CXCR6 Plays a Critical Role in Angiotensin II–Induced Renal Injury and Fibrosis

Yunfeng Xia, Xiaogao Jin, Jingyin Yan, Mark L. Entman, Yanlin Wang

Objective—Recent studies have shown that angiotensin II (Ang II) plays a critical role in the pathogenesis and progression of hypertensive kidney disease. The knowledge of diverse aspects related to CKD, the pathogenesis and the initial molecular events leading to chronic renal fibrosis and eventually chronic renal failure remain elusive.

Approach and Results—Wild-type and CXCR6-green fluorescent protein (GFP) knockin mice were treated with Ang II via subcutaneous osmotic minipumps at 1500 ng/kg per minute after unilateral nephrectomy for ≤4 weeks. Wild-type and CXCR6-GFP knockin mice had virtually identical blood pressure at baseline. Ang II treatment led to an increase in blood pressure that was similar between wild-type and CXCR6-GFP knockin mice. CXCR6-GFP knockin mice were protected from Ang II–induced renal dysfunction, proteinuria, and fibrosis. CXCR6-GFP knockin mice accumulated fewer bone marrow–derived fibroblasts and myofibroblasts and produced less extracellular matrix protein in the kidneys after Ang II treatment. Furthermore, CXCR6-GFP knockin mice exhibited fewer F4/80+ macrophages and CD3+ T cells and expressed less proinflammatory cytokines in the kidneys after Ang II treatment. Finally, wild-type mice engrafted with CXCR6−/− bone marrow cells displayed fewer bone marrow–derived fibroblasts, macrophages, and T cells in the kidney after Ang II treatment when compared with wild-type mice engrafted with CXCR6+/+ bone marrow cells.

Conclusions—Our results indicate that CXCR6 plays a pivotal role in the development of Ang II–induced renal injury and fibrosis through regulation of macrophage and T-cell infiltration and bone marrow–derived fibroblast accumulation.

Key Words: angiotensin II • CC chemokine receptor • fibrosis • inflammation

Chronic kidney disease (CKD) is a growing public health problem in the world. Hypertension is a major cause of CKD. A prominent pathological feature in patients with CKD is inflammation, tubular atrophy, and interstitial fibrosis. The degree of renal fibrosis correlates well with the prognosis of kidney disease.1 Renal interstitial fibrosis is characterized by massive fibroblast activation and excessive production and deposition of extracellular matrix, which leads to the destruction of renal parenchyma and progressive loss of kidney function. The current therapeutic options in the clinical settings for this devastating condition are limited and often ineffective except for dialysis or kidney transplantation, thus making chronic kidney failure one of the most expensive diseases to treat on a per-patient basis.2 Despite improvement in the knowledge of diverse aspects related to CKD, the pathogenesis and the initial molecular events leading to chronic renal fibrosis and eventually chronic renal failure remain elusive. Therefore, a better understanding of the cellular and molecular mechanisms underlying the pathogenesis of CKD is essential for developing effective strategies to treat this devastating disorder and prevent its progression.

A large body of evidence indicates that activation of the renin–angiotensin system plays a central role in initiation and progression of CKD through regulation of inflammation and fibrosis.3 The underlying mechanisms involved in angiotensin II (Ang II)–induced kidney disease are incompletely understood. Recent studies have shown that inflammatory and immune cell infiltration and altered chemokine production are characteristic for hypertensive kidney damage.4,5 The infiltration of circulating cells into sites of injury is mediated by locally produced chemokines through interaction with their respective receptors. However, the mechanism resulting in infiltration of these cells in hypertension remains incompletely understood.

We have recently shown that CXCL16 is induced in the kidney in response to Ang II, and genetic deletion of CXCL16 suppresses Ang II–induced renal injury and fibrosis.6 CXCR6 is the receptor for CXCL16, which is expressed in T cells,

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monocytes, and myeloid fibroblasts.7–9 In this study, we investigated the role of CXCR6 in leukocyte recruitment and renal injury in Ang II–induced hypertensive kidney disease.

Materials and Methods
Materials and Methods are available in the online-only Supplement.10

Results

Blood Pressure
There were no significant differences in blood pressure among the 4 groups at baseline. Ang II treatment increased blood pressure in both WT and CXCR6-green fluorescent protein (GFP) knockin mice with no differences between the 2 treatment groups (Figure 1A).

Renal Function
Treatment with Ang II for 4 weeks caused kidney dysfunction in WT mice as reflected by significant elevation of blood urea nitrogen. Kidney function was preserved in CXCR6-GFP knockin mice with blood urea nitrogen markedly lower than WT mice (Figure 1B).

Albuminuria
WT mice developed massive albuminuria after Ang II treatment for 4 weeks, whereas Ang II–treated CXCR6-GFP knockin mice produced significantly less albuminuria (Figure 1C).

Kidney Injury and Fibrosis
To assess the effect of CXCR6 disruption on Ang II–induced kidney damage, kidney sections were stained with Periodic Acid Schiff and scored for histological injury after 4 weeks of saline or Ang II infusion (Figure 2A; Table I in the online-only Data Supplement). On a semiquantitative scale that includes glomerulosclerosis, interstitial disease, fibrosis, and vascular injury, the 2 saline-infused groups had minimal kidney damage. Ang II caused a marked increase in the severity of kidney injury in the WT mice, which was substantially reduced in CXCR6-GFP knockin mice. Sirius red staining showed that Ang II–treated WT mice developed significant collagen deposition in the kidney when compared with saline-treated WT mice. These fibrotic responses were significantly reduced in CXCR6-GFP knockin mice with chronic Ang II infusion (Figure 2B and 2C).

We next examined the effect of CXCR6 disruption on the expression of collagen I and fibronectin, 2 major components of extracellular matrix. Immunofluorescence staining demonstrated that CXCR6 disruption attenuated the upregulation of collagen I (Figure 3A and 3B) and fibronectin (Figure 3C and 3D). Similar results were obtained with Western blot analysis (Figure 3E and 3F).

Myeloid Fibroblasts Accumulation and Myofibroblast Formation
Recent evidence indicates that myeloid fibroblasts contribute significantly to the pathogenesis of renal fibrosis and circulating fibroblast precursors expressed CXCR6.9,11–14 To examine the effect of CXCR6 disruption on the accumulation of myeloid fibroblasts in the kidney, WT and CXCR6-GFP knockin mice were infused with vehicle or Ang II for 2 weeks. The 2-week model of Ang II infusion was chosen because we and others have shown that cellular infiltration precedes the development of target organ injury.9,15,16 Kidney sections were stained for CD45 and pro–collagen I and examined with a fluorescence microscope. The number of bone marrow–derived fibroblasts dual positive for CD45 and pro–collagen I was significantly reduced in the kidneys of Ang II–treated CXCR6-GFP knockin mice when compared with those of WT mice (Figure 4A and 4B). These data indicate that CXCR6 has an important role in recruiting bone marrow–derived fibroblasts into the kidney in response to Ang II.

To determine whether CXCR6 disruption influences myofibroblast formation, kidney sections were stained for α-smooth muscle actin (α-SMA), a marker of myofibroblasts, and examined with a fluorescence microscope. Ang II–treated CXCR6-GFP knockin mice exhibited a significant reduction in the number of α-SMA+ myofibroblasts in the kidneys when compared with Ang II–treated WT mice (Figure 4C and 4D).

Figure 1. Effect of CXCR6 disruption on blood pressure, renal function, and proteinuria. A, Systolic blood pressure was elevated to the similar level between angiotensin II (Ang II)–treated wild-type (WT) and CXCR6-GFP (green fluorescent protein) knockin mice. **P<0.01 between Ang II–treated groups and vehicle-treated control groups. n=6 per group. B, CXCR6 disruption significantly attenuated Ang II–induced renal dysfunction. *P<0.05 vs WT controls; +P<0.05 vs knockout (KO) Ang II; and #P<0.05 vs WT Ang II. n=6 per group. C, CXCR6 disruption inhibited Ang II–induced albuminuria. **P<0.01 vs WT controls; ++P<0.01 vs KO Ang II; and #P<0.05 vs WT Ang II. n=6 per group. CON indicates control.
Consistent with these findings, Western blot analysis showed that disruption of CXCR6 significantly reduced the protein expression levels of α-SMA in the kidneys after Ang II treatment when compared with WT mice (Figure 4E and 4F).

**Macrophage and T-Cell Infiltration and Inflammatory Cytokine Production**

Recent evidence indicates that macrophages and T cells play a critical role in the pathogenesis of Ang II–induced target organ damage. We examined whether infiltrating macrophages and T cells in the kidney express CXCR6. Kidney sections were stained for CXCR6 and F4/80, a macrophage marker, or CD3, a T-cell marker. The results showed that infiltrating F4/80⁺ macrophages and CD3⁺ T cells express CXCR6 (Figure I in the online-only Data Supplement). To determine whether CXCR6 plays a role in the regulation of inflammatory cell infiltration into the kidney, WT and CXCR6-GFP knockin mice were infused with vehicle or Ang II for 2 weeks. Kidney...
sections were stained for F4/80 and CD3. Significant infiltration of macrophages and T cells was observed in the kidneys of WT mice after Ang II treatment when compared with the vehicle-treated control group. In contrast, disruption of CXCR6 significantly inhibited macrophage and T-cell infiltration into the kidneys after Ang II treatment (Figure 5A–5D). These results indicate that CXCR6 mediates inflammatory cell infiltration into the kidney in Ang II–induced hypertensive kidney disease.

We next examined the effect of CXCR6 disruption on the expression of known proinflammatory cytokines that are involved in the pathogenesis of kidney injury. The mRNA levels of interleukin-6 (IL-6), tumor necrosis factor-α, IL-1β, and transforming growth factor-β1 were increased significantly in the kidneys of WT mice after Ang II treatment (Figure 5E–5H). In contrast, the upregulation of IL-6, tumor necrosis factor-α, IL-1β, and transforming growth factor-β1 was greatly attenuated in the kidneys of CXCR6-GFP knockin mice after Ang II or saline treatment. These results indicate that CXCR6 mediates inflammatory cell infiltration into the kidney in Ang II–induced hypertensive kidney disease.

Effect of CXCR6 Disruption on Mobilization of Myeloid Fibroblasts Precursors, Monocytes, and T Cells

To determine the effect of CXCR6 disruption on mobilization of monocytes, T cells, and fibroblast precursors, peripheral nucleated cells were stained for CD11b and CD3 or CD45 and collagen I from WT and CXCR6-GFP knockin mice 2 weeks after saline or Ang II treatment. Our results showed that there were no significant differences among the 4 groups, although Ang II treatment led to a small but not significant increase in monocytes, T cells, and fibroblast precursors (Figure II in the online-only Data Supplement).

Effect of CXCR6 Disruption in Bone Marrow Cells on Accumulation of Myeloid Fibroblasts, Macrophages, and T Cells in the Kidney

To examine the role of CXCR6 in bone marrow–derived cells in recruiting myeloid fibroblasts, macrophages, and T cells further, we performed bone marrow chimera experiments by reconstitution of WT mice with CXCR6+/+ or CXCR6−/− bone marrow cells. Eight weeks after bone marrow transplantation, chimeric mice were subjected to vehicle or Ang II treatment for 2 weeks. The genotype of bone marrow–derived cells from the chimeric mice was confirmed by polymerase chain reaction of DNA extracted from peripheral blood cells (Figure III in the online-only Data Supplement). When compared with WT mice transplanted with CXCR6+/+ bone marrow cells, WT mice transplanted with CXCR6−/− bone marrow cells accumulated fewer CD45+ and PDGFR-β+ (platelet-derived growth factor receptor) fibroblasts, F4/80+ macrophages, and CD3+ T cells (Figure 6). These data indicate that CXCR6 in bone marrow–derived cells is critical for...
recruiting bone marrow–derived fibroblasts, macrophages, and T cells into the kidney in response to Ang II.

**Discussion**

In this study, we have demonstrated that disruption of CXCR6 leads to renal protection in an experimental model of hypertensive kidney disease induced by Ang II. In the Ang II–induced hypertension model, disruption of CXCR6 preserves renal function, reduces urinary albumin excretion, and attenuates tubulointerstitial disease, glomerular injury, and vascular damage.

A large body of evidence indicates that activation of renin–angiotensin system plays a central role in the initiation and progression of CKD. The underlying mechanisms involved in Ang II–induced hypertensive kidney disease are incompletely understood. Recent studies have shown that inflammatory and immune cell infiltration are characteristic for hypertensive kidney disease.6 The CXCL16 receptor, CXCR6, is expressed in T cells, monocytes, and myeloid fibroblasts.7–9 Because activated fibroblasts are the principal effector cells that are responsible for extracellular matrix production in the fibrotic kidney, their activation is regarded as a key event in the pathogenesis of renal fibrosis.10–20 However, the origin of these fibroblasts remains debatable. They are traditionally thought to arise from resident renal fibroblasts.21–23 Recent evidence suggests that they may originate from bone marrow–derived fibroblast precursors.5,11–14,24–26 We and others have shown that these cells migrate into the kidney in response to obstructive injury and contribute to the development of renal fibrosis.3,12,25–28 However, the signaling mechanisms underlying the recruitment of these bone marrow–derived fibroblast precursors into the kidney are incompletely understood.

**Figure 5.** CXCR6 disruption reduces angiotensin II (Ang II)–induced inflammation. A, Representative photomicrographs of kidney sections stained for F4/80 (a macrophage marker; brown) and counterstained with hematoxylin (blue) in wild-type (WT) and CXCR6–green fluorescent protein (GFP) knockin mice 2 weeks after Ang II or saline treatment. Scale bar, 50 μm. B, Quantitative analysis of F4/80–macrophages in the kidneys. **P<0.01 vs WT controls; ###P<0.05 vs knockout (KO) Ang II. n=8 in each group. C, Representative photomicrographs of kidney sections stained for CD3 (a T-lymphocyte marker; brown) and counterstained with hematoxylin (blue) in WT and CXCR6-GFP knockin mice 2 weeks after Ang II or saline treatment. Scale bar, 50 μm. D, Quantitative analysis of CD3+ T cells in the kidneys. *P<0.01 vs WT controls; ##P<0.01 vs WT Ang II; +P<0.05 vs KO Ang II. n=6 in each group. E, Quantitative analysis of tumor necrosis factor (TNF)-α mRNA expression in the kidneys. **P<0.01 vs WT controls; ##P<0.01 vs WT Ang II; +P<0.05 vs KO Ang II. n=5 in each group. F, Quantitative analysis of interleukin-6 (IL-6) mRNA expression in the kidneys. **P<0.01 vs WT control; ##P<0.05 vs WT Ang II; +P<0.05 vs KO Ang II. n=5 in each group. G, Quantitative analysis of IL-1β mRNA expression in the kidneys. **P<0.01 vs WT control; #P<0.05 vs WT Ang II; +P<0.05 vs KO Ang II. n=5 in each group. H, Quantitative analysis of transforming growth factor (TGF)-β1 mRNA expression in the kidneys. *P<0.01 vs WT controls; ##P<0.01 vs WT Ang II; +P<0.05 vs KO Ang II. n=5 in each group. CON indicates control; and HPF, high-power field.
In the present study, we showed that disruption of CXCR6 inhibits bone marrow–derived fibroblast accumulation and myofibroblast formation in the kidney and the development of renal fibrosis in response to Ang II. These results indicate that CXCR6 plays a key role in recruitment of bone marrow–derived fibroblasts into the kidney and development of renal fibrosis in Ang II–induced hypertensive nephropathy.

Macrophages and T cells have been shown to play an important role in the development of hypertensive nephropathy. Ang II is a key factor in the regulation of inflammatory response in hypertensive end-organ damage. CXCR6 is expressed in various leukocyte subsets, including monocytes and T cells. Recent studies have shown that CXCR6 plays an important role in inflammatory disease, such as atherosclerosis. In the present study, our results show that Ang II–induced interstitial infiltration of macrophages and T cells into the kidney is significantly attenuated in CXCR6-GFP knockin mice. These data indicate that CXCR6 signaling mediates Ang II–induced macrophage and T-cell infiltration.

Our results indicate that disruption of CXCR6 significantly attenuates proinflammatory and profibrotic cytokine expression in the kidney in response to Ang II. This is relevant because proinflammatory cytokines, tumor necrosis factor-α, IL-6, and IL-1β, have been implicated in the pathogenesis of Ang II–induced target organ damage, and transforming growth factor-β1 functions as a downstream mediator of Ang II–induced renal fibrosis.

In summary, our studies identify that CXCR6 critically regulates Ang II–induced renal injury and fibrosis. In response to Ang II, the activated CXCR6 signaling recruits myeloid fibroblasts, monocytes/macrophages, and T cells into the kidney, leading to proinflammatory and profibrotic cytokine production, renal injury, and fibrosis. These findings suggest that inhibition of CXCR6 signaling could constitute a novel therapeutic target for hypertensive kidney disease.

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Disclosures
None.

References
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**Significance**

Our study defines a critical role of CXCR6 in regulating angiotensin II–induced renal injury and fibrosis. In response to angiotensin, the activated CXCR6 signaling recruits myofibroblast fibroblasts, monocytes/macrophages, and T cells into the kidney, leading to proinflammatory and profibrotic cytokine production, renal injury, and fibrosis. These findings suggest that inhibition of CXCR6 signaling could constitute a novel therapeutic strategy for hypertensive kidney disease.
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MATERIALS AND METHODS

Animals

Wild-type C57BL/6 and CXCR6-GFP knockin mice on a C57BL/6 background were purchased from the Jackson Laboratory. Mice were bred and maintained in the animal care facility of Baylor College of Medicine and had access to food and water ad libitum. All animal procedures were in accordance with national and international animal care and ethical guidelines and have been approved by the institutional animal welfare committee.

Bone Marrow Transplantation

Bone marrow transplantation was performed as described previously. Briefly, bone marrow cells (5X10^6) were injected into irradiated WT or CXCR6-GFP knockin mice through tail vein. After transplantation, mice were allowed to recuperate for 8 weeks prior to angiotensin II or vehicle infusion (n=5 per group).

Ang II-induced Hypertension

Male mice, 8-10 weeks old, were infused with Ang II (1.5 µg/kg/min; Sigma) or vehicle (0.9% NaCl) continuously via subcutaneous osmotic minipumps (Alzet) for 14 or 28 days following uninephrectomy as described. To accelerate renal injury, Ang II-treated mice were supplied with 1% saline drinking water ad libitum.

Blood Pressure and Heart Rate

Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious mice using a tail cuff system (Visitech Systems) as reported.

Renal Function

Blood urea nitrogen was determined fluorometrically as described.

Histopathologic Analysis

At the end of experiments, mice (n=6 per group) were perfused with PBS to remove the blood. A portion of kidney tissue was fixed in 10% buffered formalin, embedded in paraffin, and cut at 5-µm thickness. After deparaffinization and rehydration, sections were stained with Periodic Acid Schiff (PAS) and Sirius red. The pathological abnormalities in the kidney were graded on the basis of the presence and severity of component abnormalities, including glomerulosclerosis, epithelial reactivity, chronic inflammation, tubular casts, fibrosis, and vascular injury. Grading for each component was performed using a semiquantitative scale, as described, where 0 represented no abnormality and where 1, 2, 3, and 4 represented mild, moderate, moderately severe, and severe abnormalities, respectively. The total injury score for each kidney was a summation of these component injury scores. The Sirius red-stained sections were scanned using a microscope equipped with a digital camera (Nikon Instruments Inc., Melville, NY), and quantitative evaluation was performed using NIS-Elements Br 3.0 software as described. The Sirius red-stained area was calculated as a percentage of the total area.
To assess monocyte/macrophage and T lymphocyte infiltration in the kidneys, sections were stained with antibodies against F/4/80 (Serotec) and CD3 (Calbiochem) respectively. Interstitial infiltrating F4/80-positive macrophages and CD3-positive T cells were counted in the cortex under x400 magnification observing 10 consecutive non-overlapping fields per animal.

**Immunofluorescence**

Renal tissues from WT and CXCR6-GFP knockin mice treated with saline or Ang II (n=6) were embedded in OCT compound, snap-frozen on dry ice, cut at 5 µm thickness, and mounted. After fixation, nonspecific binding was blocked with protein block (Dako, Carpinteria, CA). Kidney sections were then incubated with rabbit anti-collagen I antibody (Rockland Immunochemicals, Gilbertsville, PA) followed by Alexa-488 conjugated donkey anti-rabbit antibody (Invitrogen, Carlsbad, CA), rabbit anti-fibronectin antibody (Sigma-Aldrich, St. Louis, MO) followed by Alexa-488 conjugated donkey anti-rabbit antibody (Invitrogen, Carlsbad, CA), or rabbit anti-α-SMA antibody (Abcam, Cambridge, MA) followed by Alexa-488 conjugated donkey anti-rabbit antibody (Invitrogen, Carlsbad, CA). For double immunofluorescence, kidney sections were fixed and stained with rat anti-CD45 (BD Biosciences) and mouse anti-procollagen I (Santa Cruz Biotechnology) followed by appropriate secondary antibodies sequentially. Slides were mounted with mounting medium containing DAPI. Fluorescence intensity was visualized using a microscope equipped with a digital camera (Nikon Instruments Inc., Melville, NY). Quantitative evaluation of sections stained with antibodies to α-SMA, collagen I and fibronectin was performed using NIS-Elements Br 3.0 software. The fluorescence positive area was calculated as a percentage of the total area as described.

**Albuminuria**

Mice were placed into metabolic cages for 12-hour urine collection. Albumin and creatinine in the urine were measured using commercially available kits (EXOCCELL, Inc).

**Quantitative Real-Time RT-PCR**

Total RNA was extracted from kidney tissues with TRIzol reagent (Invitrogen). Aliquots (1 µg) of total RNA were reverse transcribed using SuperScript II reverse transcriptase. Real-time PCR was performed using IQ SYBR green supermix reagent (Bio-Rad, Hercules, CA) with a Bio-Rad real-time PCR machine according to the manufacturer’s instructions. The specificity of real-time PCR was confirmed via melting-curve analysis. The comparative Ct method (ΔΔCt) was used to quantify gene expression, and the relative quantification was calculated as $2^{-\Delta\Delta Ct}$. The expression levels of the target genes were normalized to GAPDH level in each sample. The primer sequences were: IL-6 - forward, 5′-GAGGATAACCACCCTCCACAGACACC-3′, reverse, 5′-AAGTGTCATCGTTGTTCTACACA-3′; TNF-α - forward, 5′-CATGAGCAGCAAAACAGATGGATCC-3′, reverse, 5′-AAGCAGGAATGAGAGGCTGAG-3′; IL-1β - forward, 5′-CTTCCAGGGGAGTACATCATC-3′, reverse, 5′-TCTAAATGGGAAACGTCACACACACAGC-3′; TGF-β1 - forward, 5′-CAACAATTCTGGCGTACCTTGG-3′, reverse, 5′-
Western Blot Analysis

Protein was extracted using the RIPA buffer containing a cocktail of proteinase inhibitors (Thermo Fisher Scientific Inc., Rockford, IL) and quantified with Bio-Rad protein assay. Equal amounts of protein were separated on SDS–polyacrylamide gels in a Tris/glycine buffer system, transferred onto nitrocellulose membranes, and blotted according to standard procedures with primary antibodies (collagen I, fibronectin, and α-SMA) followed by appropriate secondary antibodies as described. Membranes were rebotted with anti-GAPDH antibody (Millipore, Billerica, CA). The specific bands of target proteins were analyzed using an Odyssey IR scanner (LI-COR Bioscience, Lincoln, NE) and band intensities were quantified using NIH Image/J.

Flow Cytometry

Flow cytometry were performed as described. Briefly, Venous blood were collected with EDTA as anticoagulant from WT and KO mice treated with saline or Ang II for two weeks (n=4-5 per group). Red blood cells were lysed with lysis buffer. The cells were washed in PBS and resuspended in FACS buffer. To quantify T lymphocytes and monocytes, cells (5 x 10^5) were stained with PE-Cy5-anti-CD3 and PE-anti-CD11b antibodies (BD Biosciences, San Jose, CA). Granulocytes were removed from CD11b positive cell population based on side scatter analysis. To identify circulating fibroblast precursors, cells (5 x 10^5) were incubated with PE-anti-CD45 (BD Biosciences, San Jose, CA), then fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences, San Jose, CA) in accordance with the manufacturer's protocol, and incubated with biotin-anti-collagen I (Rockland, Gilbertsville, PA)/streptavidin-APC (BD Biosciences, San Jose, CA). Cells incubated with irrelevant isotype-matched antibodies (BD Biosciences, San Jose, CA) and unstained cells were used as controls. The cutoffs were set according to results of controls. The fluorescence intensities were measured using a BD LSR II flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed using BD FACSDiva software.

Statistical Analysis

All data were expressed as mean ± SEM. Multiple group comparisons were performed by one-way ANOVA followed by the Bonferroni procedure for comparison of means. P < 0.05 was considered statistically significant.

References


Supplementary Figure I

A. Macrophages in the kidney express CXCR6. Kidney sections were stained for F4/80 and CXCR6 followed by appropriate secondary antibodies, counterstained with DAPI, and examined with the fluorescence microscope.

B. T cells in the kidney express CXCR6. Kidney sections were stained for CD3 and CXCR6 followed by appropriate secondary antibodies, counterstained with DAPI, and examined with the fluorescence microscope.
Supplementary Figure II: Disruption of CXCR6 does not affect the mobilization of circulating monocytes, T cells, and fibroblast precursors. A. Representative cytometric diagrams showing the effect of CXCR6 disruption on circulating CD11b⁺ monocytes. B. Quantitative analysis of circulating CD11b⁺ monocytes. n=4-5 per group. C. Representative cytometric diagrams showing the effect of CXCR6 disruption on circulating CD11b⁺ granulocytes. D. Quantitative analysis of circulating CD11b⁺ granulocytes. n=4-5 per group. E. Representative cytometric diagrams showing the effect of CXCR6 disruption on circulating T cells. F. Quantitative analysis of circulating T cells. n=4-5 per group. G. Representative cytometric diagrams showing effect of CXCR6 disruption on fibroblast precursors in the circulation. H. Quantitative analysis of CD45⁺ and collagen⁺ fibroblast precursors in the circulation. n=4-5 per group.
**Supplementary Figure III:** Replacement of hematopoietic cells in the chimeric mice is confirmed by genotyping of genomic DNA from whole blood using PCR. PCR products are shown on ethidium bromide-stained agarose gel with the WT allele DNA at 220 bp and the mutated allele DNA at 173 bp.

**Supplementary Table I: Disruption of CXCR6 Prevents Ang II-induced Kidney Injury**

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**p<0.01 versus WT-CON; # p<0.05 versus WT-Ang II; **p<0.01 versus KO-Ang II. n=6 per group.