetables, in addition to baby foods with blueberry and spinach (Natural for Baby, Gerber Products Company) were acquired for digestion and fermentation experiments. Caco-2 cell experiments with these foods are ongoing. We expect Caco-2 monolayer incubated with fermenta from human milk and fruit or vegetables will have greater TEER values due to increased integrity of the cell layer as compared to those with breast milk alone. We also expect that exposure to fruit and vegetable fermenta will increase gene expression of tight junctions compared to exposure to media and human milk. DISCUSSION/SIGNIFICANCE OF IMPACT: Using an in vitro digestion and fermentation system coupled with cell culture studies, we are identifying cellular mechanisms that link individual fruits and vegetables to gut barrier function. This will support translational work focused on mitigating obesity development in vulnerable populations.

470

#### Senolytic therapies as treatments for posttraumatic epilepsy\*

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OBJECTIVES/GOALS: Increased numbers of senescent cells have been detected in both traumatic brain injury and epilepsy, suggesting them as targets for therapeutic intervention for treating posttraumatic epilepsy (PTE) and underscoring the need for innovative methods to identify and target senescent cells as a means of alleviating pathology. METHODS/STUDY POPULATION: C57BL/6 mice will receive a single controlled cortical impact (CCI) before having their brains removed at 1 week, 2 weeks, 4 weeks, 1 month, 2 months, and 4 months post injury (n = 5 per time point). Brain sections will then be co-labelled for glial and senescent markers to observe which cells begin to express senescent markers at various time points. We will also perform single-cell RNA sequencing to observe genetic changes associated with both TBI and epileptogenesis. Mice will also be treated with navitoclax, a BCL2 inhibitor being investigated as a senolytic agent, to determine if treatment results in decreased senescence and epileptogenesis, as well as improved behavioral outcomes. RESULTS/ANTICIPATED RESULTS: Preliminary data revealed that senescent microglia begin to arise in the mouse hippocampus as early as 1 week post injury and continue to increase in concentration over the course of the following month, with up to 25% of microglia expressing p16, a known marker of senescence. We anticipate that further staining will reveal senescent astrocytes and neurons in a similar time-dependent manner. Further, we hypothesize that the single-cell sequencing of microglia from injured mice will reveal alterations to the expression of genes associated with neuronal

excitability, inflammation, and/or synaptic modeling, features known to be associated with epilepsy. Finally, we anticipate treatment with navitoclax will alleviate the senescent phenotype, resulting in decreased epileptogenesis and improved behavioral outcomes. DISCUSSION/SIGNIFICANCE OF IMPACT: Considering the lack of any studies examining senescent cell prevalence in PTE, these data will be the first to identify these cells as etiological factors in PTE onset, as well as druggable targets for improving pathological outcomes in PTE patients.

471

#### Defining proteomic and cellular elements of the pancreatic ductal adenocarcinoma (PDAC) tumor microenvironment with mass spectrometry imaging

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OBJECTIVES/GOALS: Currently, a lack of screening markers and targeted therapies prevent clinicians from successfully treating PDAC. Precision medicine may allow oncologists to better combat this disease. To personalize care, knowledge of tumor protein post-translational modifications, extracellular matrix makeup, and infiltrating immune cells is imperative. METHODS/STUDY POPULATION: Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) was employed to characterize the N glycosylation state, the ECM composition, and immune cell populations present within 10 formalin fixed paraffin embedded PDAC patient samples. Molecular dry spray of PNGase F and Collagenase III followed by enzymatic digestion allowed for the release of N glycans and ECM peptides from the tissue. Multiplex immunohistochemistry with photocleavable, mass-tagged probes was also performed on each tissue. This analysis produced a spatial map of N glycans, ECM peptides and immune cells with their distribution and abundance color-coded as a heat map of each tissue. RESULTS/ANTICIPATED RESULTS: This analysis produced a unique N-glycan signature associated with specific tumor regions (necrosis, invasive margin, etc.) and immune cell clusters. Additionally, immune cells within the PDAC tumor microenvironment were found to be organized into immature tertiary lymphoid structures composed primarily of CD20+ B cells. Finally, a distinct distribution of ECM peptides within and surrounding tumor tissue was visualized, and putative identifications have been assigned to these stromal elements. DISCUSSION/SIGNIFICANCE OF IMPACT: In the future, insights from this hypothesis-generating study may be leveraged to identify diagnostic and prognostic biomarkers for PDAC to improve early diagnosis and treatment response rates. The N glycan signature, ECM composition, and immune activation state in liquid biopsies including serum and PBMCs will be compared to data from this study.

472

#### Deciphering the role of Dnmbp in kidney development: Implications for CAKUT

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