Child Health Research Institute

University of Nebraska Medical Center

Children's Hospital & Medical Center

Acute and chronic transcriptome changes during S. epidermidis CSF shunt infection

Matthew Beaver, Department of Pediatrics University of Nebraska Medical Center

Samar Chehimi, Department of Psychiatry University of Pennsylvania Perelman School of Medicine

Richard Crist III, Department of Psychiatry University of Pennsylvania Perelman School of Medicine

Benjamin Reiner, Department of Psychiatry University of Pennsylvania Perelman School of Medicine

Gwenn Skar, Department of Pediatrics University of Nebraska Medical Center & Children's Hospital & Medical Center

CSF shunt placement is the most common pediatric neurosurgical procedure in the United States with tens of thousands of shunts placed every year. However, while this is a lifesaving intervention, shunt placement is often complicated by infection. Tragically, these infections are associated with significant long-term neurologic morbidity including impaired cognition, impaired school performance, memory deficits, and seizures. The cellular mechanisms underlying these chronic neurological issues and the relevant neural cell types involved are currently poorly understood. Recent technology has provided ways to analyze transcriptomics at the level of single cells, allowing us to get a better understanding of the cell type-specific biological mechanisms involved in pediatric infections. The goal of the current study is to identify both the acute and chronic transcriptomic changes that occur in the brain following CNS infection using single nucleus RNA sequencing (snRNAseq). SF shunt placement is the most common pediatric neurosurgical procedure in the United States with tens of thousands of shunts placed each year. While these shunts are a life-saving intervention, they are complicated by infection in 5-30% of patients, who then require onerous treatment. Surgical removal and shunt replacement accompanied by weeks of intravenous antibiotics. Furthermore, shunt infections are associated with significant long-term neurologic sequelae. Studies have demonstrated that children with previous shunt infection have lower verbal and performance IQ, lower intelligence, and significantly impaired school performance compared to their peers without a previous shunt infection. Shunt infection also increases seizure risk, which has been reported to be as high as 47% after infection. The deleterious neurologic effects associated with infection are compounded by the fact that children who have had one shunt infection are at a significantly higher risk of repeated infections. The mechanisms responsible for the severe neurologic damage associated with shunt infection are unknown. Therefore, studies designed to elucidate the underlying mechanisms of central nervous system (CNS) damage resulting from shunt infection are critical for identifying therapeutic strategies to improve life-long neurologic outcomes of the thousands of children with shunt infection annually. Cell type-specific transcriptome alterations during S. epidermidis shunt infection will illuminate mechanisms responsible for the neurologic morbidity associated with CSF shunt infections. We utilized male C57/BL6 mice (n-4/group) infected with *S.* epidermidis, the most common cause of shunt infection. To establish CNS infection, silicone catheters were precoated with *S. epidermidis* and implanted into the lateral ventricle of the brain using a stereotactic apparatus. Control animals underwent sterile catheter placement. At days 3 and 56 post-infection animals were sacrificed using and overdose of isoflurane and brain tissue immediately surrounding the catheter (1mm3) was removed and snap

frozen for storage and later analysis. SnRNAseq libraries were generated using the 10x Genomics Chromium Controller and sequenced on a NovaSeq 6000. The R package *Seurat* was used for quality control and to cluster nuclei based on transcriptomic data. Identities of clusters were determined using expression of known cell-specific marker genes. Pseudobulk analysis was performed in the R package Libra, allowing identification of differentially expressed genes between cases and controls in each cell cluster. Ingenuity Pathway Analysis was used for analysis of canonical pathways and upstream regulators. Twenty-two transcriptomically distinct clusters of nuclei were identified. In these clusters, differentially expressed genes (DEGs) at day 3 post-infection were observed in both neuronal and glial populations. The top DEGs included genes with known connections to regulation of inflammation and infection (e.g. *Saa3*, *Acod1*, *Atp5g1*). These genes were not differentially expressed at day 56 post-infection. At day 56 post-infection group, the majority of DEGs were identified in neuronal clusters. Genes encoding components of the complement system (e.g. *C4b*, *C1qa*, *C1qc*) were upregulated in specific neuronal subtypes. Complement components, most notably C1q, are involved in synaptic pruning and C1q-deficient mice show reductions in cognitive decline during aging. Pathway analysis of DEGS observed at day 56 post-infection also revealed overrepresentation of genes involved in neuron development. These data indicate that pediatric S. epidermidis CSF shunt infection results in both short-term and long-term transcriptomic changes in the frontal cortex and that those changes differ by cellular population. The identification of DEGs and activated pathways with prior connections to cognitive function and neuronal development provides validity for our findings and suggests that future work must focus on the roles specific cell types play in the long-term consequences of pediatric CNS infection.