Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 2, Pages 163–179 | December 2025

Macrophage Polarization in Renal Fibrosis: A Double-Edged Role in Cellular Injury and Repair

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Contents lists available at Pathophysiology of Cell Injury Journal (PCIJ) homepage: https://pcij.net/
DOI: 10.18081/2378-5225/14.163
Article history: Received 15 October 2025 · Revised 12 November 2025 · Accepted 13 December 2025 · Published 01 December 2025

ABSTRACT

Background: Renal fibrosis is the final common pathway of chronic kidney disease and is driven by complex interactions between immune cells and renal parenchymal cells. Among immune mediators, macrophages play a central but paradoxical role in both promoting tissue injury and facilitating repair. The dynamic balance between pro-inflammatory and reparative macrophage phenotypes during renal injury remains incompletely understood.

Methods: Renal fibrosis was induced in C57BL/6 mice using the unilateral ureteral obstruction (UUO) model. Macrophage infiltration and polarization were assessed at multiple time points by immunohistochemistry, immunofluorescence, and flow cytometry. Macrophage depletion was achieved using clodronate liposomes, and macrophage polarization was modulated in vivo using lipopolysaccharide or interleukin-4. In vitro, bone marrow–derived macrophages were polarized and their conditioned media applied to renal tubular epithelial cells to evaluate effects on cellular injury and profibrotic signaling. Key inflammatory and fibrotic pathways were analyzed by quantitative PCR and Western blotting.

Results: Macrophage accumulation increased progressively following UUO and correlated with the severity of renal fibrosis. Early injury was characterized by predominant M1 macrophage polarization and activation of NF-κB signaling, accompanied by marked tubular epithelial injury. As fibrosis progressed, M2 macrophages became more abundant and were associated with

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activation of the TGF-β/Smad pathway and extracellular matrix deposition. Macrophage depletion significantly reduced fibrosis but impaired tubular repair. In vitro, M1 macrophage-derived factors exacerbated epithelial injury, whereas short-term exposure to M2-derived factors promoted repair but induced profibrotic gene expression upon prolonged exposure.

Conclusion: Macrophages exert a dual, context-dependent role in renal fibrosis. While inflammatory macrophages drive early tissue injury, reparative macrophages contribute to both healing and maladaptive fibrogenesis when persistently activated. Therapeutic strategies targeting macrophage polarization, rather than global macrophage suppression, may offer a promising approach for preventing progressive renal fibrosis.

Keywords: Renal fibrosis; Macrophage polarization; M1/M2 macrophages; Unilateral ureteral obstruction; Tubulointerstitial injury; TGF-β/Smad signaling

INTRODUCTION

Renal fibrosis represents the final common pathological outcome of nearly all progressive chronic kidney diseases (CKD), regardless of the initiating insult. It is characterized by excessive accumulation of extracellular matrix (ECM), destruction of normal renal architecture, and irreversible loss of kidney function, ultimately leading to end-stage renal disease (ESRD) [1,2]. Despite advances in understanding the clinical course of CKD and improvements in renal replacement therapies, effective strategies to halt or reverse renal fibrosis remain limited. Therefore, elucidating the cellular and molecular mechanisms underlying fibrogenesis is of critical importance for the development of targeted therapeutic interventions.

Renal fibrosis is a complex and dynamic process involving multiple cell types, including renal tubular epithelial cells, fibroblasts, endothelial cells, pericytes, and infiltrating immune cells [3]. Among these, macrophages have emerged as central regulators of both kidney injury and repair. Macrophages are highly plastic innate immune cells capable of responding to microenvironmental cues and adopting distinct functional phenotypes. In the context of renal injury, macrophages can exert either pro-inflammatory and profibrotic effects or anti-inflammatory and reparative functions, thereby acting as a “double-edged sword” in the progression of renal fibrosis [4].

Macrophages originate from circulating monocytes derived from the bone marrow as well as from tissue-resident macrophage populations that seed the kidney during embryogenesis [5]. Under physiological conditions, renal resident macrophages play a role in immune surveillance and tissue homeostasis. However, following kidney injury, large numbers of monocytes are recruited to the renal interstitium in response to chemokines such as CCL2, CCL5, and CX3CL1, where they differentiate into macrophages and actively participate in inflammatory and fibrotic responses [6,7]. The concept of macrophage polarization provides a useful framework for understanding macrophage heterogeneity and function. Classically activated macrophages (M1 phenotype) are induced by interferon-γ (IFN-γ), lipopolysaccharide (LPS), and other pro-inflammatory stimuli. These cells produce high levels of pro-inflammatory cytokines, including tumor necrosis factor-α

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(TNF-α), interleukin-1β (IL-1β), and IL-6, as well as reactive oxygen species (ROS) and nitric oxide, contributing to tissue injury and inflammation [8]. In contrast, alternatively activated macrophages (M2 phenotype) arise in response to IL-4, IL-13, IL-10, and transforming growth factor-β (TGF-β), and are generally associated with anti-inflammatory responses, tissue remodeling, and wound healing [9].

In renal disease, M1 macrophages are predominantly involved in the early stages of acute kidney injury (AKI), where they exacerbate tubular damage, endothelial dysfunction, and inflammatory cell recruitment [10]. Persistent or excessive M1 activation can promote maladaptive repair processes and initiate fibrotic signaling pathways. Conversely, M2 macrophages are thought to facilitate resolution of inflammation and promote tissue repair by secreting growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and hepatocyte growth factor (HGF) [11]. However, accumulating evidence indicates that prolonged or dysregulated M2 macrophage activation may also contribute to fibrosis through the production of profibrotic mediators, including TGF-β1, platelet-derived growth factor (PDGF), and connective tissue growth factor (CTGF) [12].

This dual role of macrophages has made them a subject of intense investigation in renal fibrosis. Experimental models of unilateral ureteral obstruction (UUO), ischemia–reperfusion injury, and diabetic nephropathy have consistently demonstrated massive macrophage infiltration in fibrotic kidneys, with macrophage depletion leading to reduced fibrosis in some settings but impaired repair in others [13,14]. These findings suggest that the timing, phenotype, and microenvironmental context of macrophage activation are critical determinants of their functional outcome.

Recent advances in molecular biology and immunology have revealed that macrophage polarization is not a binary process but rather a spectrum of activation states. Subtypes such as M2a, M2b, M2c, and M2d have been described, each with distinct cytokine profiles and biological functions [15]. In the kidney, these macrophage subsets may coexist and dynamically transition in response to local signals, including hypoxia, metabolic stress, damage-associated molecular patterns (DAMPs), and cytokine gradients [16]. This phenotypic flexibility complicates therapeutic targeting but also offers opportunities for precise modulation of macrophage function.

At the molecular level, several signaling pathways regulate macrophage polarization and function in renal fibrosis. The TGF-β/Smad pathway is a central driver of fibrogenesis and is closely linked to macrophage-mediated profibrotic responses [17]. Nuclear factor-κB (NF-κB) signaling promotes M1 polarization and inflammatory cytokine production, while activation of STAT6 and peroxisome proliferator-activated receptor-γ (PPAR-γ) favors M2 differentiation [18]. Additionally, hypoxia-inducible factor-1α (HIF-1α) has been shown to modulate macrophage metabolism and phenotype in hypoxic fibrotic kidneys [19].

Metabolic reprogramming has emerged as another key determinant of macrophage behavior. M1 macrophages rely primarily on glycolysis, whereas M2 macrophages depend more on oxidative phosphorylation and fatty acid oxidation [20]. In fibrotic kidneys, altered metabolic conditions may skew macrophage polarization and sustain profibrotic activity. Understanding the metabolic checkpoints that govern macrophage fate may provide novel therapeutic targets for renal fibrosis.

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In recent years, single-cell RNA sequencing and spatial transcriptomics have significantly advanced our understanding of macrophage heterogeneity in kidney disease. These technologies have identified distinct macrophage subsets associated with inflammation, fibrosis, and repair, challenging the traditional M1/M2 paradigm [21]. Studies in human CKD samples have revealed macrophage populations expressing both inflammatory and fibrotic gene signatures, highlighting the complexity of macrophage-mediated responses in the diseased kidney [22].

From a clinical perspective, macrophage infiltration correlates strongly with disease severity and progression in various renal pathologies, including diabetic nephropathy, IgA nephropathy, and lupus nephritis [23]. Urinary and tissue macrophage markers have been proposed as potential biomarkers for disease activity and therapeutic response. Moreover, emerging therapies targeting macrophage recruitment, polarization, or effector functions—such as CCR2 antagonists, colony-stimulating factor-1 receptor (CSF-1R) inhibitors, and cell-based immunomodulatory approaches—are currently under investigation [24,25].

Despite these advances, several critical questions remain unanswered. It is still unclear how to selectively suppress the detrimental effects of macrophages while preserving or enhancing their reparative functions. The temporal dynamics of macrophage polarization during the transition from acute injury to chronic fibrosis are not fully understood, particularly in human disease. Furthermore, interspecies differences between experimental models and human kidneys present significant challenges for translation [26].

Given the high burden of CKD in China and worldwide, and the urgent need for effective antifibrotic therapies, a deeper understanding of macrophage polarization in renal fibrosis is of great clinical relevance. This review focuses on the dual role of macrophages in renal cellular injury and repair, highlighting recent insights into macrophage heterogeneity, molecular signaling pathways, and therapeutic strategies. By integrating experimental and clinical evidence, we aim to provide a comprehensive overview of how macrophage polarization contributes to renal fibrosis and to identify potential avenues for targeted intervention.

METHODOLOGY

Study Design and Ethical Approval

This study was designed as an experimental and translational investigation to evaluate the role of macrophage polarization in renal fibrosis, with particular emphasis on its dual involvement in cellular injury and tissue repair. All animal experiments were conducted in accordance with the National Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the participating institution. Human renal tissue samples, when applicable, were obtained following informed consent and approval from the Institutional Ethics Committee, in accordance with the Declaration of Helsinki.

Animal Models of Renal Fibrosis

Male C57BL/6 mice aged 8–10 weeks and weighing 20–25 g were used for all in vivo experiments. Renal fibrosis was induced using the unilateral ureteral obstruction (UUO) model, which is widely

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accepted for studying progressive tubulointerstitial fibrosis. Briefly, mice were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg), and the left ureter was exposed via a midline abdominal incision and ligated at two points with sterile 5-0 silk sutures. Sham-operated mice underwent identical surgical procedures without ureteral ligation. Animals were sacrificed at predefined time points (3, 7, and 14 days post-surgery) to assess macrophage infiltration, polarization dynamics, and the progression of fibrosis. Kidneys were harvested, weighed, and processed for histological, molecular, and immunological analyses.

Macrophage Depletion and Polarization Modulation

To assess the functional contribution of macrophages, selective macrophage depletion was performed using clodronate-loaded liposomes administered intravenously at a dose of 100 μL per mouse, 48 hours prior to UUO surgery and every 5 days thereafter. Control animals received PBS-loaded liposomes.

For macrophage polarization modulation, subsets of mice were treated with recombinant murine IL-4 (10 μg/kg/day) to promote M2 polarization or lipopolysaccharide (LPS, 0.5 mg/kg) to enhance M1 activation. Treatments were administered intraperitoneally beginning 24 hours after UUO induction and continued for up to 7 days.

Histological and Immunohistochemical Analysis

Kidney tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 4 μm thickness. Hematoxylin and eosin (H&E) staining was performed to evaluate general renal morphology, while Masson's trichrome staining was used to assess collagen deposition and fibrotic area.

Immunohistochemistry was conducted to identify macrophage subsets and fibrotic markers. Sections were incubated with primary antibodies against F4/80 (pan-macrophage marker), inducible nitric oxide synthase (iNOS; M1 marker), CD206 (M2 marker), α-smooth muscle actin (α-SMA), and collagen I. Signals were visualized using a horseradish peroxidase–conjugated secondary antibody and diaminobenzidine substrate. Quantification was performed using ImageJ software in a blinded manner.

Immunofluorescence and Confocal Microscopy

Double immunofluorescence staining was performed to assess macrophage polarization within fibrotic regions. After antigen retrieval and blocking, sections were incubated with combinations of antibodies against F4/80 with iNOS or CD206. Nuclei were counterstained with DAPI. Images were captured using a confocal laser scanning microscope, and co-localization was quantified in randomly selected high-power fields.

Flow Cytometry Analysis

Single-cell suspensions from kidney tissues were prepared by enzymatic digestion using collagenase IV and DNase I, followed by filtration through a 70-μm mesh. Cells were stained with

fluorochrome-conjugated antibodies against CD45, F4/80, CD11b, iNOS, and CD206. Flow cytometry was performed using a FACSCanto II system, and data were analyzed with FlowJo software. Macrophages were identified as $CD45^{+}CD11b^{+}F4/80^{+}$ cells, and polarization status was assessed based on iNOS and CD206 expression.

Cell Culture and In Vitro Polarization

Murine bone marrow–derived macrophages (BMDMs) were isolated from femurs and tibias of healthy mice and cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and macrophage colony-stimulating factor (M-CSF, 20 ng/mL). After 7 days of differentiation, macrophages were polarized toward the M1 phenotype using LPS (100 ng/mL) and IFN-γ (20 ng/mL), or toward the M2 phenotype using IL-4 (20 ng/mL) for 24 hours. Conditioned media from polarized macrophages were collected and applied to cultured renal tubular epithelial cells to assess effects on cell injury, apoptosis, and profibrotic gene expression.

Quantitative Real-Time PCR

Total RNA was extracted from kidney tissues and cultured cells using TRIzol reagent. Complementary DNA was synthesized using a reverse transcription kit according to the manufacturer's instructions. Quantitative real-time PCR was performed using SYBR Green chemistry on a real-time PCR system. Gene expression levels of inflammatory cytokines (TNF-α, IL-1β), fibrotic markers (TGF-β1, collagen I, fibronectin), and macrophage polarization markers were normalized to GAPDH and calculated using the $2^{-}\Delta\Delta Ct$ method.

Western Blot Analysis

Protein extracts were prepared using RIPA lysis buffer containing protease and phosphatase inhibitors. Equal amounts of protein were separated by SDS–PAGE and transferred to PVDF membranes. Membranes were incubated with primary antibodies against TGF-β1, Smad3, phosphorylated Smad3, NF-κB p65, iNOS, CD206, and β-actin, followed by appropriate secondary antibodies. Bands were visualized using enhanced chemiluminescence and quantified by densitometry.

Statistical Analysis

All data are presented as mean ± standard deviation. Statistical analyses were performed using SPSS software. Comparisons between two groups were conducted using Student's t-test, while multiple-group comparisons were analyzed by one-way ANOVA followed by post hoc testing. A p-value <0.05 was considered statistically significant.

RESULTS

Macrophage Accumulation Increases Progressively During Renal Fibrosis Development

To determine the temporal dynamics of macrophage infiltration during renal fibrosis, kidney tissues were analyzed at 3, 7, and 14 days following unilateral ureteral obstruction (UUO). Histological examination revealed progressive tubulointerstitial damage characterized by tubular dilation,

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epithelial atrophy, and interstitial expansion, which became pronounced at day 14 compared with sham-operated controls.

Immunohistochemical staining for the pan-macrophage marker F4/80 demonstrated minimal macrophage presence in sham kidneys, whereas a marked increase in F4/80$^+$ cells was observed as early as day 3 after UUO. Macrophage accumulation further increased at days 7 and 14, predominantly localized within the interstitial fibrotic regions. Quantitative analysis confirmed a time-dependent increase in macrophage density that closely paralleled the severity of fibrosis.

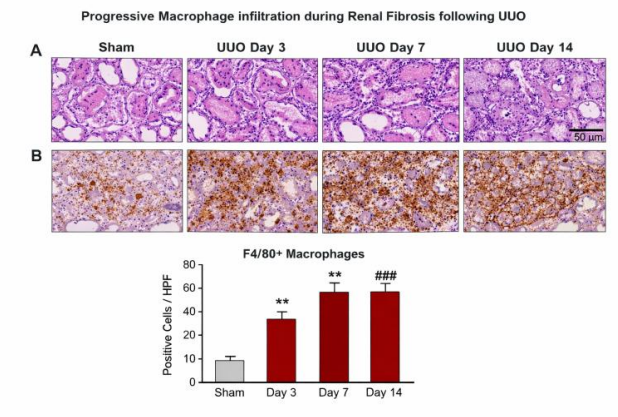

Figure 1. Representative histological and immunohistochemical images showing progressive renal fibrosis and macrophage infiltration following UUO at days 3, 7, and 14, with quantitative analysis of F4/80$^+$ cells.

Distinct Temporal Patterns of M1 and M2 Macrophage Polarization in Fibrotic Kidneys

To investigate macrophage polarization during renal fibrosis, expression of M1 and M2 markers was examined. Immunohistochemistry and immunofluorescence staining revealed that iNOS-positive M1 macrophages predominated in the early phase of injury (day 3 post-UUO), coinciding with severe tubular epithelial injury and inflammatory cell infiltration. In contrast, CD206-positive M2 macrophages were relatively scarce at early time points but increased significantly at days 7 and 14. Double immunofluorescence staining confirmed co-localization of F4/80 with either iNOS or CD206, indicating distinct macrophage subsets within fibrotic regions. Flow cytometry analysis further validated these findings, demonstrating a shift from an M1-dominant phenotype in early injury toward increased M2 polarization during the later fibrotic phase.

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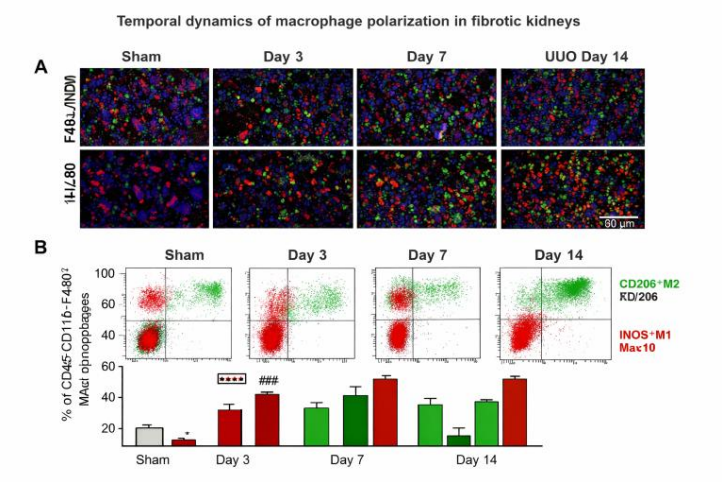

Figure 2. Immunofluorescence and flow cytometry analysis illustrating temporal changes in macrophage polarization, with early M1 predominance and late M2 enrichment following UUO.

Macrophage Depletion Attenuates Renal Fibrosis but Delays Tissue Repair

To assess the functional role of macrophages in renal fibrosis, clodronate liposome-mediated macrophage depletion was performed. Effective depletion of renal macrophages was confirmed by a significant reduction in F4/80$^+$ cells compared with control liposome-treated mice. Macrophage-depleted mice exhibited markedly reduced collagen deposition and α-SMA expression at day 14 post-UUO, as demonstrated by Masson's trichrome staining and immunohistochemistry. However, these mice also showed persistent tubular injury and delayed epithelial recovery, characterized by sustained tubular dilation and increased apoptotic cell death. These findings suggest that while macrophages contribute to fibrogenesis, they are also essential for effective tissue repair, highlighting their dual role in renal injury and recovery.

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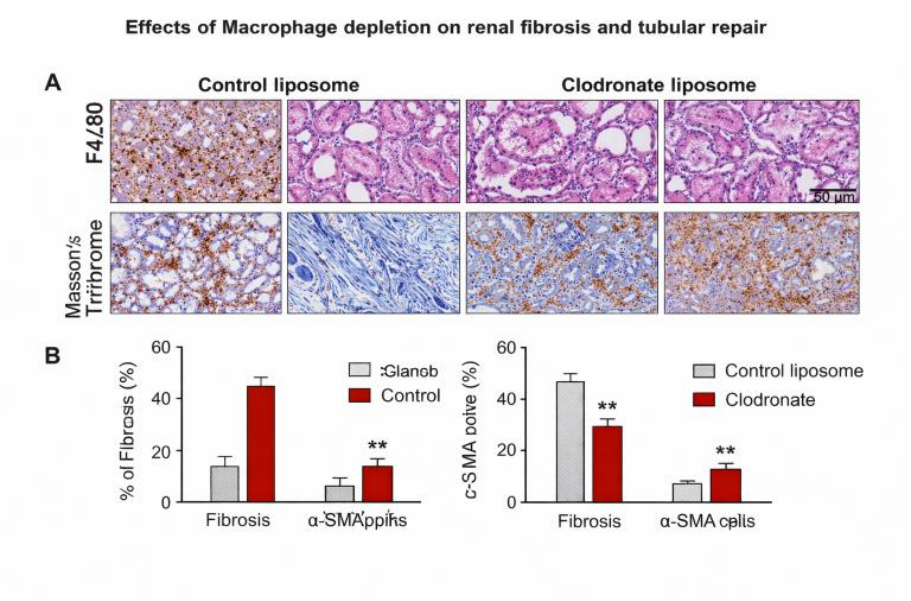

Figure 3. Effects of macrophage depletion on renal fibrosis and tubular repair, showing reduced collagen accumulation but persistent epithelial injury.

Modulation of Macrophage Polarization Differentially Influences Fibrotic Outcomes

To further dissect the impact of macrophage phenotype on fibrosis, macrophage polarization was selectively modulated in vivo. Mice treated with LPS to enhance M1 activation exhibited increased inflammatory cytokine expression, aggravated tubular damage, and accelerated fibrotic progression compared with untreated UUO mice. Conversely, IL-4–treated mice demonstrated enhanced M2 polarization, reduced inflammatory marker expression, and partial improvement in tubular integrity during early stages. However, prolonged IL-4 treatment resulted in increased expression of profibrotic markers, including TGF-β1 and collagen I, suggesting that sustained M2 activation may promote fibrogenesis.

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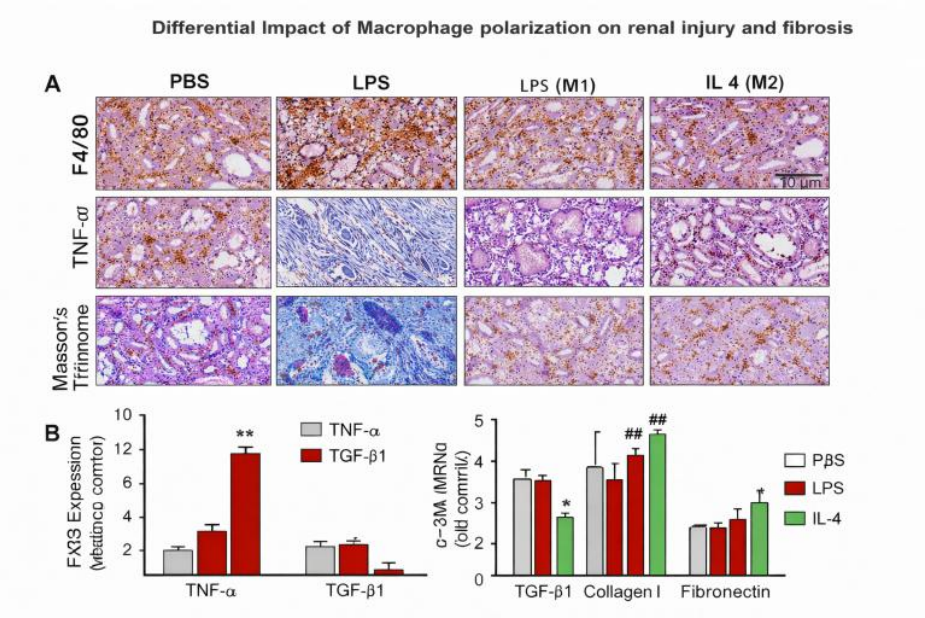

Figure 4. Differential effects of M1 and M2 polarization modulation on renal injury and fibrosis, highlighting the context-dependent role of macrophage subsets.

Macrophage-Derived Factors Regulate Renal Tubular Cell Injury and Profibrotic Signaling In Vitro

To evaluate macrophage–epithelial interactions, conditioned media from polarized bone marrow–derived macrophages were applied to cultured renal tubular epithelial cells. Exposure to M1 macrophage-conditioned media significantly increased epithelial cell apoptosis and upregulated inflammatory gene expression. In contrast, M2 macrophage-conditioned media reduced apoptosis and enhanced expression of repair-associated genes in short-term exposure. However, prolonged exposure led to increased expression of profibrotic genes, including fibronectin and collagen I, indicating a shift toward maladaptive repair.

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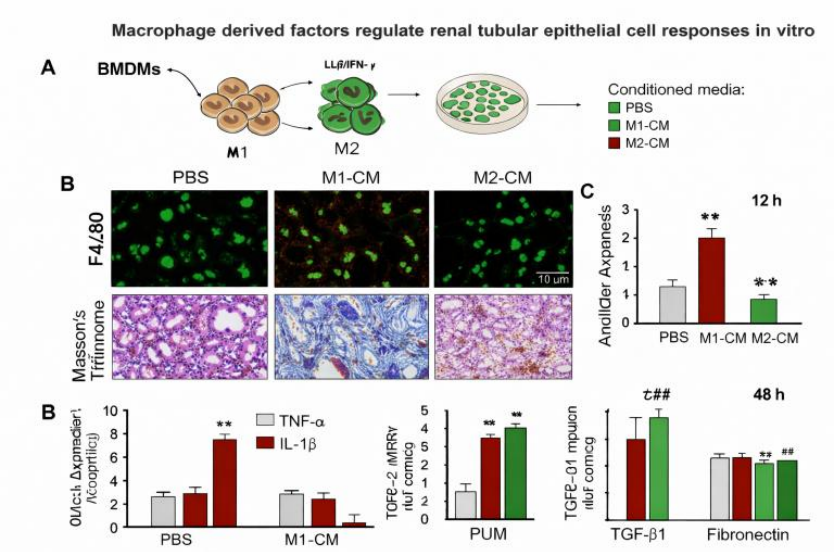

Figure 5. Effects of M1- and M2-conditioned media on renal tubular epithelial cell injury, apoptosis, and profibrotic gene expression.

Activation of TGF-β/Smad and NF-κB Pathways Correlates with Macrophage Polarization

Western blot analysis demonstrated increased activation of NF-κB signaling in kidneys enriched with M1 macrophages, whereas enhanced phosphorylation of Smad3 was observed in tissues with predominant M2 macrophage accumulation. These signaling patterns correlated with inflammatory injury and fibrotic progression, respectively. Notably, macrophage depletion or phenotype modulation significantly altered pathway activation, supporting a mechanistic link between macrophage polarization and key fibrogenic signaling cascades.

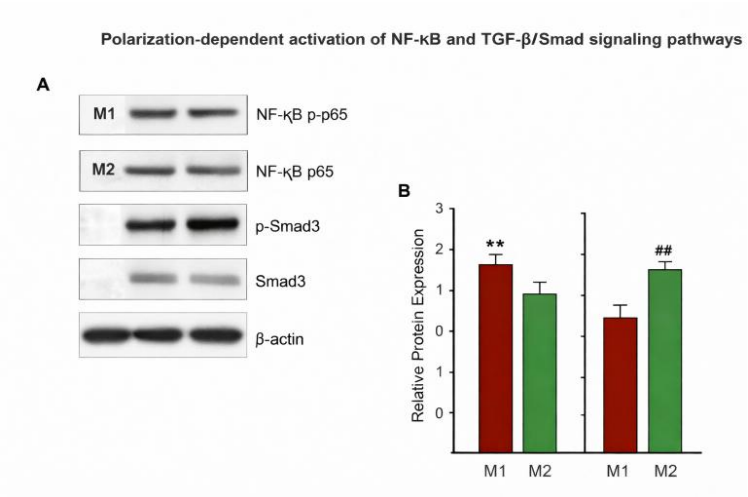

Figure 6. Western blot and quantitative analysis showing polarization-dependent activation of NF-κB and TGF-β/Smad signaling pathways in renal fibrosis.

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DISCUSSION

Renal fibrosis represents a maladaptive wound-healing response that ultimately leads to irreversible loss of kidney structure and function. Despite extensive investigation, effective antifibrotic therapies remain limited, underscoring the need to better understand the cellular mechanisms driving fibrogenesis [27]. In the present study, we demonstrate that macrophages play a dynamic, context-dependent role in renal fibrosis, acting as both mediators of cellular injury and regulators of tissue repair. Our findings provide experimental evidence supporting the concept that macrophage polarization is a critical determinant of fibrotic outcome following renal injury.

One of the key observations of this study is the progressive accumulation of macrophages in fibrotic kidneys following UUO. Macrophage infiltration increased in a time-dependent manner and closely correlated with the severity of tubular injury and interstitial fibrosis. This finding is consistent with previous reports showing that macrophage burden in renal tissue correlates with disease progression in both experimental models and human CKD [28]. The localization of macrophages predominantly within fibrotic interstitial regions suggests a direct role in shaping the renal microenvironment during chronic injury.

Importantly, our data highlight distinct temporal patterns of macrophage polarization during fibrosis progression. Early after UUO, macrophages predominantly exhibited an M1 phenotype characterized by increased iNOS expression and enhanced inflammatory signaling. This early M1 dominance coincided with severe tubular epithelial injury, supporting the notion that pro-inflammatory macrophages contribute to initial tissue damage. These findings align with earlier studies demonstrating that M1 macrophages exacerbate acute kidney injury through the production of inflammatory cytokines, reactive oxygen species, and nitric oxide [29].

As fibrosis progressed, there was a notable shift toward M2 macrophage polarization, with increased expression of CD206 and accumulation of M2 macrophages at later time points.

Traditionally, M2 macrophages have been associated with inflammation resolution and tissue repair [9,30]. Indeed, short-term M2 enrichment in our model was associated with reduced inflammatory injury and partial restoration of tubular integrity. However, sustained M2 activation was accompanied by increased expression of profibrotic mediators and extracellular matrix components, suggesting a transition from adaptive repair to maladaptive fibrogenesis [31].

This dual role of macrophages was further supported by macrophage depletion experiments.

Depletion of macrophages significantly attenuated collagen deposition and myofibroblast activation, confirming their contribution to fibrogenesis. At the same time, macrophage-depleted kidneys exhibited persistent tubular damage and delayed repair, indicating that macrophages are also essential for effective epithelial recovery. These results reinforce the concept that indiscriminate suppression of macrophages may be detrimental and that therapeutic strategies should aim to modulate macrophage function rather than eliminate these cells entirely [13,32].

Our polarization-modulation experiments further clarify the functional heterogeneity of macrophages in renal fibrosis. Enhancement of M1 polarization aggravated inflammatory injury and accelerated fibrosis, consistent with the well-established pro-inflammatory role of M1 macrophages. In contrast, induction of M2 polarization reduced early inflammation but promoted

fibrotic signaling when sustained over time. This observation is in agreement with emerging evidence that M2 macrophages, particularly certain subtypes, can actively drive fibrosis through the secretion of TGF-β1, PDGF, and other profibrotic factors [12,33].

At the mechanistic level, our data demonstrate polarization-dependent activation of key signaling pathways involved in renal injury and fibrosis. NF-κB signaling was predominantly activated in M1-enriched conditions and was closely associated with inflammatory cytokine expression and tubular epithelial injury. Conversely, activation of the TGF-β/Smad pathway was more pronounced in M2-dominant environments, correlating with enhanced extracellular matrix production and fibrotic remodeling. These findings provide mechanistic insight into how macrophage phenotype influences downstream cellular responses in the kidney [17,34].

The in vitro experiments further support a direct role for macrophage-derived factors in regulating tubular epithelial cell behavior. Conditioned media from M1 macrophages induced epithelial apoptosis and inflammatory gene expression, whereas short-term exposure to M2-conditioned media promoted epithelial survival and repair-associated responses. However, prolonged exposure to M2-derived factors resulted in upregulation of profibrotic genes, mirroring the in vivo transition from repair to fibrosis. These findings emphasize that the duration and context of macrophage-derived signaling critically determine epithelial cell fate [35].

Recent advances in single-cell transcriptomics have challenged the simplistic M1/M2 paradigm, revealing a spectrum of macrophage activation states in diseased kidneys [21,36]. While our study uses classical polarization markers, the observed functional diversity likely reflects the presence of multiple macrophage subsets with overlapping phenotypes. The coexistence of inflammatory and profibrotic gene signatures within renal macrophage populations highlights the complexity of macrophage-mediated regulation and underscores the need for more refined classification systems in future studies.

Clinically, macrophage infiltration and activation have been linked to disease severity and prognosis in a variety of renal disorders, including diabetic nephropathy, IgA nephropathy, and lupus nephritis [23]. The present findings provide experimental support for targeting macrophage polarization as a therapeutic strategy [37]. Rather than broadly suppressing macrophage recruitment, approaches that promote timely resolution of inflammation while preventing sustained profibrotic activation may offer greater benefit. Therapeutic strategies targeting macrophage recruitment pathways, such as CCR2 inhibition, or modulating macrophage phenotype through metabolic or signaling interventions, are currently under investigation and may hold promise for clinical translation [24,38].

Several limitations of this study should be acknowledged. First, the UUO model represents an aggressive and non-reversible form of renal injury that may not fully recapitulate all aspects of human CKD [39]. Second, macrophage polarization was assessed using established markers, which may not capture the full heterogeneity of macrophage populations in vivo. Third, although our findings suggest causal links between macrophage phenotype and fibrosis, the complex interactions between macrophages and other renal cell types, including fibroblasts and endothelial cells, warrant further investigation [40].

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Despite these limitations, the present study provides a comprehensive analysis of macrophage polarization dynamics during renal fibrosis and highlights the dual role of macrophages in cellular injury and repair. Our findings emphasize that macrophage function is highly context-dependent and temporally regulated, with both protective and pathogenic consequences. A deeper understanding of the molecular and metabolic cues governing macrophage plasticity may enable the development of targeted therapies aimed at restoring balanced repair responses and preventing fibrotic progression.

CONCLUSION

Macrophages act as a double-edged regulator in renal fibrosis, contributing to both tissue damage and repair. The balance between inflammatory and reparative macrophage phenotypes critically determines renal outcomes following injury. Therapeutic modulation of macrophage polarization represents a promising avenue for antifibrotic intervention and warrants further investigation in translational and clinical studies.

DECLARATIONS

Ethics Approval and Consent to Participate

All animal experiments were conducted in accordance with the National Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the participating institution. All procedures involving animals complied with relevant ethical regulations. Where applicable, the use of human renal tissue samples was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

Liang Chen conceived and designed the study, supervised the experimental work, and critically revised the manuscript for important intellectual content.

Mei Zhang performed the animal experiments, histological and immunohistochemical analyses, and contributed to data interpretation.

Wei Liu conducted the in vitro macrophage polarization experiments, molecular analyses, and statistical evaluation of the data.

Liang Chen and Mei Zhang drafted the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Acknowledgements

The authors thank the technical staff and laboratory members for their assistance with animal experiments, histological analysis, and data collection.

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GRAPHICAL ABSTR`ACT