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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83.5\%$) of murine renal mononuclear phagocyte (unpublished data).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 M2caccording 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 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]

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.

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