Analysis of TNF-mediated recruitment and activation of glomerular dendritic cells in mouse kidneys by compartment-specific flow cytometry

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Renal dendritic cells (DCs) form an interstitial network contributing to inflammatory and adaptive immune responses in the kidney. The presence and functional role of DC-like glomerular CD11c$^+$ mononuclear phagocytes is a matter of debate. Using compartment-specific flow cytometry we found that healthy mouse kidneys contained 1.3 CD11c$^+$ cells per 100 glomeruli and these increased by 4.6-fold and 13-fold after TNF stimulation and immune complex deposition, respectively. Compartment-specific mRNA expression revealed a predominantly glomerular expression of TNF receptors, chemokines, and adhesion molecules; all upregulated after TNF exposure. Intraperitoneal TNF injection induced influx of neutrophils and mononuclear phagocytes including DC-like CD11c$^+$ cells into both the glomerular and tubulointerstitial compartments, but reduced in TNF receptor (Tnfr) 1-deficient mice. Additionally, Tnfr2 deficiency impaired glomerular infiltration of CD11c$^+$ cells, but not neutrophils. Interstitial CD11c$^+$ cells infiltrated in the presence of Tnfr1 or Tnfr2. TNF exposure also induced similar maturation of glomerular and interstitial CD11c$^+$ cells as demonstrated by increased surface expression of MHC II, CD54, and costimulatory molecules CD40, CD80, and CD86. Thus, by compartment-specific flow cytometry we could demonstrate the constitutive presence of DC-like CD11c$^+$ mononuclear phagocytes in normal mouse glomeruli and their TNF-induced accumulation and activation.

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Dendritic cells (DCs) and macrophages are constitutively present in all tissues. In the kidney these cells form a mononuclear phagocytic system that coordinates innate and adaptive immune responses.[1] Early studies identified cells with DC-like characteristics in rodent kidneys by immunohistochemistry,[2] electron microscopy,[3,4] and flow cytometry.[5,6] Subsequently, it was demonstrated that these cells express the murine DC marker CD11c and possess functional properties of DCs, despite their expression of macrophage-associated molecules such as F4/80.[7,8] Indeed, mononuclear leukocytes present in healthy and diseased kidneys show a substantial phenotypic overlap in their expression of conventional DC and macrophage markers, and their functions.[7,9] These renal mononuclear phagocytes form a cellular system characterized by high plasticity and the capability of phenotypic reprogramming.[1]

CD11c$^+$ renal mononuclear phagocytes have been most frequently, but not exclusively, associated with typical DC-like markers and functions, including the expression of major histocompatibility complex (MHC) class II and costimulatory molecules, and the capacity to locally capture antigens and migrate to local renal lymph nodes, inducing T-cell responses.[10–12] Recent work in mice identified a network of DC-like resident mononuclear phagocytes within the renal interstitium,[8] where they may continuously probe the environment to alert the immune system against infectious or inflammatory injury.[9] Moreover, resident interstitial DC-like CD11c$^+$ cells are an important early source of proinflammatory mediators like tumor necrosis factor (TNF) after acute ischemia/reperfusion damage or obstructive nephropathy.[13,14]

The contribution of renal DCs to glomerular disease is less clear, although they have been shown to modulate disease in models of primary glomerular injury.[15,16] The presence of DC-like CD11c$^+$ mononuclear phagocytes in healthy or diseased glomeruli and their potential role in mediating glomerular injury locally is a matter of debate.[7,9] Several studies reported an occasional presence of DCs in inflamed mouse glomeruli [7,12,17] and in human glomeruli with lupus nephritis,[18] whereas other human renal biopsy studies could

not conclusively demonstrate the presence of glomerular DCs in a variety of glomerular diseases.[19,20]

Given the recently emerged role of renal CD11c$^+$ DCs in interstitial disease, glomerular CD11c$^+$ mononuclear phagocytes may contribute to glomerular inflammation, and their therapeutic modulation may be beneficial in glomerulonephritis (GN). For example, TNF, a potential therapeutic target, has been identified as a crucial factor in the recruitment and activation of DCs, thereby enhancing T-cell activation *in situ*.[21] In murine models of GN, TNF and TNF receptor 2 (Tnfr2) were described as proinflammatory mediators of disease.[22,23] Here, we demonstrate the constitutive presence of DC-like CD11c$^+$ mononuclear phagocytes in normal mouse glomeruli and their TNF-induced accumulation and activation by compartment-specific flow cytometry.

RESULTS

Separation of glomerular and tubulointerstitial tissue from mouse kidneys

We used a magnetic bead–based procedure to isolate mouse glomeruli after perfusion with paramagnetic Dynabeads that accumulate in glomerular capillaries.[24] Microscopically isolated glomeruli were lacking the Bowman's capsule (Figure 1a), as described.[24] The first supernatant obtained during the washing procedure was free of glomeruli, but contained tubular fragments, single tubular cells, and a variety of polymorphic interstitial cells. The second supernatant contained tubular fragments only (Figure 1b and c). This technique allowed a highly efficient separation of renal tissue into glomeruli and tubulointerstitial fractions.

To proof the glomerular and tubulointerstitial origin of separated tissue fractions, we examined mRNA expression of glomerular and tubular marker genes. The glomerular marker gene nephrin was prominently expressed in isolated glomeruli, but not in the tubulointerstitial or tubular fraction (Figure 1d). In contrast, expression of Fxyd2, the γ-subunit of the tubular Na,K-ATPase, was found in the tubulointerstitial and tubular tissue, but not in the glomerular preparation (Figure 1e).

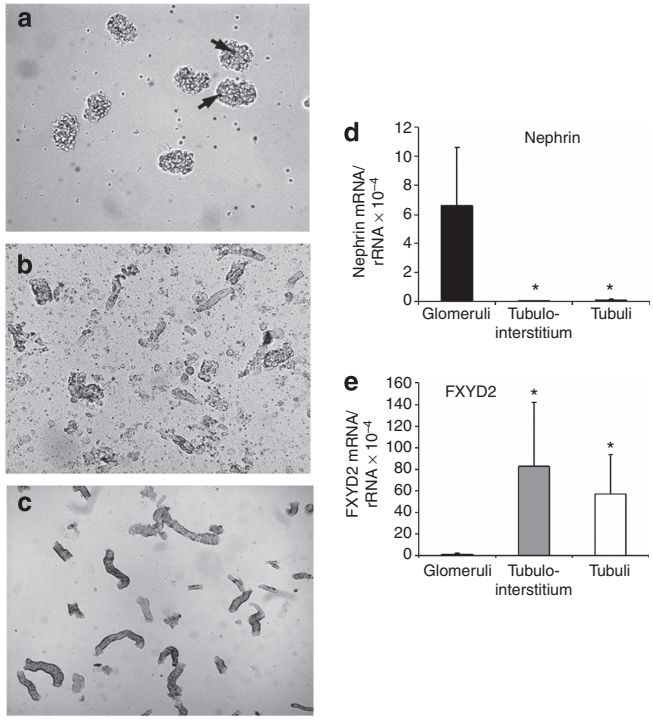

Figure 1 | Separation of glomerular, tubulointerstitial, and tubular tissue fractions from mouse kidneys. (**a**) Microscopic appearance of mouse glomeruli isolated after magnetic bead perfusion. Arrowheads indicate magnetic Dynabeads within glomerular capillaries. (**b**) Microscopic appearance of the tubulointerstitial tissue fraction obtained during the glomerular isolation procedure as first wash. This fraction contained tubular fragments, tubular cells, and polymorphic interstitial cells, but no glomeruli. (**c**) Microscopic examination of the tubular fraction obtained from the second wash. This fraction contained tubular fragments only, but no glomeruli or interstitial cells. (**a**–**c**) Original magnification ×100. Expression of glomerular (nephrin) and tubulointerstitial (FXYD2) marker genes was analyzed by quantitative real-time PCR. (**d**) Nephrin expression was detectable in the glomeruli but not tubulointerstitial and tubular fractions. (**e**) In contrast, mRNA expression of FXYD2, the γ-subunit of the tubular Na,K-ATPase, was barely present in the glomeruli, but was abundant in the tubulointerstitial and tubular tissue. Values were normalized to ribosomal RNA (rRNA) expression used as reference gene. Data represent mean ± s.d. of four mice. *$P < 0.05$.

Quantification of glomerular and interstitial leukocyte populations in mice with unilateral ureteral obstruction (UUO) and nephrotoxic serum nephritis (NTN) by flow cytometry

Separated tissue fractions of individual mice were used to simultaneously quantify glomerular and tubulointerstitial leukocyte numbers by compartment-specific flow cytometry. Supplementary Figure S1 online illustrates the gating strategy for the different leukocyte subpopulations.

To validate the compartment specificity of the flow cytometric technique, we isolated glomerular and tubulointerstitial tissue from obstructed kidneys of wild-type mice 5 days after UUO. Obstructed kidneys are characterized by interstitial nephritis, but lack glomerular pathology. UUO resulted in increased interstitial but not glomerular infiltration of CD45$^+$ leukocytes in obstructed kidneys at day 5 when compared with unobstructed contralateral kidneys of the same mice or untreated control kidneys (Supplementary Figure S2a online). Numbers of all tubulointerstitial leukocyte subsets significantly increased in UUO kidneys compared with contralateral kidneys (Supplementary Figure S2b–h online). In comparison with naive wild-type mice, contralateral unobstructed kidneys also showed a moderate increase in interstitial leukocytes, possibly because of a systemic inflammatory response in mice subjected to UUO (Supplementary Figure S2a–h online). Glomerular leukocyte infiltrates were not different between obstructed and contralateral unobstructed kidneys.