CR2-Crry (C3 inhibitor that blocks all activation products), anti-C5 mAb (blocks C5a and MAC), C5aRA (blocks C5a-C5a receptor interaction), or anti-C7 mAb (blocks MAC). Study endpoints were P7 or P14. RESULTS/ANTICIPATED RESULTS: Following GMH, CR2-Crry treatment decreased MAC deposition on RBC and additionally decreased heme oxygenase-1 expression, heme deposition, and iron-induced inflammation measured at P7. In support of a specific role for the MAC, anti-C7 mAb treatment resulted in similar outcomes and was similarly protective. Anti-C7 mAb treatment also reduced hydrocephalus development at a later time point (P14). A similar result was obtained using C7 deficient mice and with anti-C5 mAb treatment. On the other hand, no protective effect was seen with C5aR blockade, and double knock out of C3aR/C5aR also did not provide protection, indicating no role for the anaphylatoxins C3a and C5a and their receptors expressed on leukocytes and endothelial cells in exacerbating deteriorating outcomes. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data indicate a key role for the MAC in RBC induced hemolysis after GMH which serves as a driver of inflammation and early GMH pathogenesis. We further show that we can effectively increase precision by targeting solely the MAC complex acutely. Future work will be undertaken to determine temporal roles of individual complement activation products.

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Defining the impact of short-chain fatty acids on the gut–urinary axis in a naturally occurring canine model of calcium oxalate urolithiasis

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OBJECTIVES/GOALS: Short-chain fatty acids (SCFAs) exert protective effects against calcium oxalate (CaOx) urinary stone formation in experimental rodent models, yet these effects are not understood in natural stone formers. This study will define the impact of SCFAs on stone risk factors along the gut–kidney axis in a natural canine model of CaOx stone disease. METHODS/STUDY POPULATION: A randomized, placebo-controlled, clinical trial will be performed using a crossover study design. Twenty dogs that are natural CaOx stone formers will be fed a standardized diet and randomized to receive either a daily prebiotic fiber (inulin) that stimulates SCFA production or a placebo. We will perform fecal shotgun metagenomics and SCFA quantification before and after each intervention (four timepoints) to identify how inulin and SCFAs enrich or deplete pathways relevant to stone formation within the gut microbiome. RT-qPCR will be performed to determine the effects of SCFAs on intestinal oxalate transporter gene expression (SLC26A3 and SLC26A6). At each timepoint, urinary shotgun metagenomics and quantification of urine biochemical profiles used to predict stone risk will also be performed. RESULTS/ANTICIPATED RESULTS: We anticipate that prebiotic stimulation of SCFAs with inulin will reduce stone risk factors along the gut–urinary axis in a natural canine model of urinary stone disease. Specifically, we anticipate that prebiotic stimulation of SCFAs will 1) modify gut and urinary microbial communities to promote pathways considered protective against stone formation, 2) alter the expression of oxalate transporters (SLC26A3, SLC26A6) to reduce net oxalate absorption, and 3) reduce stone-promoting metabolites (e.g., oxalate) in the urine. DISCUSSION/SIGNIFICANCE OF IMPACT: By defining the impact of prebiotic fibers and SCFAs on the gut–urinary axis in a natural animal model of CaOx urolithiasis, we will lay the foundation for novel nutritional strategies to prevent CaOx stone disease in both humans and animals.

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Development of small molecules targeting an epigenetic modulator for pediatric neuroblastoma

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OBJECTIVES/GOALS: The bromodomain PHD finger transcription factor (BPTF) is an oncogenic driver of neuroblastoma. Our objective is to pioneer the discovery of the first class of chemical compounds that engage the PHD finger of BPTF and inhibit its biological function in cellulo, thereby establishing first-in-class chemical probes for this epigenetic reader. METHODS/STUDY POPULATION: Our previous work has identified a collection of small molecules that engage BPTF PHD in vitro. Following structure–activity relationships analysis, candidates will be used in a neuroblastoma cell model to validate BPTF PHD interaction in cellulo and predict therapeutic potential. Nanoluciferase bioluminescence resonance energy transfer (NanoBRET) will be used to confirm BPTF PHD engagement by compounds. Selective toxicity in neuroblastoma cells upon inhibitor treatment will be gauged by comparing cell growth and viability in the IMR-32 cell line against the HEK293 cell line. Treated HEK293 cells will be subjected to the assay for transposase-accessible chromatin (ATAC) and RNA sequencing methods to monitor changes in chromatin structure and transcriptional signatures against untreated cells. RESULTS/ANTICIPATED RESULTS: We hypothesize that compounds with low micromolar potency for BPTF PHD in vitro will engage the target in cellulo and displace NanoLuciferase tagged protein from its HaloTagged® peptide binding partner. Additionally, we anticipate that our inhibitors will show cytotoxicity for IMR-32 cells with limited effects on HEK293 cells. We envision that inhibitor treatment in HEK293 cells will correlate with reduced chromatin exposure, suggesting that blocking the BPTF–histone interaction via PHD finger inhibition hinders the remodeling of transcriptionally silent heterochromatin into a transcriptionally active state. Finally, we expect that inhibitor treatment will result in diminished gene expression of oncogenic transcription factors, including N-Myc, a biomarker of neuroblastoma. DISCUSSION/SIGNIFICANCE OF IMPACT: These first-in-class chemical probes for BPTF PHD will enable further investigation of BPTF and high-risk neuroblastoma progression, as well as its role in other diseases. In addition, these compounds will serve as a platform for the development of new anticancer agents that may improve outcomes for children that suffer from high-risk neuroblastoma.

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Fetal natural killer cells play an essential immunoprotective role in preventing the onset of symptomatic congenital cytomegalovirus

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OBJECTIVES/GOALS: Congenital cytomegalovirus (cCMV) continues to be the primary infectious cause of fetal anomalies. The role

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of fetal natural killer (NK) cells in response to cCMV remains largely unexplored. This study seeks to investigate how fetal NK cells respond to human cytomegalovirus (HCMV) during gestation. METHODS/STUDY POPULATION: Umbilical cord blood and corresponding umbilical cord tissues were collected from fetuses that had no complications during gestation. These samples, provided by the Medical College of Wisconsin Tissue Bank, were processed within 24 hours after live birth. Single-cell suspensions were prepared from the samples, and fetal NK cells were isolated and exposed to HCMV antigen peptides VMAPRTLFL, VMAPRTLIL, VMAPQSLLL, and the human self-peptide ALALVRMLI. These peptides were presented on HLA-E*01:03 BV421-conjugated tetramers produced by the National Institutes of Health Tetramer Core Facility. Additionally, fetal NK cells were also prepared for single-cell RNA sequencing (scRNA-seq), and cells were filtered and clustered based on the number of uniquely expressed genes. RESULTS/ANTICIPATED RESULTS: Through unbiased clustering, our scRNA-seq analysis identified five unique fetal NK cell subsets in umbilical cord blood and four in the corresponding umbilical cord tissue. Notably, fetal NK cells exposed to HCMV during gestation were primarily mature NK cell subsets, while those from unexposed fetuses were mostly immature subsets. Additionally, HCMV-exposed fetal NK cells exhibited a strong recall response to the HCMV antigen, with a notably higher frequency and elevated production of IFN-γ. Conversely, naïve fetal NK cells from fetuses unexposed to HCMV produced significantly lower levels of IFN-γ. Finally, we identified a distinct subset of fetal NK cells that emerge following exposure to the HCMV antigen. DISCUSSION/SIGNIFICANCE OF IMPACT: In this study, we show that HCMV infection can influence the formation of specific NK cell subsets and re-exposure to the HCMV antigen can trigger a recall response. These insights could pave the way for the development of innovative NK cell-based immunotherapies aimed at preventing fetuses from developing symptomatic cCMV.

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Parental involvement is related to parental resilience and offspring neural reward prediction error signaling

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OBJECTIVES/GOALS: Our goal was to investigate the associations between parental resilience and parenting behaviors, and their relationship to their offspring's reward neurocircuitry function; in particular, the reward prediction error (RPE) circuit, a transdiagnostic marker of psychopathology. METHODS/STUDY POPULATION: N = 26 parent–child dyads (children ages 10–14) were recruited. Parents reported on parenting behaviors using the Alabama Parenting Questionnaire (APQ), and resilience using the Connor-Davidson Resilience Scale (CD-RISC). Children performed the Novelty task, a reward learning task, during fMRI scanning. Trial-by-trial RPEs were calculated based on a reinforcement learning model. Brain regions of interest (ROIs) including the nucleus accumbens, anterior putamen, and ventromedial prefrontal cortex were created (regions implicated in RPE representation). RESULTS/ANTICIPATED RESULTS: The APQ parental involvement subscale was associated with increased negative affect tolerance (r = 0.40, p DISCUSSION/SIGNIFICANCE OF IMPACT: Findings suggest that parental factors may impact neurocircuitries underlying psychopathology in offspring, and consequently, risk for offspring psychopathology. Interventions designed to increase parental resiliency may reduce risk for psychopathology in offspring, perhaps by increasing parental involvement and neural RPE sensitivity.

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Identification of molecular and cellular events during recurrence of focal segmental glomerulosclerosis in human allografts

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OBJECTIVES/GOALS: The identification of the cascade of molecular and cellular events occurring during the progression of focal segmental glomerulosclerosis in human kidney biopsies from kidney transplant (KTx) recipients (KTR) with normal function or recurrent FSGS to determine potential targets of intervention and therapy. METHODS/STUDY POPULATION: In this study, we evaluate the molecular and cellular events associated with primary FSGS in both native and transplant kidneys. We collected biopsy samples from the native normal kidney (nNK, n = 3), normal functioning allografts (NKTx, n = 3), primary FSGS in the native kidney (nFSGS, n = 1), recurrent FSGS (KTxFSGS, n = 5). KTxFSGS comprises a collection of longitudinal samples with biopsy also collected at the subsequent recurrence. Blood samples were collected during biopsy collection. Biopsies were preserved in RNAlater at the time of collection. 10X genomics chromium single nuclei RNA sequencing (snRNAseq) was performed using isolated nuclei. Data was analyzed using Seurat on R. Conditionally immortalized podocytes were treated with a patient serum to determine the change in expression observed in snRNAseq data. RESULTS/ANTICIPATED RESULTS: Recurrence rates of primary FSGS are high in kidney allograft recipients up to 25–50% in first, and up to 80% in second transplants, often leading to graft loss. Our findings reveal that podocyte detachment is driven by metabolic and structural dysregulation rather than cell death, increasing VEGFA expression and disrupting glomerular endothelial cell growth and permeability. Parietal epithelial cells initially compensate by dedifferentiating toward podocytes but later increase collagen deposition, contributing to glomerular sclerosis. Increased interactions of glomerular cells with B cells exacerbate extracellular matrix deposition and scarring. We also observed tubular sclerosis and disruption of the regenerative potential of proximal tubular cells, with increased interaction with T cells. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings offer new insights into the pathogenesis of recurrent FSGS and suggest potential therapeutic targets and establishes a foundation for future studies to further evaluate the role of metabolic dysfunction as the cause of podocyte injury and loss.

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