handling to injury, enabling safer, personalized therapy to slow kidney damage in patients.

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Sepsis-associated PARDS is associated with an exhausted neutrophil phenotype in the lower respiratory tract

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OBJECTIVES/GOALS: This study aims to uncover immuno-endotypes in sepsis-associated pediatric acute respiratory distress syndrome (SA-PARDS) using single-cell RNA sequencing (scRNA seq) to analyze the immune cell populations of the respiratory tract of intubated pediatric subjects with SA-PARDS. METHODS/STUDY POPULATION: The inclusion criteria are an age of less than 18 years, admission to the PICU, a diagnosis of SA-PARDS, and intubation. Both sepsis and PARDS will be defined using the most recent consensus definitions. Exclusion criteria include an order of limited resuscitation and clinician discretion. A tracheal aspirate and blood sample will be obtained on days 1, 3, and 7. Both samples will be processed for single-cell RNA sequencing via the HIVE platform according to the manufacturer's protocol. cDNA libraries will be generated and undergo 150 bp sequencing, quality control, and alignment. The Seurat package in R will be used for cell-type annotation and analysis of differential gene expression. Clinical variables, labs, and outcomes will be recorded in REDCap. RESULTS/ANTICIPATED RESULTS: We evaluated five samples from two subjects. After quality control, 31,330 cells remained for analysis. UMAP projections were created, and we annotated cell types. We found that most of the cells were immune cells with very few epithelial cells. There were two groups of neutrophils, which we termed classic and migratory. Classical neutrophils expressed classic neutrophil markers, and migratory neutrophils expressed the classical markers plus increased adhesion markers. We identified a third group of cells that were neutrophil-like, and these were isolated for further analysis. These cells exhibited characteristics of exhausted neutrophils, including downregulation of CXCR1 and CXCR2, and upregulation of PI3 and SLIPI. In each subject, the proportion of exhausted neutrophils increased over time. DISCUSSION/SIGNIFICANCE OF IMPACT: We found that in our subjects with SA-PARDS, there were a subset of neutrophils characterized by down-regulation of classical neutrophil markers and up-regulation of neutrophil stress markers, suggested an exhausted phenotype.

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Structure-function-migration in the aging lymph node

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OBJECTIVES/GOALS: The aging (65+) population is rapidly growing and faces unique health issues exacerbated by immune function decline. However, the driving mechanisms behind this decline are poorly understood. We will use a mouse model to study the relationship between stiffness and cell mobility within the lymph node (LN) as one potential driving mechanism. METHODS/STUDY POPULATION: We will collect inguinal LNs from young (6–8 weeks) and aged (18+ months) female mice and section them for *ex vivo* analysis, including quantification of proteins in the fibroblastic reticular network (collagen I, collagen III, collagen VI, laminin, and fibronectin) using immunofluorescent staining and Python scripts; analysis of biomechanical properties (elastic modulus, loss modulus, and pore size) using multiple particle tracking (MPT); and migration analysis of adoptively transferred B- and T-cells using live imaging and MATLAB scripts. We will use ANOVA to compare abundance and organization of proteins, biomechanical properties, and cell migration between young and aged LNs, as well as using Pearson's correlation coefficients to identify relationships between characteristics. RESULTS/ANTICIPATED RESULTS: We hypothesize a positive correlation between fibroblastic reticular network protein deposits, especially collagens, and stiffness (elastic modulus) within murine LNs due to known mechanisms underlying age-related fibrosis. We also hypothesize that areas of increased stiffness (as revealed by MPT) will exhibit decreased cell migration due to physical hindrance to B- and T-cell mobilization. Furthermore, we hypothesize that aged murine LNs will exhibit a significant increase in stiffness and resultant decreased cell mobility when compared to young murine LN, particularly in areas with increased collagen localization. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies will elucidate structure–function relationships driving age-associated LN fibrosis and stiffness, and the resultant effect on cell migration, thus clarifying some of the potential driving mechanisms behind immune aging and providing data capable of informing the development of relevant models, therapeutics, and interventions.

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Prenatal family and community exposures and associations with postnatal epigenetic signatures of child metabolic health

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OBJECTIVES/GOALS: The purpose of this study was to examine associations between prenatal family and community exposures and early childhood DNA methylation (DNAm) at genomic regions related to energy regulation or fat deposition. Results could contribute to the understanding of disparities in metabolic health risk among children from disadvantaged communities. METHODS/STUDY POPULATION: This is part of a cross-sectional study of mother–child dyads recruited from a previous RCT with pregnant women in California who had a pre-pregnancy BMI of 25–40. DNA was extracted from saliva of 49 child participants between 1 and 4 years old and is undergoing whole-genome bisulfite sequencing. Outcomes are percent DNAm, both genome-wide and at genomic regions related to energy regulation or fat deposition. Prenatal exposures were measured by the Geospatial Research, Analysis, and Services Program (GRASP) Place & Health burden percentile ranking, based on census tract of residence during pregnancy, with higher percentiles indicating greater burden. We will

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