Proximal tubule-derived colony stimulating factor-1 mediates polarization of renal macrophages and dendritic cells, and recovery in acute kidney injury

Yinqiu Wang[1], Jian Chang[1], Bing Yao[1], Aolei Niu[1], Emily Kelly[1], Matthew C. Breeggemann[1], Sherry L. Abboud Werner[2], Raymond C. Harris[1,3,4] and Ming-Zhi Zhang[1,5]

[1]Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA; [2]University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA; [3]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee, USA; [4]Nashville Veterans Affairs Hospital, Nashville, Tennessee, USA and [5]Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

Infiltrating cells play an important role in both the development of and recovery from acute kidney injury (AKI). Macrophages and renal dendritic cells are of particular interest because they can exhibit distinctly different functional phenotypes, broadly characterized as proinflammatory (M1) or tissue reparative (M2). Resident renal macrophages and dendritic cells participate in recovery from AKI in response to either ischemia/reperfusion or a model of selective proximal tubule injury induced by diphtheria-toxin-induced apoptosis in transgenic mice expressing the human diphtheria toxin receptor on proximal tubule cells. Colony-stimulating factor-1 (CSF-1) is an important factor mediating the recovery from AKI, and CSF-1 can stimulate macrophage and dendritic cell proliferation and polarization during the recovery phase of AKI. The kidney, and specifically the proximal tubule, is a major source of intrarenal CSF-1 production in response to AKI. We induced selective deletion of proximal tubule CSF-1 to determine its role in expansion and proliferation of renal macrophages and dendritic cells and in recovery from AKI. In both models of AKI, there was decreased M2 polarization, delayed functional and structural recovery, and increased tubulointerstitial fibrosis. Thus, intrarenal CSF-1 is an important mediator of macrophage/dendritic cell polarization and recovery from AKI.

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Correspondence: *Raymond C. Harris, Division of Nephrology, C3121 MCN, Vanderbilt University School of Medicine and Nashville Veterans Affairs Hospital, Nashville, Tennessee 37232, USA. E-mail: ray.harris@vanderbilt.edu or Ming-Zhi Zhang, Division of Nephrology S-3223, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA.*
E-mail: ming-zhi.zhang@vanderbilt.edu

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Acute kidney injury (AKI) is defined as an abrupt decrease in renal function. The reported incidence of AKI varies from 5% in all hospitalized patients to 30–50% in intensive care units.[1] Renal tubule epithelia, and especially cells of the proximal tubule, are the primary targets for injury. Depending upon the nature and extent of the injurious stimuli, tubular cells lose functional integrity transiently or die by necrosis or apoptosis. It is currently thought that epithelial cell repair in response to AKI is accomplished primarily by the de-differentiation, migration, and proliferation of surviving tubular cells, with ultimate restoration of tissue integrity.[2,3] However, it remains uncertain for the major factor to stimulate the epithelial repair. Also unclear is the cell type(s) generating the stimulating factor in response to AKI.

There is increasing evidence that infiltrating cells play an important role in the initiation and propagation of the tubule dysfunction and structural injury.[4–12] The role of macrophages is of particular interest because they can exhibit distinctly different functional phenotypes, broadly characterized as proinflammatory (M1 or 'classically activated') and tissue reparative (M2 or 'alternatively activated') phenotypes.[13,14]

Recent studies have indicated that colony-stimulating factor-1 (CSF-1) is an important factor mediating the recovery from AKI.[15,16] CSF-1 can stimulate macrophage and dendritic cell proliferation and polarization,[16–21] and recent studies have indicated an important role for these cells in recovery from AKI.[16,20,22]

Previous studies[15,16,23] have also indicated that the kidney, and specifically the proximal tubule, is a major source of intrarenal CSF-1 production in response to AKI. However, CSF-1 has also been shown to stimulate renal epithelial cell proliferation directly after ischemia/reperfusion (I/R) injury to the kidney.[15] Therefore, to determine the role of proximal tubule CSF-1 expression in macrophage/dendritic-cell-mediated proliferation and differentiation following acute tubule injury, we utilized a previously described model of selective proximal tubule injury (proximal tubule DTR

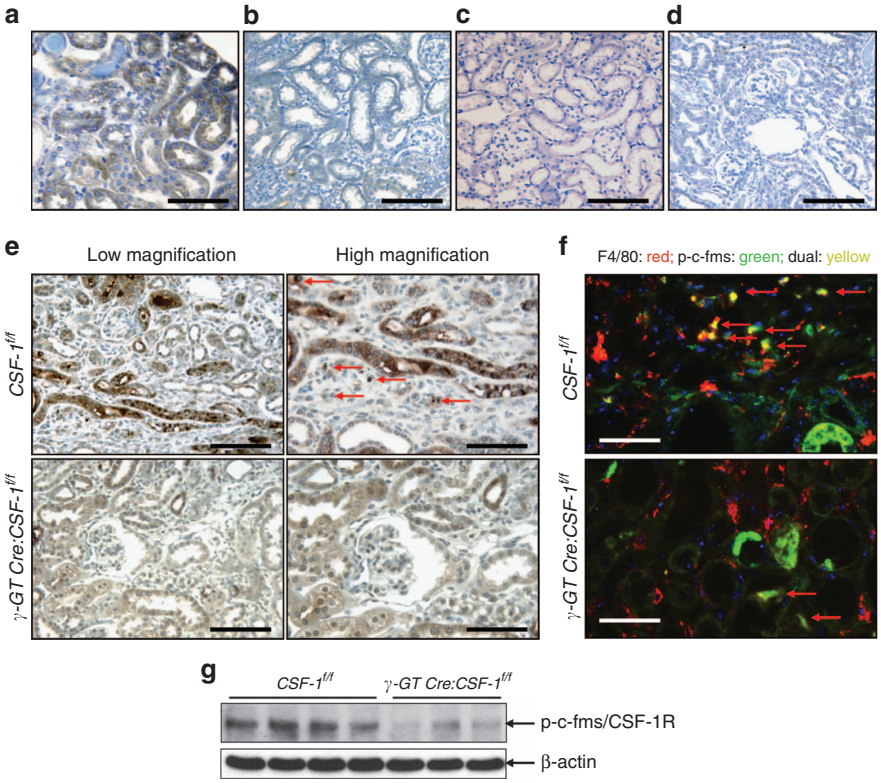

Figure 1 | The renal CSF-1-CSF-1R pathway was blunted in *γ – GT Cre:CSF-1$^{f/f}$* mice. (**a**–**d**) CSF-1 expression in the renal proximal tubule was decreased in *γ-GT Cre:CSF-1$^{f/f}$* mice (CSF-1 knockout in the renal proximal tubule). Immunohistochemical staining indicated strong CSF-1 immunostaining in tubule epithelia in the kidney of *CSF-1$^{f/f}$* mice (wild-type control) at 6 days after DT injection (**a**), which was eliminated by preincubation of primary antibody with CSF-1 peptide (**b**) or omission of primary antibody (**c**). (**d**) The effectiveness of CSF-1 deletion in the proximal tubule was confirmed by very weak renal CSF-1 immunostaining in *γ-GT Cre:CSF-1$^{f/f}$* mice 6 days after DT injection. (**e**) Kidney p-c-fms/CSF-1R immunostaining in both epithelial cells and interstitial cells (arrows) was much lower in *γ-GT Cre:CSF-1$^{f/f}$* mouse than in *CSF-1$^{f/f}$* mice at 6 days after DT injection. (**f**) Double immunofluorescent staining indicated that activation of c-fms/CSF-1R in macrophages (F4/80^{+} and p-c-fms/CSF-1R^{+} cells) was attenuated in *γ-GT Cre:CSF-1$^{f/f}$* mouse (arrows). (**g**) Immunoblotting indicated reduced protein levels of phosphorylated c-fms/CSF-1R in kidneys from *γ-GT Cre:CSF-1$^{f/f}$* mice at 6 days after DT injection. Each lane represented a sample from individual animal. Scale bar: 62 μm in (**a**) and (**e**) high magnification, 96 μm in (**b**–**d**) and (**e**) low magnification, and 32 μm in (**f**). CSF-1, colony-stimulating factor-1; DT, diphtheria toxin.

transgenic mice[16]) as well as —I/R-induced AKI. For both models of AKI, we induced selective deletion of proximal tubule CSF-1 in order to determine the role of proximal-tubule-generated CSF-1 in expansion and proliferation of renal macrophages and dendritic cells and in recovery from acute injury.

RESULTS

To determine the effect of proximal tubule CSF-1 on recovery from acute proximal tubule injury, we generated transgenic mice expressing the human diphtheria toxin receptor (DTR) in the proximal tubule with selective proximal tubule CSF-1 deletion (*γ-GT Cre:CSF-1$^{f/f}$* with littermate CSF-*1$^{f/f}$* as wild-type control) as described in Materials and Methods. In *CSF-1$^{f/f}$* mice, immunoreactive CSF-1 expression was detected primarily in proximal tubules (Figure 1a); immunostaining was inhibited by preincubation of the antibody with a blocking peptide or in the absence of primary antibody (Figure 1b and c). In *γ-GT Cre:CSF-1$^{f/f}$* mice, CSF-1 immunoreactivity was markedly decreased (Figure 1d).

As described previously, administration of diphtheria toxin (DT, 200 ng/g, intraperitoneally) resulted in functional renal dysfunction, as manifested by increased blood urea nitrogen (BUN), which peaked at 4 days after DT administration (Figure 2a). Six days after DT administration, *CSF-1$^{f/f}$* mice demonstrated phosphorylation of the CSF-1 receptor (c-fms) in tubules and interstitial cells, which was markedly decreased in *γ-GT Cre:CSF-1$^{f/f}$* mice (Figure 1e–g). Immunofluorescent staining showed that there was a marked increase in F4/80 and p-c-fms/CSF-1R double-positive cells (monocytes/macrophages/dendritic cells) in *CSF-1$^{f/f}$* mice than in *γ-GT Cre:CSF-1$^{f/f}$* mice (Figure 1f). The *γ-GT Cre:CSF-1$^{f/f}$* mice also had delayed functional recovery (Figure 2a) and increased expression of the proximal injury marker (Kim-1) (Figure 2b). In addition, *γ-GT Cre:CSF-1$^{f/f}$* mice had increased evidence of oxidative stress, as indicated by nitrotyrosine staining (Figure 2c).