Type 1 angiotensin receptors on macrophages ameliorate IL-1 receptor–mediated kidney fibrosis

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In a wide array of kidney diseases, type 1 angiotensin (AT1) receptors are present on the immune cells that infiltrate the renal interstitium. Here, we examined the actions of AT1 receptors on macrophages in progressive renal fibrosis and found that macrophage-specific AT1 receptor deficiency exacerbates kidney fibrosis induced by unilateral ureteral obstruction (UUO). Macrophages isolated from obstructed kidneys of mice lacking AT1 receptors solely on macrophages had heightened expression of proinflammatory M1 cytokines, including IL-1. Evaluation of isolated AT1 receptor–deficient macrophages confirmed the propensity of these cells to produce exaggerated levels of M1 cytokines, which led to more severe renal epithelial cell damage via IL-1 receptor activation in coculture compared with WT macrophages. A murine kidney crosstransplantation concomitant with UUO model revealed that augmentation of renal fibrosis instigated by AT1 receptor–deficient macrophages is mediated by IL-1 receptor stimulation in the kidney. This study indicates that a key role of AT1 receptors on macrophages is to protect the kidney from fibrosis by limiting activation of IL-1 receptors in the kidney.

Introduction

Chronic kidney disease (CKD) constitutes a major public health concern. Hematopoietic cells infiltrate the injured kidney and modulate the progression of immune-mediated glomerulonephritides and even “nonimmune” forms of kidney damage related to hypertension and ischemia-reperfusion (1–3). Among BM-derived cell lineages, macrophages play a prominent role in regulating kidney fibrosis (4), and the extent of fibrosis predicts organ failure in chronic diseases of the kidney and other tissues (5).

After infiltrating the kidney, macrophages can promote inflammatory injury or direct tissue repair depending on their phenotype, summarized in a dichotomous “M1 versus M2” paradigm (6). In this vernacular, proinflammatory M1 macrophages induce damage by secreting cytokines such as TNF-α and IL-1β, whereas reparative M2 macrophages secrete the decoy IL-1 receptor (IL-1R2) and other immunosuppressive mediators. However, the mechanisms through which key drivers of kidney fibrosis contribute to macrophage polarization require elucidation.

As the principal effector molecule of the renin-angiotensin system (RAS), Ang II is a fundamental driver of renal fibrosis (7). Accordingly, treatment with type 1 angiotensin (AT1) receptor blockers has achieved considerable success in managing CKD patients (8). Ang II is a fundamental driver of renal fibrosis (7). Accordionngly, treatment with type 1 angiotensin (AT1) receptor blockers has achieved considerable success in managing CKD patients (8). Ang II is a fundamental driver of renal fibrosis (7). Activation on macrophages exacerbates CKD by promoting a proinflammatory M1 phenotype. We employed the unilateral ureteral obstruction–induced (UUO-induced) kidney fibrosis model of CKD because UUO stimulates the RAS and macrophages are critical to the pathogenesis of renal fibrosis (12, 13). However, we found that AT1 receptors on macrophages protect the kidney from fibrosis and suppress M1 cytokine production.

Results and Discussion

AT1A receptor deficiency on macrophages exacerbates kidney fibrosis. To examine infiltration of LysM-expressing macrophages in the kidney during renal fibrosis, we subjected LysM-Cre−/−/mT/mG reporter mice to UUO. By day 7 of UUO, robust accumulation of GFP+ macrophages was detected in the interstitial areas of obstructed kidneys, but not the contralateral, unobstructed kidneys (Figure 1A), confirming that LysM-Cre−/−/mT/mG gene excision affects macrophages infiltrating the diseased kidney. To evaluate the actions of AT1 receptors on macrophages that infiltrate the kidney undergoing fibrosis, we harvested kidneys from mice with macrophage-specific deletion of Agtr1a (LysM-Cre+ Agtr1afl/flx, herein referred to as Macro KO) and WT mice 7 days following UUO. Obstructed kidneys from Macro KO mice contained 64%

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more interstitial fibrosis (Figure 1B and Supplemental Figure 5), 80% more collagen-producing myofibroblasts (Supplemental Figure 6), and 80% more collagen I (Col I) protein (Figure 1C) than in WT mice. Moreover, at day 7 after UUO, mRNA expressions of Col I, PAI-1, and TGF-β1 were enhanced by 129%, 47%, and 40%, respectively, in Macro KO kidneys compared with WTs (Figure 1D). Additional experiments further confirmed our results (Supplemental Paragraph 2 and Supplemental Figures 7 and 8). These findings contrast starkly with studies in which global AT1A receptor deficiency ameliorates kidney fibrosis (14), but align with reports of exaggerated tissue damage in AT1A receptor–deficient BM chimeras (15, 16). Collectively, our data indicate that activation of AT1 receptors on infiltrating macrophages protects the kidney from progressive fibrosis.

The heterogeneity of macrophage polarization plays a critical role in the pathogenesis of kidney damage and fibrosis (6). In simplified terms, the balance between proinflammatory M1 and reparative M2 macrophages determines the local composition of cytokine reservoirs that orchestrate disruptions in renal architecture following an insult. Therefore, on days 3 and 7 after UUO, we profiled the renal expression of M1 and M2 markers, including M1 cytokines IL-1β and TNF-α, which have been implicated in the progression of renal fibrosis (Figure 1E). Obstructed Macro KO kidneys had significantly higher expression of M1 markers, including IL-1β, IL-1R1, TNF-α, IL-12p40, and CCL2, compared with obstructed WT kidneys 3 days after UUO, whereas expression of M2 markers Arg-1, FIZZ-1, YM-1, and IL-1R2 was virtually identical between cohorts. A similar pattern was noted at day 7 after UUO (Figure 1E), suggesting that activation of AT1 receptors on macrophages represses M1 cytokine expression during kidney fibrosis.

AT1A receptors on macrophages limit their expression of M1 cytokines. We next examined the effects of macrophage AT1 receptor activation on the phenotype of infiltrating macrophages at day 7 after UUO by histologic staining (Supplemental Paragraph 2 and Supplemental Figures 9 and 10) and, more precisely, by isolating CD45+CD11b+Ly6Chi and CD45 +CD11b +Ly6Clo macrophages directly from the obstructed kidney (Supplemental Figure 11). Based on reports that CD45+CD11b+Ly6Chi macrophages promote tissue damage and fibrosis (4, 17), TaqMan low-density

Figure 1
AT1A receptor deficiency on macrophages exacerbates kidney fibrosis induced by UUO. (A) Representative kidney sections from LysM-Cre+ mT/mG mice 7 days after UUO with contralateral unobstructed kidney on left and obstructed kidney on right. (B) Polarized images of representative sections from obstructed WT and Macro KO kidneys stained with picrosirius red for collagen fibrils at day 7 after UUO. Original magnification, ×20. (C) Western blot for Col I in whole kidney at day 7 after UUO. (D) mRNA expression of Coll, PAI1, and TGFb1 in obstructed kidney at day 7 after UUO. (E) Gene expression of M1 markers Il1b, Il1r1, Tnfa, Il12b, and Ccl2 and M2 markers Arg1, Fizz1, Ym1, and Il1r2 in obstructed kidneys at 3 and 7 days after UUO. At day 3, *P < 0.02 vs. WT, †P = 0.02 vs. WT, ‡P = 0.01 vs. WT, §P = 0.03, ¶P < 0.02. At day 7, *P < 0.03 vs. WT, †P = 0.02 vs. WT, ‡P = 0.05, §P < 0.001.
array (TLDA) card–based microarray was performed to survey gene expression for inflammatory mediators and signaling molecules in the Ly6Ch-activated macrophages (Figure 2A and Supplemental Table 1). Among the upregulated genes, IL-1β but not TNF-α showed enhanced expression in Macro KO kidneys at both days 3 and 7 of UUO (Figure 1E). Moreover, by real-time PCR, Ly6C hi and Ly6Clo macrophages from the Macro KO kidneys had higher IL-1β expression levels than found in WT controls (Figure 2B).

These data indicate that AT1A receptor activation constrains infiltrating macrophage activation by suppressing key inflammatory cytokines including IL-1 during kidney fibrosis.

To explore in vitro the role of the macrophage AT1A receptor in regulating macrophage activation, we cultured Macro KO and WT peritoneal macrophages and subjected them to M1- or M2-polarizing stimuli or vehicle (M0). M1 stimulation increased mRNA expression of M1 markers in both groups, confirming that AT1A receptor activation reduces M1 polarization. (C) mRNA levels of proinflammatory cytokines Il1b, Il1a, Ccl5, Ccl2, Il12a, and Il12b in WT and Macro KO peritoneal macrophages following M1 stimulation. (D) Coll and Ngal gene expression in WT (Il1r1 WT) or IL-1R–deficient (Il1r1 KO) renal tubular epithelial cells cocultured with M0- or M1-activated macrophages from WT or Macro KO (MKO) animals.
M1 polarization (Figure 2C). However, M1 macrophages lacking the AT1A receptor showed exaggerated expression levels for each marker. These findings together with M2 polarization studies (Supplemental Paragraph 4 and Supplemental Figures 12 and 13) suggest that AT1A receptor activation during M1 differentiation constrains M1 cytokine production without altering the macrophage’s susceptibility to M2 differentiation.

Enhanced IL-1 production by AT1 receptor–deficient macrophages drives renal cell injury in coculture. Proinflammatory cytokines including IL-1β have been implicated in renal tubular cell (RTC) damage (18). We therefore hypothesized that exaggerated production of inflammatory cytokines by AT1A receptor–deficient macrophages could alter gene expression programs for injury within the kidney tubular cell. Testing this hypothesis in vitro, we measured expres-

Figure 3
Activation of the kidney IL-1R mediates exaggerated renal damage induced by AT1A receptor–deficient macrophages. (A) Right panel shows section of a WT kidney transplanted into a LysM-Cre+ mT/mG recipient and subjected to ureteral obstruction (KT-UO). Green fluorescence marks infiltrating LysM+ myeloid cells from recipient. Left panel shows section of an unobstructed native contralateral kidney from the same LysM-Cre+ mT/mG recipient. (B) Right panel shows section of a LysM-Cre+ mT/mG kidney transplanted into a non-Cre mT/mG recipient and subjected to ureteral obstruction. Green fluorescence marks LysM+ macrophages resident in KT-UO kidney. Left panel shows section of an unobstructed native contralateral kidney from the same non-Cre mT/mG recipient. Blue fluorescence is a nuclear DAPI stain. Original magnification, ×20. (C–E) Use of KT-UO model to dissect the contribution of WT vs. AT1 receptor–deficient macrophages to kidney damage mediated through renal IL-1R activation. WT (Macro AT1A WT) or Macro KO (KO) recipients were transplanted with an IL-1R WT or KO kidney subjected to UUO (groups I–IV as in Supplemental Table 2; n = 6–7 per group). (C) Representative polarized images of obstructed kidneys stained with picrosirius red at day 7 KT-UO. Original magnification, ×20. Quantitation is shown on the right. Excessive fibrosis due to AT1 receptor deficiency on macrophages in Macro KO recipient is abrogated in IL-1R–deficient donor kidneys. mRNA expression of (D) Col I and (E) NGAL in obstructed kidneys 7 days after KT-UO.
Brief report

A brief report on the role of infiltrating macrophages in kidney injury and fibrosis.

In the context of kidney transplantation, we observed that the donor kidney in the KT-UO model is characterized by a dense infiltrate of macrophages, as evidenced by GFP labeling. In contrast, the native unobstructed kidney in the KT-UO model is characterized by a sparse infiltrate of macrophages. These observations suggest that the donor kidney is not simply a transplantation site for macrophages, but rather a source of inflammation, driven by the innate immune response.

The infiltration of macrophages into the transplant kidney is regulated by the IL-1 receptor pathway. In macrophage-specific IL-1 receptor-deficient (KO) RTCs, the levels of fibrosis in the KT-UO kidney are significantly reduced compared with their counterparts with WT IL-1R1. This finding suggests that IL-1R1 plays a critical role in mediating fibrosis in the KT-UO model.

To further explore the role of IL-1R1 in fibrosis, we used a kidney transplantation–ureteral obstruction (KT-UO) model. In this model, we observed a significant increase in the expression of extracellular matrix proteins, such as collagen I and NGAL, in the macrophage-deficient group. These findings suggest that macrophages play a crucial role in mediating renal fibrosis in the KT-UO model.

In summary, our results demonstrate that macrophage infiltration is a key factor in mediating renal fibrosis in the KT-UO model. These findings have important implications for the development of targeted therapies for kidney fibrosis.

Methods

Detailed Supplemental Methods are available online.

Statistics. Data are expressed as mean ± SEM and analyzed by 2-tailed unpaired t test or ANOVA according to statistical methods described in detail in the Supplemental Methods. P < 0.05 was considered significant.

Study approval. Mice were bred and maintained in the Association for Assessment and Accreditation of Laboratory Animal Care–accredited animal facilities at the Durham Veterans’ Affairs Medical Center (DVAMC) per NIH guidelines. The animal studies were approved by the DVAMC Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.
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