Macrophage heterogeneity, phenotypes, and roles in renal fibrosis

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Macrophages (MΦ) are highly heterogeneous cells that exhibit distinct phenotypic and functional characteristics depending on their microenvironment and the disease type and stage. MΦ are distributed throughout normal and diseased kidney tissue, where they have been recognized as key factors in renal fibrosis. Recent studies have identified switch of phenotype and diverse roles for MΦ in several murine models of kidney disease. In this review, we discuss macrophage heterogeneity and their involvement in renal fibrosis.

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MACROPHAGE HETEROGENEITY AND PHENOTYPES

Macrophages (MΦ) comprise a heterogeneous population of cells, with diverse functions and phenotypic plasticity. MΦ belong to the family of mononuclear phagocytes and are known to have a central role in promoting progression or resolution of renal inflammation and fibrosis.[1] However, lack of specific markers to differentiate dendritic cells from MΦ has generated confusion regarding their exact function in kidney diseases.[2] Moreover, MΦ are highly heterogeneous cells whose subsets exhibit varying activities in different kidney diseases. The existing simplistic definitions of MΦ, based largely on *in vitro* observations, are not sufficient to allow conclusions about the role of sub-populations of MΦ. In light of the importance of accurate characterization of MΦ subsets, recently we have re-examined their classification and identified four subsets of renal mononuclear phagocytes of which two subsets displayed MΦ-like properties and accounted for the great majority (>83.5%) of murine renal mononuclear phagocyte (unpublished data). Of these two subsets, one expressed the typical MΦ marker F4/80 without CD11c, and the other also expressed CD11c, a classical marker for dendritic cells. In healthy and diseased kidney, both subsets displayed typical MΦ-like properties including morphology, *in vitro* functions, expression of specific surface markers and transcription factors, and ontogeny. However, the role of these two subsets in renal fibrosis is unknown.

Although MΦ were recognized commonly for their pathogenic role in renal inflammation and fibrosis, MΦ also have critical roles in wound healing, in tissue remodeling and repair, and in immune regulation. MΦ *in vitro* have been classified into classically activated macrophages (M1) and alternatively activated macrophages (M2), which have been subdivided further into M2a, M2b, and M2c according to their response to different modulators.[3,4] However, this classification does not reflect adequately their true phenotypes in *in vivo* tissue environments. Recently, Anders and Ryu[5] have proposed four types of *in vivo* MΦ, defined according to their predominant roles in phases of wound healing, namely pro-inflammatory, anti-inflammatory, profibrotic and fibrolytic MΦ. MΦ of M1- and M2-like (i.e., pro-inflammatory and anti-inflammatory) phenotypes have been demonstrated in acute ischemia-reperfusion injury and unilateral ureteral obstruction (UUO) models.[6–9]

Phenotypic switch of MΦ from M1 to M2 has been shown accompanying a change in the microenvironment.[7,10] Lee et al.[7] found that kidney MΦ expressed pro-inflammatory markers during the initial phase of ischemia-reperfusion injury, whereas MΦ displayed an alternatively activated phenotype during the repair phase. When M1 MΦ were adoptively transferred early after injury, they switched to an M2 phenotype within the kidney during the later recovery phase. Colony-stimulating factor-1 has been reported to induce resident MΦ expansion and direct them toward an M2 phenotype, which mediated renal tubule epithelial regeneration after acute kidney injury.[10] Moreover, C-C chemokine receptor 5 and Kruppel-like factor 4 have been identified as key regulators controlling M1 vs. M2 MΦ phenotypes, respectively, in kidney transplantation and wound healing.[11,12]

In our previous studies, adoptive transfer of M1 MΦ, but not resting MΦ, increased renal injury and fibrosis in murine adriamycin nephropathy (AN), highlighting the importance of MΦ activation status in causing renal injury.[13] In contrast, M2a MΦ protected against renal structural and functional injury in immunodeficient (severe combined immunodeficiency) mice with AN.[14] Recently, we compared the effectiveness of different subsets of M2 MΦ in protecting against renal injury in AN mice (Table 1).[15,16] Both transfused M2a and M2c MΦ significantly reduced glomerulosclerosis, tubular atrophy, interstitial expansion, and renal fibrosis in AN mice. M2a and M2c MΦ localized preferentially to the area of injury and kidney-draining lymph nodes, and their protective effect was associated with deactivation of endogenous renal MΦ and inhibition of CD4 T-cell proliferation. It appeared that M2c were more effective than M2a in reducing renal histological and functional injury with less proteinuria, tubular atrophy, intestinal volume expansion, and CD4 T-cell infiltration.[15,16] The greater potency of M2c than M2a could relate to the high-level expression of the regulatory co-stimulatory molecule B7-H4 on M2c that mediates Treg production.[15]

M2a MΦ have also been investigated in murine streptozotocin-induced diabetes.[17] Transfused M2a MΦ accumulated progressively in kidneys for at least 10 weeks after streptozotocin and significantly reduced renal interstitial fibrosis and islet injury. Similarly, M2a MΦ transfusion of diabetic endothelial nitric oxide synthase knockout (eNOS$^{-/-}$) mice resulted in less renal fibrosis and glomerulosclerosis than in untransfused diabetic eNOS$^{-/-}$ mice (unpublished data). M2 MΦ also can be induced *in vivo*. Our group found that interleukin (IL)-25, by increasing Th2 cell IL-4 and IL-13 production, induced M2 MΦ and attenuated kidney injury in AN mice, providing a possible strategy to induce M2 MΦ *in vivo* to limit renal inflammation.[18]

A large proportion of renal MΦ during inflammation and fibrosis originate from bone marrow (BM). We found that BM–derived MΦ have greater proliferative ability and less phenotypic stability *in vitro* than splenic (SP) and peritoneal MΦ.[19] Unlike SP-M2a, BM-M2a did not protect against renal structural or functional injury in murine AN. The failed

Table 1 | Protective effect of M2a and M2c in AN mice[14–16]

	M2a	M2c
Cytokine expression	IL-10, TGF-β	IL-10, TGF-β
Surface molecules	MR, arginase, FIZZ-1	MR, arginase, B7-H4
Inhibit T-cell proliferation	+	+
Inhibit MΦ activation	+	+
Induce Tregs	−	+
Reduce renal injury	+	+ +
Reduce renal fibrosis	+	+ +

Abbreviations: AN, adriamycin nephropathy; IL, interleukin; MΦ, macrophage; TGF-β, transforming growth factor-beta; Tregs, regulatory T cells.

renoprotection of BM-M2a was linked to their proliferation within inflamed kidney. BM-M2a MΦ, but not SP-M2a, proliferated strongly in kidney, and divided cells did not express the regulatory phenotype of M2. The likely explanation for the increased proliferation of BM-M2a, but not SP-M2a MΦ, was their increased expression of macrophage-colony–stimulating factor receptor in comparison with SP-M2a MΦ. Blockade of macrophage-colony–stimulating factor by a c-fms inhibitor not only limited BM-M2a MΦ proliferation, but also prevented phenotype shift. These data suggest that proliferation-dependent shift of phenotype could be limited by targeting macrophage-colony–stimulating factor.[20]

MΦ display pro-inflammatory and anti-inflammatory phenotypes *in vitro* and *in vitro*. Our studies have demonstrated that they can be used as potential therapeutic tools to regulate inflammation and promote tissue repair in chronic kidney diseases. The antifibrotic effect of transfused M2 MΦ observed in AN mice could be explained by their production of anti-inflammatory cytokines and reduction of local inflammation, resulting in less renal injury and consequently less fibrosis.

ROLES OF MACROPHAGES IN RENAL FIBROSIS

Traditionally, MΦ have been recognized as key factors that may promote renal fibrosis. However, several recent studies have suggested an antifibrotic role of infiltrating MΦ in obstructive nephropathy. Triggers of renal cell damage recruit circulating monocytes into interstitial compartments where they differentiate into M1 or M2 MΦ phenotypes depending on the local tissue environment. Interferon-related factor 4 and 5 have been found to be involved in macrophage activation.[21,22] Pro-inflammatory M1 MΦ release pro-inflammatory mediators including tumor necrosis factor-α and reactive oxygen species, which cause tissue inflammation and subsequent renal fibrosis. In contrast, anti-inflammatory M2 MΦ release anti-inflammatory mediators including IL-10 and transforming growth factor-beta; the latter suppresses renal inflammation yet promotes renal fibrosis.[4,5,23,24]

Systemic MΦ depletion 1 day before UUO resulted in reduced initial interstitial MΦ infiltration and also decreased renal fibrosis, suggesting that the initial phase of MΦ infiltration may promote subsequent renal fibrosis.[25] In the same way, administration of liposomal clodronate selectively depleted both F4/80+ MΦ and F4/80+ dendritic cells in mice with UUO, but not F4/80− dendritic cells, resulting in

Table 2 | Profibrotic and antifibrotic effects of macrophages in UUO

MΦ phenotype	Stage of UUO	Mediators	Citation
Profibrotic	Early phase (days 1–7)	Direct: TGF-β, MMP-9. Indirect: CCL2, TNF-α, ROS	23–28
Antifibrotic	Late phase (days 7–14)	Direct: Agtr1, Mrc2, uPAR Indirect: IL-10	8–9, 29–31

Abbreviations: Agtr1, angiotensin II type 1 receptor; CCL2, chemokine ligand 2; IL, interleukin; MΦ, macrophage; MMP, matrix metalloprotease; Mrc2, mannose receptor 2; ROS, reactive oxygen species; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; uPAR, urokinase receptor; UUO, unilateral ureteral obstruction.

attenuated tubular apoptosis and renal fibrosis and decreased level of the profibrotic cytokine transforming growth factor-beta.[26] Braga et al.[27] found that M2 MΦ contributed to renal fibrosis of UUO in a MyD88-dependent manner. Mediators released by injured tissue can activate infiltration MΦ through toll-like receptors and MyD88 signaling pathways, which promote renal fibrosis. These results suggest that targeting innate immune response signaling pathways of MΦ could be a possible therapeutic strategy against renal fibrosis. Our group found that matrix metalloprotease (MMP)-9 was involved in epithelial mesenchymal transition and thereby contributed to renal fibrosis.[28–30] Lipopolysaccharide/interferon-γ-activated M1 MΦ produced a large amount of MMP-9, which increased tubular cell epithelial mesenchymal transition via the beta-catenin pathway. Tubular epithelial cells were the predominant source of MMP-9 during the early stage of UUO, whereas tubular epithelial cells, MΦ, and myofibroblasts produced MMP-9 during late-stage UUO. Blockade of MMP-2/MMP-9 or MMP-9 alone significantly reduced tubular cell epithelial mesenchymal transition and renal fibrosis in UUO.[30] In contrast, an inverse correlation between the number of interstitial MΦ and the degree of fibrosis has been found recently in UUO, suggesting an antifibrotic role of infiltrating MΦ in the later recovery phase of obstructive nephropathy.[8,9,31-33] Nishida et al.[8] demonstrated that interstitial MΦ display an antifibrotic role at day 14, but not day 5 after UUO. They found that the angiotensin II type 1 receptor on MΦ functions to attenuate renal fibrosis in vivo. Their data suggest that angiotensin II affects the quantity and phagocytic activity of MΦ through the angiotensin II type 1 receptor. The inverse correlation between interstitial MΦ number and interstitial fibrosis at late stage (day 14) of UUO was confirmed using cyclophosphamide-mediated MΦ depletion.[32] Mannose receptor 2 (Mrc2) contains an extracellular fibronectin type II domain that binds to and internalizes collagen. Upregulated Mrc2 expression by MΦ and myofibroblasts has been shown in UUO, and reduced Mrc2 expression significantly worsened fibrosis in Mrc2-deficient mice. This study demonstrated a fibrosis-attenuating role of Mrc2-expressing MΦ, involving a lysosomal collagen turnover pathway.[9] Zhang et al.[31] showed that absence of scavenging receptors on uPAR$^{-/-}$ MΦ led to delayed clearance of profibrotic molecules, resulting in renal fibrosis in the late stage of the UUO model. Taken together, current data suggest a phase-dependent balance of profibrotic and antifibrotic effects of MΦ in UUO (Table 2). MΦ undergo a switch from a pro-inflammatory to a trophic phenotype that supports the transition from kidney injury to
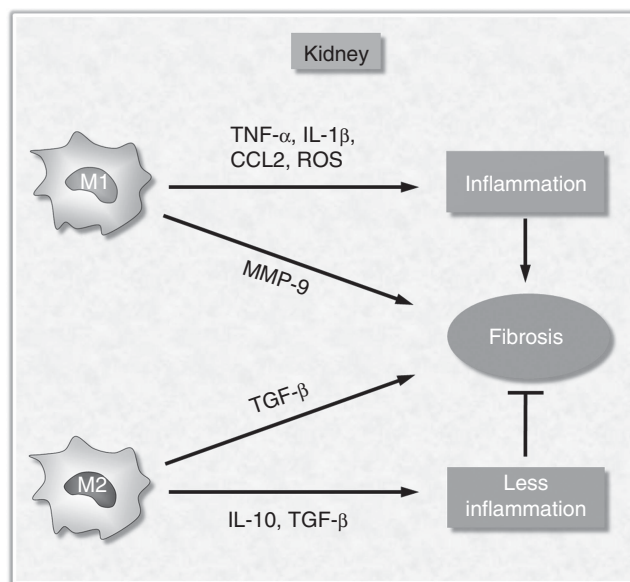
Figure 1 | Macrophage phenotype and function are critical determinants of kidney fibrosis. In response to ongoing injury, activated pro-inflammatory macrophages (M1) enhance kidney inflammation by secreting pathogenic mediators, resulting in kidney fibrosis in the late stage of disease. M1 macrophages also directly induce kidney fibrosis by secreting profibrotic factors, such as matrix metalloprotease (MMP)-9. In contrast, anti-inflammatory macrophages (M2) suppress kidney inflammation by releasing anti-inflammatory mediators interleukin (IL)-10 and transforming growth factor-beta (TGF-β), resulting in reduced kidney fibrosis. In addition, TGF-β produced by M2 macrophages promotes kidney fibrosis directly. Therefore, the net effect of M2 macrophages on kidney fibrosis is uncertain. CCL2, chemokine ligand 2; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha.

kidney repair. MΦ phenotypes depend on the influence of tissue microenvironments and are subject to change depending on the stage of disease, from early tissue injury to late wound repair. Renal fibrosis could be a consequence of renal injury, which involves MΦ infiltration. Inflammatory (M1) and anti-inflammatory (M2) MΦ will accelerate or reduce kidney injury, respectively, to impact indirectly on the degree of renal fibrosis (Figure 1). In contrast, MΦ at the later stage of repair may become profibrotic or fibrolytic to respectively induce or resolve renal fibrosis directly.[5] However, existence of profibrotic and fibrolytic MΦ has yet to be demonstrated unequivocally in vivo CKD.

In summary, although classification of MΦ into M1 and M2 phenotypes is based primarily on in vitro studies and does not fully mirror MΦ phenotype in vivo, yet M1-like and

M2-like MΦ have been demonstrated in kidney disease models. Functions of M1 and M2 MΦ have been demonstrated to be inflammatory and anti-inflammatory, respectively, which respond to and directly impact kidney injury. Modulation of MΦ *ex vitro* or *in vivo* into an anti-inflammatory phenotype presents a potential approach to limiting kidney injury by reducing inflammation. However, the exact phenotype and roles of MΦ in renal fibrosis are complex and uncertain. Profibrotic and fibrolytic MΦ have been defined *in vitro*, but their presence and function *in vivo* need to be verified in various types of kidney disease.

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