Spatiotemporal immune zonation of the human kidney

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T he kidneys play a vital role in organism homeostasis but can be affected by a number of prevalent and life-limiting conditions for which recognition and response to pathogen and danger signals is critical (1). These responses are mediated by a network of immune cells (1), but our understanding of human kidney-resident immune cells and their cell-signaling circuitry is limited. Anatomically, each kidney is composed of the cortex, containing glomeruli where filtrate is generated, and the medulla, where urine is concentrated (Fig. 1A). The functional subunit of the kidney is the nephron, which is made up of a glomerulus; a proximal tubule (PT), where filtered electrolytes are reabsorbed; the loop of Henle (LOH), which generates the intrarenal sodium gradient required for urine concentration; and collecting ducts (CDs), which coalesce in the kidney pelvis. Urine subsequently flows into the ureter and on to the bladder (2) (Fig. 1A). The principal infectious challenge arises from bacteria ascending the ureter into the kidney pelvis, and we have previously shown that the hypersaline environment within the medulla may promote antimicrobial responses (3).

Here, we used droplet encapsulation high-throughput single-cell RNA sequencing (scRNA-seq) (10x Genomics platform) and flow and mass cytometry to define the global immune landscape of the human kidney. We studied single-cell suspensions from 14 mature human kidneys and six fetal kidneys (tables S1 and S2 and fig. S1). We captured 114,113 droplets from mature kidneys, yielding 40,268 cells after rigorous quality control, as described previously (4, 5) (fig. S2A). We loaded 739 highly variable genes into a principal component analysis and identified clusters that were manually curated into four major cellular compartments on the basis of canonical marker expression: endothelial, immune, fibroblast and myofibroblast, and epithelium (Fig. 1B and fig. S3, A to F).

Within the endothelial cell clusters, glomuerular endothelial (GE) cells, vasa recta (VR) cells, and peritubular capillaries (PCaps) were identified (Fig. 1B and fig. S3, E and F). Nephron epithelial cells were evident, including podocytes (Podos), PTs, LOHs, connecting nephron tubules (CNTs), intercalated cells (ICs), and principal cells (PCs) of the CD, as well as pelvic epithelium (PE) (fig. 1B). Immune cell populations included mononuclear phagocytes (MNPs), B cells, T cells, and natural killer (NK) cells (fig. 1B), and their presence was confirmed by mass cytometry in three additional adult kidney samples (fig. S4) that were flushed to minimize intravascular contamination.

To explore the spatial distribution of these cells across the kidney, we sought to assign a depth estimate of the sample from which the cells originated, termed “pseudodepth” (fig. S5, table S3, and materials and methods). We observed enrichment of GE, Podo, and PT cells in samples predicted to be cortical or corticomedullary in pseudodepth, whereas PE and transitional epithelium were limited to medulla or pelvic pseudodepth (Fig. 1C), as predicted by their known arrangement. This analysis revealed an asymmetric distribution of immune cells; B cells were almost exclusively located in cortical samples, whereas MNPs were enriched in deeper samples (Fig. 1, C and D, and figs. S4G and S5D).

We next analyzed single-cell transcriptomes from fetal kidneys obtained at 7 to 16 post-conception weeks (PCW). We analyzed 33,865 droplets, yields 27,203 annotated cells after rigorous quality control (figs. S1A and S2B), and identified immune, endothelial, developing nephron epithelial, and stromal cell clusters by canonical marker expression and informed by previous transcriptional analyses of fetal kidney (6, 7) (Fig. 1E, figs. S6 and S7, and table S10). Cells from various developmental stages of nephrogenesis (8) (Fig. 1F) were evident in our single-cell data (Fig. 1, E, G, and H), with cap mesenchyme dominating at 7 to 8 PCW (Fig. 1, G and H). From 9 PCW, Podos were more evident, and by 12 PCW, cells from across nephrogenesis were present, including PT, LOH, CD, and PE cells (fig. 1, E, G, and H).

Much of the knowledge about the developing immune system in the human fetus is inferred from murine studies. Fetal kidneys that gestational age captured in our study (7 to 16 PCW) would be expected to contain macrophages with potentially three developmental origins: yolk sac progenitors, aorta-gonad-mesonephros (AGM) hematopoietic stem cells (HSCs), and fetal liver HSCs (9, 10). The extent to which other myeloid and lymphoid cells populate the human fetal kidney is unclear. In our fetal kidney dataset, several immune cell clusters were evident (Fig. 1E). Macrophages and some dendritic cells (DCs) were present at the earliest developmental stages of nephrogenesis.
stage (Fig. 1H). Monocytes, T cells, and NK cells appeared from 9 PCW, whereas B cells were present at later developmental stages from 12 PCW (Fig. 1H). These data demonstrate that immune cell subsets exhibit different temporal patterns of localization to the human fetal kidney.

Terminally differentiated fetal nephron epithelial cells (fig. S7) showed transcriptional similarity to their mature counterparts (figs. S8 and S9), particularly in proximal nephron components, whereas CNT and PE showed less similarity (Fig. 2A). Immune gene ontology (GO) terms were enriched across the mature nephron, particularly in the PE, including “innate-immune” and “antimicrobial-response” genes (Fig. 2B). We verified this pattern of gene expression in bulk transcriptomic data, which similarly demonstrated the highest expression of immune genes in the pelvis (fig. S10C). By contrast, there was little or no expression of immune genes in fetal kidney epithelium (Fig. 2B).

We hypothesized that these spatially distinct immune gene expression patterns may be related to the dominant infection threat in the postnatal renal tract, which occurs by bacteria ascending the ureter from the bladder, most commonly uropathogenic Escherichia coli (UPEC). UPEC-associated molecules such as flagellin are sensed by extracellular toll-like receptors (TLRs) (II). In mature kidneys, we observed higher expression of the flagellin receptor TLR5, and its downstream signaling molecule MYD88, in the PE compared with the proximal nephron epithelium (fig. S10D). Epithelial cells may directly contribute to organ defense by secreting antimicrobial peptides (AMPs). AMP expression was highest in the mature PE (Fig. 2B), including serum amyloid A1 (SAA1), which inhibits biofilm formation in UPEC (II2), and Lipocalin 2 (LCN2), an iron chelator with bacteriostatic effects (I3), the deficiency of which results in susceptibility to recurrent urinary tract infections (UTIs) (I4).

To validate the differential expression and functional importance of these AMPs in kidney epithelium, we measured transcripts in bulk human kidney samples ex vivo and demonstrated high expression of LCN2 and SAA1 in medulla and pelvis samples that increased after the addition of UPEC (Fig. 2C). Similarly, in vivo in a murine model of pyelonephritis (I5), Lcn2 and Stat1 were more highly expressed in the medulla and pelvis compared with cortex, and their expression was up-regulated 24 hours after urethral challenge with UPEC (Fig. 2C). Thus, the distinct expression patterns of AMPs in human kidney likely facilitate protective epithelial responses in the region most vulnerable to ascending bacterial infection. This zonated epithelial innate immune capability is acquired postnatally and was not evident in fetal kidney.

Analysis of the immune compartment in mature kidney identified, and delineated the defining markers of, resident MNP, neutrophil, mast, pDC, B, CD4 T, CD8 T, NK, and NKT cell clusters (Fig. 3A, fig. S11, and supplementary data files 7 and 8). Lymphocyte subsets expressed molecules associated with tissue residency (I5) (fig. S12A) and a recently defined Hobit/Blimp1-containing murine-resident lymphocyte signature (I6) (fig. S12B), consistent with the conclusion that the mature human kidney houses bona fide tissue-resident lymphocytes. The B cell cluster included immunoglobulin M (IgM)-, IgG-, and IgA-expressing cells (fig. S12C). Cytokine and transcription factor expression did not indicate any specific polarization of CD4 T cells (fig. S12D). Within the NK cluster, there were cells with dual expression of γ- and δ-T cell receptor and markers typically...

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**Fig. 1.** Mapping the spatial and temporal architecture of the mature and developing human kidney. (A) Anatomy of the human kidney. (B) UMAP plot of 40,268 human mature kidney cells. Compartments are indicated by different colors (red, immune; blue, vasculature; green, nephron; mauve, stroma). Annotations are derived from compartment-specific analysis (figs. S8, S11, and S13). (C) UMAP plots illustrating the contribution made by cells from biopsies at inferred biopsy depths, with density contours colored according to compartments in (B). (D) Bar plots showing the proportion of immune cells at each inferred biopsy depth. (E) UMAP plot of 27,203 human fetal kidney cells. Compartments are indicated by different colors (red, immune; blue, vasculature; green, developing nephron; mauve, stroma). Annotations are derived from compartment-specific analysis (figs. S7 and S15). (F) Diagram illustrating steps in development of the nephron through early fetal life. The ureteric bud (UB) undergoes branching and instructs development of cap mesenchyme (CM) into renal vesicle (RV) and subsequently the S-shaped body (SSB). UB forms distal nephron structures, whereas SSB forms proximal structures. (G) Proportional contribution of fetal developing nephron cell types at distinct developmental time points. Cell types are colored as in fig. S7F. (H) UMAP plots illustrating the contribution made by cells from kidneys at discrete developmental time points, with density contours colored according to compartments in (E). PCW, postconception weeks; MNP, mononuclear phagocyte; MPhage, macrophage; Na, neutrophil; Mast, mast cell; pDC, plasmacytoid dendritic cell; B, B cell; NK, natural killer cell; NKT, natural killer T cell; CD4 T, CD4 T cell; CD8 T, CD8 T cell; MK, megakaryocyte; AVRE, ascending vasa recta endothelium; DVRE, descending vasa recta endothelium; PCE, peritubular capillary endothelium; GE, glomerular endothelium; PE, pelvic epithelium; TE, transitional epithelium of ureter; LOH, loop of Henle; CNT, connecting tubule; PC, principal cell; IC (A+B), type A and B intercalated cells; Podo, podocyte; PT, proximal tubule; dPT, distinct proximal tubule; EPC, epithelial progenitor cell; Fib, fibroblast; MFib, myofibroblast; Prl-CM, proliferating cap mesenchyme; RV, renal vesicle; UB, ureteric bud.

associated with mucosal-associated invariant T cells (fig. S12D).

Within the myeloid compartment, we identified four distinct clusters of MNPs (Fig. 3B and fig. S13, A to D). MNPs comprise monocytes, macrophages, and DCs, with several subsets described on the basis of surface markers, function, and ontogeny (17). Expression of CD11c, major histocompatibility complex (MHC) class II molecules, and CD14 identifies monocyte-derived macrophages with an avid phagocytic capacity, whereas CD11c+/MHCII+/CD14+ are classical myeloid DCs (cDCs) with the ability to migrate and present or cross-present antigen to T cells (17, 18). cDCs can be further subdivided into cDC1, which are CD141(+) and present antigen to CD4 T cells, respectively. In mature kidneys, “antigen processing and presentation” and “T cell costimulation” were the top GO terms associated with MNPs (fig. S13J), consistent with their identity as cDCs. “Defense response to bacterium” and “neutrophil degranulation” genes were expressed by monocyte-derived MNP subsets, MNPs and MNPb (fig. S13I), including antimicrobial genes such as S100A8 and S100A9, IL1B, and lysozyme (LYZ) (fig. S13J).

CD1c+/MHCII+CD14+ MNPs in mature human kidney are enriched in inner regions of the kidney and specialized for defense against UPEC (17). However, in the current scRNAseq experiment, two subsets of MNP were found to express CD14, MNPa and MNPd (Fig. 3C), with the single-cell transcriptomes indicating functional diversity and MNPa alone specialized in antibacterial activities (fig. S13I). Using marker genes identified by our scRNAseq dataset (table S6), we confirmed the presence of MNPa and MNPd in adult kidney by flow cytometry (fig. S14, A and B), and compared the phagocytic capacity of MNPa and MNPd (fig. S14B). MNPa demonstrated avid uptake of fluorescently labeled UPEC, in contrast to MNPd (Fig. 3D), in keeping with the GO term analysis.

In the fetal kidney, the lymphoid compartment contained CD4+ T cells, with few CD8 T cells detectable (in contrast to the mature kidney), as well as B, NK, and innate lymphoid cells (Fig. 3E). There were several subsets of myeloid cells, including monocytes, two subsets of macrophages (MPhage1 and MPhage2), cDC1 and cDC2, pDCs, and mast cells (Fig. 3E). We also observed proliferating counterparts of MPhage1, cDC2, monocytes, B cells, and NK cells (Fig. 3E and fig. S15). The MPhage1 population showed transcriptional similarity to murine kidney F4/80(high) yolk sac–derived macrophages (21) (fig. S16A). The MPhage2 subset had transcriptional overlap with MPhage1 but also expressed proinflammatory genes (Fig. 3C, E, and fig. S16B). MPhage1 dominated the resident-immune cell population in fetal kidneys at 7 to 10 PCW (Fig. 3F), but at later stages, MPhage2 increased in number along with other immune cells (Fig. 3E). The emergence of MPhage2 at later time points may reflect a different origin or increasing exposure of the same precursor cell to a proinflammatory stimulus. Fetal kidney DCs were predominantly cDC2 with few cDC1 (Fig. 3F), as observed in mature kidneys (Fig. 3B and fig. S13, E and F). The emergence of kidney CD4, CD8 T cells, and B cells at >10 PCW mirrors the development of fetal thymus and spleen.

To investigate the relationship between immune cell subsets in fetal and mature human kidney, we quantified their transcriptional similarity (figs. S16C and S17A). MNPd was the only mature kidney MNP cluster with similarity to fetal kidney MPhage1 (fig. S16C), and both clusters expressed MRC1, CLEC7A, CD163, and MAFA (Fig. 3C and fig. S16D). Trajectory analysis demonstrated a temporal progression of the MPhage1 transcriptome toward that of MNPd (fig. S16F). Mature kidney MNPs and MNPb were highly transcriptionally similar to fetal kidney monocytes (Fig. 3C and fig. S16C).

**Fig. 2. Gene expression patterns in the developing and mature nephron.** (A) Heat map of mean similarity scores between fetal and mature nephron cell types. (B) Upper panel: heat map of mean scaled scores for immune process gene sets (innate immune response, G0:0045087; defense response, G0:0006952; immune response, G0:0006955; antimicrobial humoral response, G0:0019730). Lower panel: heat map of mean expression values of antimicrobial peptides (AMPs) among pelvic epithelium marker genes. Point size shows the fraction of cells with nonzero expression. (C) Log10-transformed relative expression levels of LCN2 and SAA1 (human) and Lcn2 and Saa1/2 (murine) in kidneys after UPEC challenge measured by quantitative polymerase chain reaction (QPCR); values are relative to unstimulated cortical samples (n = 3 human samples, n = 6 murine samples). ***p < 0.0005, **p < 0.005, *p < 0.05, ANOVA; NS, not significant. Boxplots show median values and interquartile range. C, cortex; M/P, medulla/pelvis.
We next asked how the transcriptome of kidney immune cells changed over developmental time. Previous studies indicate that fetal monocytes may be less “proinflammatory” (22) and fetal DCs less effective at stimulating CD4 T cells than their mature counterparts (23). We found that fetal kidney monocytes were less enriched for proinflammatory M1 gene expression than mature kidney MNPs and MNPb (Fig. 3G). Both fetal macrophage subsets, as well as MNPd in the mature kidney, were skewed toward an anti-inflammatory M2 transcriptome (Fig. 3G). Monocyte-derived macrophages in the mature kidney showed increased expression of “phagocytosis” and “defense response to bacterium” genes compared with fetal monocytes (fig. S16G). Similarly, “antigen processing and presentation” genes and HLA-DRA were more highly expressed in mature kidney DCs compared with those in the fetus (Fig. 3C and fig. S16, G and F). In the lymphoid compartment, fetal kidney B cells showed no evidence of class switching (in contrast to their mature counterparts) (fig. S17B), whereas fetal CD8 T cells expressed little GZMH, a cytotoxic effector molecule (fig. S17B). Functionally, fetal kidney CD8 T cells and NK cells showed reduced enrichment for “T cell receptor signaling” and “NK cell–mediated immunity” relative to mature kidney CD8 and NK cells, respectively (fig. S17C).

Epithelial and endothelial cells can communicate with immune cells by means of chemokines that orchestrate immune cell position and cytokines that promote immune cell function. To investigate epithelial-immune cross-talk in the kidney, we assessed chemokine ligand-receptor interactions (Fig. 4A and fig. S18A) (24). CX3CL1 was expressed in CNT and PE, and its receptor CX3CR1, on monocyte-derived macrophages (MNPa-b) and DCs. Analysis of bulk RNA-sequencing data demonstrated two peaks of CX3CL1 expression across kidney pseudodepth, in keeping with the single-cell analysis (Fig. 4A and fig. S18B). Using a CX3CL1 reporter mouse, we confirmed high expression of CX3CL1 protein in the kidney pelvis, with the potential to position CX3CR1-expressing MNPs (fig. S18C). Analysis of CX3CR1 expression across kidney pseudodepth similarly showed some transcripts in the cortex, but greatest expression in the deeper regions of the kidney (fig. S18B). Given the marked bacterial phagocytic capacity of MNPs (Fig. 3D), this pattern of chemokine expression would place them in a pelvic position to combat ascending UPEC. Notably, MNPd showed little expression of CX3CR1, and in human kidney sections, there were few CD206/163+ cells in the medulla and pelvis compared with the cortex (fig. S18D). The ligand-receptor analysis also highlighted interactions between PE and neutrophils by expression of CXCL1-3, CXCL5-6, and CXCL8 and their receptors on neutrophils (Fig. 4A). We validated this, demonstrating that CK17 (KRT17+) cells (a marker of PE) in human kidney pelvis express CXCL8 and LCN2 (Fig. 4B and fig. S19A). This finding is clinically important because genetic variants of CXCL8 and CXCR1 are associated with susceptibility to pyelonephritis in humans (25). To determine whether this epithelial-immune cross-talk may promote neutrophil recruitment to the pelvis during UTI, samples of human kidney were incubated with UPEC. At baseline, CXCL8 levels were significantly higher in the medulla and pelvis samples compared with that in the cortex (Fig. 4C), with a marked increase observed after UPEC challenge (Fig. 4C). Similarly, in vivo, in a mouse model of pyelonephritis, Cxcl1 and Cxcl2 (murine orthologs of human CXCL8) were higher in the medulla and pelvis samples and in PE at baseline and further increased during infection (Fig. 4C and fig. S19B). The functional importance of this response to promote neutrophil chemotaxis during infection was evident by the accumulation of LysMhigh/CD11b+ neutrophils in the PE (Fig. 4D and fig. S19C). Overall, expression of neutrophil-recruiting chemokines was highest in PE, with some expression of CXCL2/CXCL3 in principal cells, consistent with previous murine studies (26).
In the fetal kidney, there was little expression of any neutrophil-recruiting chemokine in the distal nephron and PE (Fig. 4E).

Here, we investigated immune capability in the human kidney and determined how it changes over developmental time and anatomical space. We found that antimicrobial immunity is spatially zonated but this feature was not evident prenatally. Fetal kidney epithelium showed little immune gene expression, consistent with the view that it occupies a relatively sterile environment, where anatomically polarized antimicrobial defense is redundant. We show that a variety of immune cell populations are established in the human kidney in the first trimester with distinct temporal patterns, but they differ from mature kidney immune cells, with postnatal acquisition of transcriptional programs that promote proinflammatory and infection defense capabilities. The mature kidney MNP compartment was dominated by two monocyte-derived macrophage populations specialized for antibacterial function, but also contained a smaller, M2-enriched macrophage population that was transcriptionally similar to fetal kidney macrophages, potentially indicating prenatal seeding, consistent with mouse studies (21).

Our study provides a comprehensive description of immune topology in the human kidney, providing a resource that adds to the recently published murine kidney dataset (27), which will facilitate the future study of pathogenic mechanisms and the identification of therapeutic targets in immune and infectious kidney diseases.

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REFERENCES AND NOTES

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**Data and materials availability:** Sequencing data and metadata are available through the Human Cell Atlas Data Portal (https://data.humancellatlas.org/explore/projects/abe1a013-af7a-45ed-8c26-f3793c24a1f4). Data are available to download and in an interactive browser format at www.kidneycellatlas.org. Code is uploaded to https://github.com/bjstewart1/kidney_sc_immune and archived at Zenodo (28).

**SUPPLEMENTARY MATERIALS**

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Materials and Methods
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Spatiotemporal immune zonation of the human kidney

Immune landscape of the human kidney
Single-cell RNA sequencing has begun to shed light on the full cellular diversity of specific organs. However, these studies rarely examine organ-specific immune cells. Stewart et al. sequenced healthy adult and fetal kidney samples at a single-cell level to define the heterogeneity in epithelial, myeloid, and lymphoid cells. From this dataset, they identified zonation of cells, with relevance to disease and the varied perturbations that occur in different tumor settings. This profiling of the human kidney generates a comprehensive census of existing cell populations that will help inform the diagnosis and treatment of kidney-related diseases.

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