The immune cell landscape in kidneys of patients with lupus nephritis

Researchers from the Broad Institute of MIT and Harvard, Harvard Medical School, and the University of Michigan performed single cell analysis of immune cells in human kidneys affected by lupus nephritis. They identified 21 subsets of leukocytes active in disease, and noted heightened expression of two chemokine receptors that may be involved in cell trafficking. Common gene expression between immune cells in urine and kidney samples also suggests urine might serve as a surrogate for kidney biopsies.[1]

Snapshot	10x Genomics product
Research area: Autoimmune Disease **Organism:** Homo sapiens **Sample type:** Kidney, urine, and blood samples from patients with lupus nephritis and healthy controls **Reasearch question:** What infiltrating leukocytes are active in human lupus nephritis kidneys? What are the immune mechanisms that drive lupus nephritis?	**Chromium Single Cell Gene Expression Solution** • Chromium Single Cell 3′ Library & Gel Bead Kit v2 • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines

Experiment Overview

Isolation and processing of kidney cells in preparation for single cell RNA sequencing

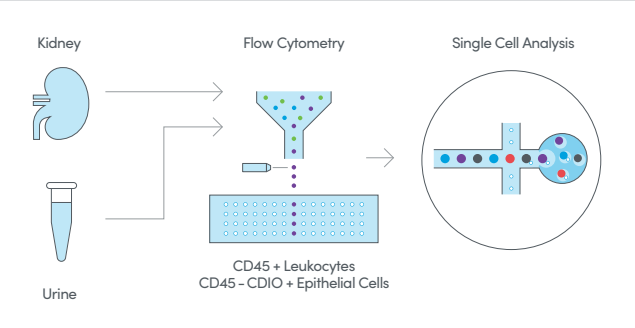

Figure 1. Workflow diagram. Overview of methods used to process and study the cellular contents and molecular states of single cells from kidney and urine samples.

- Kidney biopsies and urine samples from 24 patients with lupus nephritis and 10 control samples, acquired from living donors
- Cryopreservation and tissue processing, followed by flow cytometry to identify CD45-CD10+ epithelial cells and CD45+ leukocyte populations within dissociated kidney cells
- Viable cells sorted into 384-well plates for scRNA-seq analysis

Library construction and RNA sequencing for kidney and urine samples

- scRNA-seq performed using a modified CEL-Seq2 protocol
- Paired-end sequencing was performed on the Illumina HiSeq 2500
- Two additional healthy donor kidney samples were processed, and single cell libraries prepared using the Chromium Single Cell Gene Expression Solution from 10x Genomics
- Additional control samples used the Illumina HiSeq X for paired-end sequencing

Processing and sequencing blood samples

- Acquired blood from 10 patients with LN before kidney biopsy
- Cells were stained with antibodies against immune markers, identifying 8 of 10 samples that had enough CD8+ T cells to allow sequencing
- Sequencing libraries created using both Smart-seq2 methods and the Chromium Single Cell Gene Expression Solution
- All paired-end sequencing was performed on the Illumina NextSeq 500

Why single cell?

Lupus nephritis is a consequence of autoimmunity: immune cells infiltrate the kidney, causing inflammation and organ dysfunction. In order to fully characterize the heterogeneous cellular landscape of the diseased organ and develop a mechanistic hypothesis of disease, the authors examined single cell gene expression from traditional biopsy samples, including kidney tissue, blood, and urine. This allowed them to link specific cellular populations with genes and molecular pathways that potentially drive disease severity and progression.

Computational analysis

Researchers used Seurat (v.1.4.0.8) software to perform stepwise clustering of kidney cells, categorizing cells by canonical lineage markers and gene expression data.

Figure 2. Stepwise clustering of kidney cells. Low-resolution clustering reveals major cell types, while finer clustering distinguishes cells by subset and activation state.

They performed low-resolution clustering, analyzing all cells together, then labeled each of the resulting clusters as myeloid cells, T/NK cells, B cells, dividing cells, or epithelial cells. The cells of each general class were then analyzed separately to identify finer clusters. This analysis identified 21 immune cell clusters and a single epithelial cell cluster.

Seurat also aided identification of genes differentially expressed between patients with LN and living donor controls, and between individual cell clusters. This gene expression data was used for several additional modes of statistical analysis, including:

- Identifying cell-type-specific inflammatory signaling with interferon response scores
- Mapping cell lineage trajectories between clusters
- Correlating cell subset-specific gene expression dynamics with GWAS lupus datasets
- Gauging expression of specific immune receptors in each cell cluster

Results

Using single cell transcriptional profiling, researchers were able to reveal the complexity of immune populations in LN kidneys. They identified disease-specific subsets of myeloid, NK, and T and B cells. Additionally, they leveraged differential gene expression data to determine functional capabilities of cell clusters and define the immune mechanisms underlying disease.

For example, they noted heightened expression of chemokine receptors CXCR4 and CX3CR1 on immune cells from kidneys affected by lupus. Corresponding expression patterns of the CXCR4 ligand showed that it derived mainly from epithelial cells and M2-like macrophages, suggesting these cell types coordinate traffic of immune cells infiltrating the kidney with a targetable chemokine signaling pathway.

Finally, gene expression data revealed a high degree of correlation in urine immune cells and corresponding kidney leukocytes, suggesting that urine may present an alternative to kidney biopsies.

Reference

1. A Arazi, D Rao, C Berthier et al., The immune cell landscape in kidneys of patients with lupus nephritis. *Nature Immunology.* 20, 902-914 (2019).

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