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

Jian-dong Zhang, … , Daian Chen, Steven D. Crowley


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.

Find the latest version:

https://jci.me/61368/pdf
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 recep-

tors in the kidney.

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+ Agtr1alox/lox 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+-mediated 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+ Agtr1alox/lox, herein referred to as Macro KO) and WT mice 7 days following UUO. Obstructed kidneys from Macro KO mice contained 64%
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 Arg1, FIZZ1, 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 3 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+Ly6Clo macrophages promote tissue damage and fibrosis (4, 17), TaqMan low-density
array (TLDA) card–based microarray was performed to survey gene expression for inflammatory mediators and signaling molecules in the Ly6C<sup>hi</sup>-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<sub>hi</sub> and Ly6C<sub>Clo</sub> macrophages from the Macro KO kidneys had higher IL-1β expression levels than found in WT controls (Figure 2B).

**Figure 2**

AT1A receptor activation on macrophages suppresses their M1 proinflammatory polarization. (A) Heat map showing hierarchical cluster analysis of ΔCT values of CD11b<sup>+</sup>Ly6C<sup>hi</sup> macrophages isolated from obstructed WT and Macro KO kidneys. (B) mRNA expression of IL-1β measured by real-time RT-PCR in Ly6C<sup>hi</sup> and Ly6C<sup>Clo</sup> macrophages isolated from obstructed kidneys. (C) mRNA levels of proinflammatory cytokines IL1b, IL1a, Ccl5, Ccl2, IL12a, and IL12b in WT and Macro KO peritoneal macrophages following M1 stimulation. (D) ColⅠ and Ngal gene expression in WT (Il1r1 WT) or IL1R–deficient (Il1r1 KO) renal tubular epithelial cells cocultured with M0- or M1-activated macrophages from WT or Macro KO (MKO) animals.

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
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-
sion of Col I and kidney injury marker neutrophil gelatinase–asso-
ciated lipocalin (NGAL) in RTCs following coculture with M0- or 
M1-conditioned macrophages from our experimental animals 
(Figure 2D). At 6 hours, M1 macrophages from Macro WT or KO 
mice, but not M0 macrophages, upregulated Col I and Ngal mRNA 
expression in RTCs. Thus, cytokines from activated macrophages 
can mediate damage to kidney tubular cells without direct con-
tact. Moreover, compared with WT M1 macrophages, M1 macro-
phages lacking AT1A receptors induced an even more profound 
increase in RTC expression of Col I and NGAL (Figure 2D). To 
explore whether AT1 receptor–deficient macrophages mediate this 
exaggerated tubular cell damage through enhanced IL-1 produc-
tion acting on the renal cell IL-1 receptor (IL-1R1), we repeated our 
coculture experiments with Il1r1-deficient (KO) RTCs (Figure 2D). 
In Il1r1 KO RTCs, coincubation with M1-conditioned macroph-
ages from WT or Macro KO animals induced severely blunted 
and equivalent increases in Col I and NGAL expression. Finally, 
recombinant IL-1β induced robust expression of Col I and NGAL 
in WT but not Il1r1 KO RTCs (Supplemental Figure 14). Thus, 
activation of the IL-1R on kidney cells by IL-1 secreted from acti-
vated macrophages triggers RTC damage. We therefore posited 
that AT1 receptor activation on infiltrating macrophages limits 
their IL-1 production, which in turn suppresses gene expression 
programs for injury in kidney parenchymal cells by preventing 
renal IL-1R stimulation.

The kidney crosstransplant UUO model. Testing this possibility in 
vivo requires a system that can separate the contribution of AT1 
receptor activation on macrophages from the downstream effects 
of renal IL-1R activation during UUO-mediated renal fibrosis. We 
therefore developed a kidney transplantation–ureteral obstruction 
(KT-UO) model (Supplemental Paragraph 5 and Supplemental 
Figure 15). To examine the infiltration of macrophages from the 
kidney recipient into the donor KT-UO kidney, we transplanted 
a nonfluorescent WT kidney into a LysM-Cre+ mT/mG recipient 
mouse in which green GFP signals present in the KT-UO kidney 
represented macrophages from the kidney recipient, and red fluo-
rescent protein (RFP) signals represented other BM-derived cells 
from the recipient not of the myeloid lineage. Seven days following 
KT-UO, robust GFP signals that mark infiltrating macrophages 
were evident in the KT-UO kidneys along with a far less promi-
nent infiltrating population of red-fluorescing nonmyeloid cells 
(Figure 3A). Thus, the vast majority of BM-derived cells infil-
trating the donor kidney in the KT-UO model are macrophages. 
In contrast, the native unobstructed kidney in the LysM-Cre+ 
mT/mG recipient contained only a rare GFP fluorescing macro-
phage (Figure 3A). Next, we transplanted a kidney from a LysM-Cre+ 
mT/mG animal into a LysM-Cre+ mT/mG recipient whose native 
unobstructed kidney fluoresces red due to the absence of Cre-
mediated RFP excision (Figure 3B). Here, GFP signals in the 
KT-UO kidney represented macrophages resident in the transpl-
planted kidney that could constitute an alternative source of 
inflammatory cytokines. However, only sparse GFP signals were 
detected in LysM-Cre+ mT/mG KT-UO kidney sections (Figure 3B). 
Thus, the overwhelming majority of macrophages present in the 
KT-UO kidney arrive from the circulation of the kidney recipient.

Activation of the AT1 receptor on infiltrating macrophages ameliorates 
kidney fibrosis by limiting renal IL-1R stimulation. After validating 
our model, we transplanted Il1r1 WT or Il1r1 KO kidneys into Macro 
WT or Macro KO animals (Supplemental Paragraph 6 and Supple-
mental Table 2) to quantify the contribution of macrophage-gen-
erated IL-1 from the recipient to IL-1R–mediated fibrosis in the 
donor kidney. Seven days after KT-UO, the transplanted kidney in 
WT mice exhibited a moderate level of fibrosis similar to that seen 
earlier in nontransplanted WT mice (Figure 3C and Figure 1B). 
The extent of fibrosis in the KT-UO kidney from Macro KO Il1r1 
WT mice (Figure 3C) exceeded that in the WT KT-UO kidney by 
approximately 50%, comparable to the discrepancy seen in the 
nontransplanted WT and Macro KO groups (Supplemental Figure 
5), confirming that AT1A receptor deficiency on macrophages 
in the recipient mice exacerbates the severity of kidney fibrosis in the 
KT-UO model. The abrogation of IL-1R signaling in the KT-UO 
kidney of the Macro WT Il1r1 KO mice reduced the level of kid-
ney fibrosis by a third compared with the WT group, implicating 
the renal IL-1R as a key mediator of kidney fibrosis in our model. 
IL-1R deletion in the KT-UO kidneys of Macro KO Il1r1 KO mice 
decreased the extent of kidney fibrosis by half compared with their 
Macro KO Il1r1 WT counterparts lacking AT1A receptors on macro-
phages, but expressing IL-1Rs in the KT-UO kidney, and down 
to levels seen in the Macro WT Il1r1 KOs (Figure 3C). Col I protein 
content in the KT-UO kidney (Supplemental Figure 16) mirrored 
the levels of fibrosis across the groups. Thus, enhanced activation 
of the kidney IL-1R accounts for the exaggerated renal fibrosis that 
accrues from AT1 receptor deficiency on macrophages. As seen 
earlier in the nontransplanted groups, renal macrophage infil-
tration in the Macro KO Il1r1 WT group was numerically but not sig-
ificantly higher than in the WT group (Supplemental Figure 17). 
Levels of renal macrophage accumulation in the 2 groups lack-
ing IL-1Rs on the KT-UO kidney were similar to each other, but 
significantly reduced compared with their counterparts with Il1r1 
WT KT-UO kidneys, highlighting a role for renal IL-1R signals to 
recruit macrophages into the kidney undergoing fibrosis. Finally, 
Col I and NGAL expression (Figure 3, D and E) in the KT-UO kid-
neys followed a pattern strikingly similar to that of fibrosis across 
the transplant groups and corroborated our coculture findings.

Summary. These experiments establish a mechanism through 
which AT1 receptors on macrophages function to protect the kid-
ney from progressive fibrosis. Activation of AT1 receptors on infil-
trating macrophages suppresses their release of the proinflamma-
tory cytokine IL-1 and thereby prevents stimulation of the kidney 
IL-1R. We also establish a new KT-UO model that, in conjunction 
with conditional gene targeting, can serve as a tool for elucidating 
interactions between hematopoietic and kidney parenchymal cells. 
The ability of AT1 receptor activation on macrophages to mitigate 
kidney damage has broad implications for the design of potent 
therapies to overcome the shortcomings of global angiotensin 
receptor blockade related not only to previously recognized side 
effects but also to potentially detrimental effects of blocking angio-
tensin receptors on hematopoietic cells.

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 ani-
mal 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 accor-
dance with the NIH Guide for the Care and Use of Laboratory Animals.