# Fractalkine–CX3CR1-dependent recruitment and retention of human CD1c$^+$ myeloid dendritic cells by *in vitro*–activated proximal tubular epithelial cells

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**Chemokines play pivotal roles in tissue recruitment and retention of leukocytes, with CX3CR1 recently identified as a chemokine receptor that selectively targets mouse kidney dendritic cells (DCs). We have previously demonstrated increased tubulointerstitial recruitment of human transforming growth factor-β (TGF-β)-producing DCs in renal fibrosis and chronic kidney disease (CKD). However, little is known about the mechanism of human DC recruitment and retention within the renal interstitium. We identified CD1c$^+$ DCs as the predominant source of profibrotic TGF-β and highest expressors of the fractalkine receptor CX3CR1 within the renal DC compartment. Immunohistochemical analysis of diseased human kidney biopsies showed colocalization of CD1c$^+$ DCs with fractalkine-positive proximal tubular epithelial cells (PTECs). Human primary PTEC activation with interferon-γ and tumor necrosis factor-α induced both secreted and surface fractalkine expression. In line with this, we found fractalkine-dependent chemotaxis of CD1c$^+$ DCs to supernatant from activated PTECs. Finally, in comparison with unactivated PTECs, we showed significantly increased adhesion of CD1c$^+$ DCs to activated PTECs via a fractalkine-dependent mechanism. Thus, TGF-β-producing CD1c$^+$ DCs are recruited and retained in the renal tubulointerstitium by PTEC-derived fractalkine. These cells are then positioned to play a role in the development of fibrosis and progression of chronic kidney disease.**

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Dendritic cells (DCs) have a vital role in the induction and regulation of immune responses. Human DCs are defined as CD45$^+$ leukocytes that lack other leukocyte lineage (lin) markers and express high levels of major histocompatibility complex class II (human leukocyte antigen (HLA)-DR) (lin$^-$ HLA-DR$^+$).[1] Within this DC network are a heterogeneous population of cells, comprising multiple subsets with specialized functions.[2–5] Human DCs mobilized to peripheral tissues during disease can be categorized into the following: (1) Inflammatory or monocyte-derived DCs (MoDCs) that differentiate from peripheral blood monocytes in response to inflammation;[6] and (2) blood and lymphoid organ-resident DCs comprising CD11c$^-$ CD123$^{hi}$ plasmacytoid DCs (pDCs) and CD11c$^+$ myeloid DCs (mDCs).[7] The mDCs are further divided into CD141 (BDCA-3)$^{hi}$ and CD1c (BDCA-1)$^+$ subsets.[1,2]

We have previously demonstrated that human kidneys with tubulointerstitial fibrosis, the pathological hallmark of chronic kidney disease (CKD), have significantly elevated numbers of tubulointerstitial CD141$^{hi}$ DCs and CD1c$^+$ DCs compared with nonfibrotic renal tissue, with mDCs identified as key sources of the fibrogenic growth factor transforming growth factor-β (TGF-β).[8] However, it remains to be determined how human mDCs are recruited and retained within the renal interstitium.

Leukocyte infiltration of inflammatory sites is directed by the local expression of chemokines.[9] Among these molecules, fractalkine (chemokine (CX3C motif) ligand 1 (CX3CL1)) represents a unique class of chemokine that mediates two distinct biological actions. Soluble fractalkine is a potent chemoattractant, whereas membrane-bound fractalkine functions as an adhesion molecule to retain circulating leukocytes.[10] Both fractalkine-dependent chemotaxis and adhesion are mediated through a specific receptor, CX3CR1, that is not shared by any other chemokine.[11]

The functional impact of the fractalkine–CX3CR1 system in the progression of tubulointerstitial fibrosis has been studied in murine models of ischemia–reperfusion injury,[12]

glomerulonephritis (GN),[13] and lupus nephritis.[14] More recently, Hochheiser *et al.*[15] identified CX3CR1 as a chemokine receptor that targets mouse kidney DCs with relative specificity, promoting an influx of cortical DCs and progression of disease severity in a model of CKD. However, the role of fractalkine–CX3CR1 in the recruitment and retention of human kidney DC is unknown.

Fractalkine is induced in human renal inflammation associated with acute allograft rejection, vasculitic GN, and crescentic GN, with minimal to no expression in normal kidney tissue or noninflammatory disease such as minimal change nephropathy.[16–18] Notably, tubulointersitial fractalkine expression is significantly upregulated in biopsies from fibrotic human kidneys compared with nonfibrotic nephropathies.[19] Expression of fractalkine at tubulointerstitial sites is localized to endothelial cells of the peritubular capillary network and tubular epithelial cells, with cellular infiltrates detected adjacent to fractalkine-expressing tubular epithelial cells.[16–18,20] However, the proximal or distal origin of these fractalkine-expressing cells has not been defined.

The tubulointersitial expression of fractalkine receptor CX3CR1 is also significantly elevated in fibrotic human kidneys compared with nonfibrotic biopsies.[21] Immunohistochemical (IHC) and immunofluorescence-based studies of human diseased kidney tissue (native and renal allograft biopsies) have reported CX3CR1 expression on myeloid and lymphoid cells.[21–23] However, unlike the flow cytometric staining strategy developed in our laboratory,[8] these methods are not amenable to the multiparameter labeling required to unequivocally identify specific leukocyte subpopulations, in particular human DC subsets.

Proximal tubular epithelial cells (PTECs), in the perturbed disease state, have an established role in initiating the inflammatory influx of mononuclear cells via the secretion of chemokines. The *in vitro* expression of fractalkine by human primary PTECs can be induced by pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α).[17] Soluble fractalkine secreted from cytokine-stimulated PTECs has been shown to have a role, although minor, in the *in vitro* recruitment of peripheral blood lymphocytes.[24] Surface-expressed fractalkine on PTECs can also support the adhesion of a CX3CR1$^+$ monocytic cell line.[17] However, the role of PTEC-derived fractalkine in the recruitment and retention of human DC subsets in the interstitium during renal inflammatory responses still remains unknown.

Here, we use our novel flow cytometric–based approach to demonstrate human renal DC expression of CX3CR1 by TGF-β-producing CD1c$^+$ DCs within biopsies with tubulointerstitial fibrosis. In addition, our data indicate that these CX3CR1-expressing CD1c$^+$ DCs are recruited and retained in the renal tubulointerstitium via PTEC-derived fractalkine.

## RESULTS

### Human CD1c$^+$ DCs are the predominant source of profibrotic TGF-β within the renal DC compartment

We have previously developed a ten-color flow cytometric gating strategy to identify and phenotype infiltrating leukocyte populations in healthy and diseased kidney tissue.[8] In this study, we have expanded this methodology to 14 colors. Using a new gating strategy on diseased biopsies with interstitial fibrosis (Figure 1), we were able to separate CD45$^+$ leukocytes into granulocytes with higher side scatter and

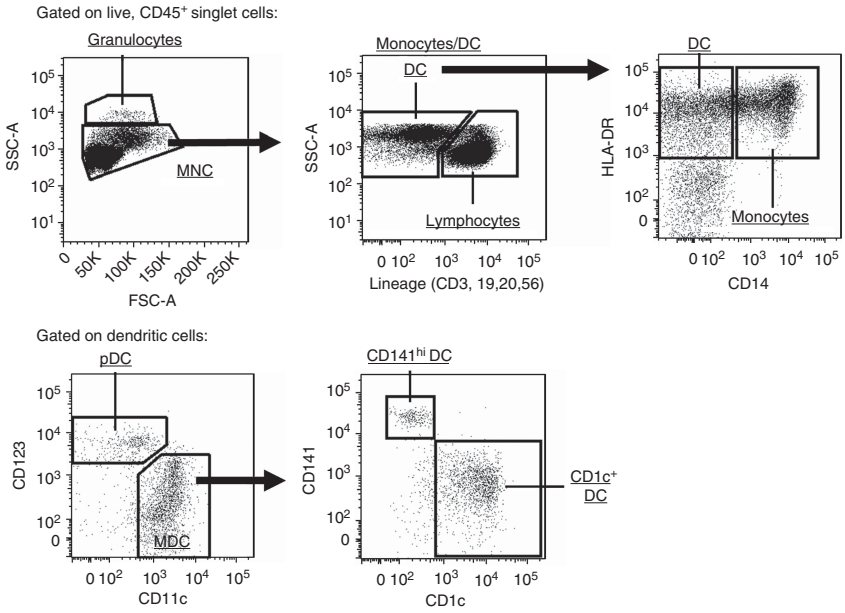


**Figure 1 | Identification of DC subsets in human fibrotic kidney tissue.** Gating strategy used to identify human dendritic cell (DC) subpopulations (CD11c$^-$CD123$^{hi}$ plasmacytoid DC (pDC), CD11c$^+$ CD141$^{hi}$, and CD11c$^+$ CD1c$^+$ myeloid DC (mDC)) within the lineage$^-$ CD14$^-$ HLA-DR$^+$ fraction in fibrotic kidney tissue. Representative flow cytometric data from 1 of 12 individual fibrotic renal biopsies are shown.