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

Kathleen Crum, Joseph Aloi, Katherine LeFevre, Mario Dzemidzic and Leslie Hulvershorn
Indiana University School of Medicine

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

Haseeb Zubair[1], Thomas V Rousselle[2], Amol C Shetty[2], Azim Shafquat[3], Akalin Enver[4], Manish Talwar[4], Daniel G Maluf[4], Gallon Lorenzo[5] and Valeria R Mas[5]
[1]University of Maryland School of Medicine, Surgical Sciences Division, Department of Surgery, University of Maryland School of Medicine, Baltimore, MD; [2]Institute for Genome Sciences, School of Medicine, University of Maryland School; Baltimore, MD 21201, USA; [3]Surgical Sciences Division, Department of Surgery, University of Maryland School of Medicine, Baltimore, MD; [4]Surgical Sciences Division, Department of Surgery, University of Maryland School of Medicine, Baltimore, MD and [5]Surgical Sciences Division, Department of Surgery, University of Maryland School of Medicine, Baltimore, MD

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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