Pathogenic and protective role of macrophages in kidney disease

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Cao Q, Wang Y, Harris DC. Pathogenic and protective role of macrophages in kidney disease. Am J Physiol Renal Physiol 305: F3–F11, 2013. First published May 1, 2013; doi:10.1152/ajprenal.00122.2013.—Macrophages (MΦ) are located throughout kidney tissue, where they play important roles in homeostasis, surveillance, tolerance, and cytoprotection. MΦ are highly heterogeneous cells and exhibit distinct phenotypic and functional characteristics depending on their microenvironment and the disease type and stage. Recent studies have identified a dual role for MΦ in several murine models of kidney disease. In this review, we discuss the pathogenic and protective roles of the various MΦ subsets in experimental and human kidney diseases and summarize current progress toward the therapeutic use of MΦ in kidney diseases.

macrophages; kidney disease; cell therapy

MACROPHAGES (MΦ) originate from a common progenitor mononuclear phagocyte system within bone marrow. MΦ are defined as tissue-resident phagocytic cells that contribute to tissue homeostasis and surveillance by clearing foreign materials, dead cells, and debris in response to inflammatory signals. During inflammation, they play an important role in initiating innate immune responses and generating adaptive immunity (23, 70). The diversity of MΦ functions has led to several classification systems. They can be classified into two major functional subsets: classically activated MΦ (M1), which after stimulation by LPS or IFN-γ are characterized by antimicrobial and cytotoxic properties; and alternatively activated MΦ (M2), which after exposure to Th2 cytokines such as IL-4 and IL-10 are involved in fibrosis, tissue repair, and resolution of inflammation (22, 57). In addition, tumor-associated MΦ have been proposed to have an M2 phenotype, emphasizing their wound-healing and regulatory functions (78). However, the M1-M2 classification is overly restrictive. Another more flexible classification is based on distinct functions of MΦ in which MΦ have a range of overlapping activities, with classically activated, wound-healing, and regulatory MΦ occupying different points along the spectrum (60).

MΦ are found in normal kidney tissue and in various kidney diseases (3). Under steady-state conditions, kidney MΦ play important roles in homeostasis, surveillance, tolerance, and cytoprotection. They clear apoptotic cells by phagocytosis and act as sentinels for the renal immune system. In inflammatory conditions, kidney MΦ are involved in initiation and progression of renal pathology (42, 77). They have been demonstrated to mediate tissue injury and disease progression in some situations, and yet to mediate renal protection and a reparative role in later stages of some kidney diseases including kidney ischemia-reperfusion injury (IRI) and unilateral ureteral obstruction (UUO) (2, 73). The functional complexities of MΦ can be explained by their phenotypic plasticity in response to varying microenvironmental stimuli determined by the type and stage of the kidney disease. MΦ infiltration is a common feature of most human chronic kidney diseases. Correlations between the degree of MΦ infiltration and severity of kidney injury in humans, suggest a pathogenic role of MΦ in kidney disease (16, 17, 25, 33, 51, 67, 98). Importantly, proof of a causal role for MΦ has to be demonstrated in animal studies. Indeed, animal studies have shown an induction of kidney injury by inflammatory mediators released from MΦ, an improvement of kidney injury and function by depletion of MΦ, and an acceleration of kidney injury by repletion of MΦ. In the past decade, the focus of MΦ studies has expanded to include their potential benefits to immune regulation and tissue repair. MΦ can be modulated to wound-healing and regulatory phenotypes, which hold therapeutic potential for kidney diseases (7, 15, 87).

In this review, we summarize current knowledge of the pathogenic and protective roles of kidney MΦ in acute and chronic kidney diseases. Specific attention will be given to the development of novel MΦ-based strategies to treat human kidney diseases.

PATHOGENIC AND PROTECTIVE ROLE OF MΦ IN ACUTE KIDNEY DISEASE

IRI. IRI is relevant to a number of clinical situations, including kidney transplantation. MΦ contribute to IRI by enhancing the inflammatory cascade through secretion of cytokines, recruitment of neutrophils, and induction of epithelial cell apoptosis (50). Early depletion and reconstitution studies have established a role of MΦ in kidney IRI (14, 35, 43, 84). Using liposomal clodronate (LC), Day’s studies demonstrated that MΦ play a critical role in mediating tissue injury from ischemia-reperfusion (IR) in a mouse model of IRI (14). Later, Kim’s group observed similar results by depleting MΦ by using systemic administration of LC in a rat IRI model (35) and demonstrated that MΦ contribute to the develop-
ment of renal inflammation and fibrosis in the long term after IRI, supporting the negative effects of MΦ on the repair of post-ischemic kidneys (43). Hypoxia occurs not only in IRI but also is present in chronic kidney diseases due to reduction of capillary density in areas of inflammation. MΦ in a hypoxic kidney microenvironment can be activated to secrete proangiogenic growth factors and proinflammatory cytokines. MΦ in response to hypoxia also increase secretion of chemokines that promote MΦ recruitment. Recruited MΦ themselves could further worsen kidney microenvironmental hypoxia (19, 61, 77). In contrast to these pro-inflammatory functions, kidney MΦ have recently been proposed to have a protective role during the repair phase. Lee found that MΦ expressed pro-inflammatory markers during the initial phase of IRI, whereas MΦ displayed an alternatively activated phenotype during the repair phase. Depletion of MΦ before IR (M1 predominance) reduced injury, whereas depletion during IR (M2 predominance) diminished tubular cell proliferation and delayed tubule repair. When M1 MΦ were adoptively transferred early after injury, they switched to an M2 phenotype within the kidney during the later recovery phase (48). These findings are consistent with several other published studies in which MΦ ablation during the repair phase of IRI resulted in deleterious outcomes (38, 52). Lin found that the mechanism underlying M2 promotion of kidney repair involves production of the Wnt pathway ligand Wnt7b, and MΦ Wnt7b promotes regeneration via epithelial cell-cycle progression and basement membrane repair (Fig. 1) (52). Also CSF-1 mediated resident MΦ expansion and polarized them toward an M2 phenotype, which resulted in renal tubule epithelial regeneration after AKI (1, 58, 101). Together, these studies show that MΦ undergo a switch from a proinflammatory to a trophic phenotype that supports the transition from tubule injury to tubule repair in acute kidney injury.

Unilateral ureteral obstruction. Unilateral ureteral obstruction (UUO) is a model of progressive renal interstitial fibrosis. The severity of fibrosis correlates with infiltration of activated MΦ (18), which secrete profibrotic and pro-inflammatory cytokines (40). 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 renal fibrosis (79). Similarly, administration of LC selectively depleted both F4/80+ MΦ and F4/80+ dendritic cells in mice with UUO but not F4/80– dendritic cells, resulting in attenuated tubular apoptosis and renal fibrosis and decreased pro-fibrotic cytokines TNF-α and TGF-β (40). Yokoyama and colleagues found that blockade of chemokine CCL2 or its receptor CCR2 impeded the initial phase of MΦ infiltration following UUO and decreased MΦ infiltration and renal fibrosis (39, 85). Our group found that MMP-2 and MMP-9 were involved in the epithelial mesenchymal transition (EMT) and thereby contributed to renal fibrosis (Fig. 1). LPS/IFN-γ-activated MΦ produce a large amount of MMP-2 and MMP-9, which increased tubular cell EMT via the β-catenin pathway. Blockade of MMP-2/MMP-9 or MMP-9 alone attenuated the renal fibrosis in UUO (80, 103). In contrast, an inverse correlation between the number of interstitial MΦ and the degree of fibrosis has been found recently in UUO, thereby suggesting an anti-fibrotic role of infiltrating MΦ in the later recovery phase of obstructive nephropathy (12, 64–66, 68, 100). Nishida demonstrated that interstitial MΦ display an anti-fibrotic role at day 14, but not day 5, after UUO. They found that angiotensin II type 1 receptor (Agtr1) on MΦ functions in vivo to attenuate renal fibrosis by using wild-type mice reconstituted with Agtr1−/− bone marrow. Their data suggest that angiotensin II affects the quantity and phagocytic activity of MΦ through Agtr1 (64).

The inverse correlation between interstitial MΦ number and interstitial fibrosis at late stage (day 14) of UUO was confirmed by using cyclophosphamide-mediated MΦ depletion and matrix metalloproteinase (MMP)-2 inhibitor studies (65, 66). These findings are consistent with those from the different groups. Zhang demonstrated that the absence of scavenging receptor on uPAR−/− MΦ resulted in the delayed clearance of pro-fibrotic molecules leading to renal fibrosis in the UUO model (100). Thus both pathogenic and protective MΦ have been reported in UUO in depletion studies. The existence of multiple roles for MΦ could be explained by depletion of MΦ of different phenotypes during different stages of UUO. Also, it cannot be excluded that incomplete depletion (usually 50–70% depletion) of kidney MΦ leaves behind a MΦ population with a different composition to that of the original population, resulting in the variable outcomes of depletion studies.

Cisplatin nephrotoxicity. Cisplatin is widely used to treat cancer, and one of its limiting side effects is acute kidney injury characterized by acute tubular necrosis and inflamma-

Fig. 1. Macrophage phenotype and function are critical determinants of kidney injury or repair. In response to ongoing injury, activated pro-inflammatory macrophages (M1) secrete pathogenic factors that induce kidney injury and fibrosis by promoting inflammation and tubular cell apoptosis. In certain microenvironments, anti-inflammatory macrophages (M2) secrete regenerative trophic factors that reduce inflammation, promote cell proliferation, and stimulate angiogenesis, which leads to wound healing and renal recovery. It is unclear whether M2 contribute to kidney fibrosis.
tion. The role of MΦ in cisplatin nephrotoxicity is uncertain. Treatment of peritoneal MΦ with cisplatin in vitro induced nitric oxide, pro-inflammatory cytokines, and activation of NF-κB (9, 81). Several recent studies showed a positive correlation between interstitial MΦ number and degree of kidney injury in cisplatin nephrotoxicity, suggesting a pathogenic role of kidney MΦ (37, 47, 49, 72). Ramesh found that cisplatin activates p38 MAPK, which leads to increased production of TNF-α in MΦ. Inhibition of p38 MAPK in cisplatin nephrotoxicity resulted in less MΦ infiltration and kidney injury (72). Other investigators found that rosiglitazone (a PPARγ agonist) and alpha-lipoic acid (an anti-oxidant) decreased infiltration of interstitial MΦ, resulting in functional and histological protection (37, 49). Moreover, Lee demonstrated that kidney MΦ accumulation was significantly reduced in mice treated with regulatory T cells, which correlated with less renal dysfunction and tubular injury (47). All of these data indicate that MΦ have a pathogenic role in cisplatin-induced kidney injury. However, Lu reported that depletion of MΦ by LC or inhibition of MΦ infiltration by CX3CL1 antibody was not sufficient to prevent cisplatin-induced renal dysfunction, suggesting that MΦ infiltration is a reflection of the severity of injury rather than its cause (54).

Taken together, current data suggest a phase-dependent balance of pro-inflammatory MΦ (M1) and anti-inflammatory MΦ (M2) in kidney diseases. M1 MΦ play a predominant pathogenic role during the early stage of acute kidney injury and contribute to acute tubular necrosis and inflammation. Subsequently, M2 MΦ predominate at a later stage of acute kidney injury and contribute to wound healing and resolution of inflammation (Table 1).

**PATHOGENIC AND PROTECTIVE ROLE OF MΦ IN CHRONIC KIDNEY DISEASE**

**Anti-glomerular basement membrane glomerulonephritis.** MΦ accumulation within the kidney is a prominent feature in most forms of human glomerulonephritis (GN). Glomerular MΦ secrete proinflammatory cytokines, reactive oxygen radicals, and nitric oxide, which are critical mediators of further glomerular damage and proteinuria (62). Early studies by Holdsworth (26, 27) demonstrated that MΦ accumulated in glomeruli in direct response to the deposition of antibody in anti-glomerular basement membrane (GBM) glomerulonephritis. Inhibition of MΦ accumulation by anti-MΦ serum largely prevented progression of experimental glomerulonephritis (26, 27). MΦ depletion by clodronate microspheres resulted in 95% depletion of MΦ from kidney and greatly reduced TNF-α and IL-1β release in anti-GBM glomerulonephritis. These data suggest that activated MΦ mediated the kidney damage in GN through release of pro-inflammatory cytokines (Fig. 1) (13). Studies by Nikolic-Paterson used adoptive transfer of MΦ to investigate their role in kidney disease. They found that adoptive transfer of bone marrow MΦ directly mediated mesangial cell proliferation and proteinuria in acute anti-GBM nephritis (30, 32). Exposure of MΦ to a specific Jun amino terminal kinase (JNK) inhibitor before adoptive transfer caused a >70% reduction in proteinuria and glomerular cell proliferation in this model (31). In the chronic anti-GBM model, early or late treatment with a specific JNK inhibitor reduced proteinuria and suppressed glomerular and tubulointerstitial damage (20, 55). These studies suggest that inhibition of JNK signaling protects against kidney injury in acute and chronic phases of anti-GBM disease, partially via suppression of the MΦ proinflammatory response. A recent study has provided clearer evidence of MΦ-mediated kidney injury. A specific MΦ colony-stimulating factor receptor (c-fms) inhibitor completely reversed MΦ infiltration and prevented kidney injury in anti-GBM disease (24).

**Lupus nephritis.** Lupus nephritis is a major cause of morbidity and mortality in patients with systemic lupus erythematosus. Progression of kidney injury is related to infiltration of MΦ and lymphocytes (45). MΦ secrete a wide array of molecules that could cause kidney injury, including reactive oxygen species, nitric oxide, pro-inflammatory cytokines, and growth factors. IFN-γ produced by infiltrating MΦ has been found to be responsible for adhesion molecule upregulation and kidney MΦ accumulation (8). CCL2 is expressed highly in lupus nephritis and is crucial for monocyte recruitment into sites of inflammation. Blockade of CCL2 or CCR2 using inhibitors or knockout mice significantly reduced MΦ infiltration with a remarkable reduction of tissue injury and improved kidney disease (44, 69). Colony stimulating factor-1 (CSF-1), a primary

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M1, pro-inflammatory macrophage; M2, anti-inflammatory macrophage.
growth factor for MΦ, was upregulated significantly in lupus nephritis. Deletion of CSF-1 reduced MΦ infiltration and protected lupus nephritis in MRL-Fas/lpr mice (59). Lena demonstrated that activated kidney MΦ are markers of disease onset and disease remission in lupus nephritis. Interestingly, a type II-activated MΦ (M2b) was identified in disease remission of lupus nephritis (76). M2 MΦ were also demonstrated in a model of poly (I:C)-accelerated lupus nephritis in NZB/W mice by their high level expression of anti-inflammatory molecules IL-10 and osteopontin, and growth factors, but not pro-inflammatory molecules TNF-α, IL-12, or inducible nitric oxide synthase. Strikingly, depletion of infiltrating kidney MΦ by liposomal clodronate resulted in a dramatic reduction of intraglomerular proliferative lesions and crescent formation. Thus these results suggest that M2 MΦ mediate a dysregulated “tissue repair” program in poly (I:C)-induced lupus nephritis (82). A recent study showed that CD11b+/CD11cint/F4/80hi MΦ are a major renal source of several proinflammatory cytokines and chemokines and also secrete molecules associated with tissue protection and repair during active lupus nephritis. This study demonstrated that infiltrating kidney MΦ exhibit a unique hybrid activation phenotype in chronic lupus nephritis (4).

Adriamycin nephropathy. Adriamycin nephropathy (AN) is an experimental model of focal segmental glomerulosclerosis. Early studies showed that blockade of CCR1, CCL2, or CCL5 by specific antagonists or DNA vaccination markedly reduced an experimental model of focal segmental glomerulosclerosis. Studies by our group found that MΦ depletion in AN protected both renal function and structure, providing direct evidence that MΦ play a pathogenic role in kidney injury of AN (88). Further studies have investigated the role of MΦ in AN by using adoptive transfer. Adoptive transfer of CpG-DNA activated MΦ (M1), but not resting MΦ (M0), exacerbated kidney injury in AN mice (86). Recently, targeting IL-18 derived from infiltrating MΦ by a neutralizing binding protein protected against the development of AN, with less proteinuria, glomerulosclerosis, and interstitial inflammation (95).

Alport nephropathy. Alport disease is induced by genetic mutations of the α3, α4, or α5 chains of type IV collagen, which impairs the proper assembly of glomerular basement membrane. Progression of Alport nephropathy is associated with MΦ infiltrates in the glomerular and the interstitial compartments. Ninichuk and colleagues (63) demonstrated that delayed CCR1 blockade improved survival of Col4α3-deficient mice, which was associated with less interstitial MΦ.

They found that BX471, a small molecule CCR1 antagonist, completely blocked CCL3-induced CCL5 production in activated murine MΦ. Reduction of interstitial MΦ was associated with decreased tubular cell apoptosis and increased tubular cell proliferation, suggesting that interstitial MΦ play a role in the balance of apoptotic cell death and tubular cell regeneration (63). However, Clauss (11) found that CCL2 blockade reduced glomerular and interstitial MΦ but did not ameliorate glomerular damage in Col4α3-deficient mice with Alport nephropathy, implying that MΦ do not contribute significantly to glomerular pathology in this model. Blockade of CCR1, but not CCR2, improved renal pathology in Col4α3-deficient mice, suggesting that CCR1+ MΦ, but not CCR2+ MΦ, are predominant pathogenic MΦ in this model of Alport nephropathy (11). A further study by the same group found that bacterial Cpg-DNA accelerated Alport nephropathy and reduced the overall lifespan of Col4α3-deficient mice. This effect was associated with a significant increase in kidney classically activated (M1) MΦ, which produce high levels of inducible nitric oxide synthase, TNF-α, and IL-12. The study suggested that activated kidney MΦ promote the progression of Alport nephropathy (74).

Thus current evidence suggests that kidney MΦ accelerate kidney injury in most types of chronic kidney disease. However, a type II-activated MΦ (M2b) was identified as a marker of disease remission in lupus nephritis, and an alternatively activated MΦ (M2a) mediated dysregulated tissue repair in poly (I:C)-induced lupus nephritis (Table 1). It is likely that, whereas pro-inflammatory and anti-inflammatory kidney MΦ coexist in chronic kidney diseases, pro-inflammatory MΦ predominate and result in long-lasting inflammation and progressive kidney injury.

**THE POTENTIAL FOR MΦ THERAPY IN KIDNEY DISEASE**

MΦ comprise a heterogeneous population of cells, with diverse functions and phenotypic plasticity. Based on a new understanding of the diverse biological functions of MΦ, they are no longer regarded merely as effectors of kidney injury but also as potential therapeutic tools that can regulate inflammation and improve renal function. MΦ that secrete trophic factors and anti-inflammatory cytokines and promote tissue repair have been referred to as regulatory MΦ. Adoptive transfer of regulatory MΦ has been applied as a treatment of experimental kidney diseases. MΦ can be modulated to a protective phenotype by either genetic manipulation or by cytokines (Table 2).

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AN, adriamycin nephropathy; DN, diabetic nephropathy; GBM, glomerular basement membrane; UUO, unilateral ureteral obstruction; IRI, ischemia reperfusion injury.
Genetic manipulation of MΦ. MΦ activity can be modulated by transfer of suppressive genes using viral vectors. Genetically engineered MΦ can deliver genes specifically to sites of inflammation by following natural chemotactic signals. For example, MΦ transduced with adenovirus expressing IL-1r1a reduced glomerular and tubulointerstitial fibrosis in both anti-GBM glomerulonephritis and obstructive nephropathy (97, 99). Kluth and colleagues showed that MΦ transfected with adenovirus expressing IL-4 or IL-10 localized efficiently to inflamed glomeruli of rats with nephrotoxic nephritis and reduced inflammatory cell infiltration and the degree of glomerular injury (41, 93). IL-10-expressing MΦ were more effective than those expressing IL-4 (92). A recent study found that MΦ transduced with IL-10 can protect against renal ischemia injury through induction of lipocalin-2 (36). Wilson showed that MΦ transduced with inhibitor of protein kB (IkB), which blocks NF-κB proinflammatory signaling, reduced IL-12 and TNF-α synthesis and NO generation of MΦ stimulated with LPS and increased IL-10 synthesis (91). These modulated MΦ also significantly reduced kidney injury in nephrotoxic nephritis. One advantage of genetically engineered MΦ is their potential for long-lasting expression of target genes. Another advantage of these MΦ is their ability to deliver genes to sites of injury and inflammation by following natural chemotactic signals. However, major obstacles to future clinical application of genetic modulated MΦ include the unproven safety of viral vectors and difficulty in controlling the level of gene expression.

Cytokine alteration of MΦ phenotype. Protective MΦ also can be induced by cytokines. Such MΦ have been subdivided into at least three groups: M2a MΦ induced by IL-4 and/or IL-13; M2b MΦ induced by immune complexes with LPS; and M2c MΦ induced by IL-10 and/or TGF-β. Our group first examined the role of M2 MΦ in mice with adriamycin nephropathy (AN). In that study, MΦ were isolated from spleen and incubated with IL-4 and IL-13 to induce an M2a phenotype. The M2a MΦ significantly protected AN mice against kidney injury that was associated with reduced accumulation and downregulated inflammatory cytokine expression of host-infiltrating MΦ (89). A further study showed the protective effects of M2a MΦ on kidney injury in another model, that of murine streptozotocin-induced diabetes nephropathy (102). Similarly, M2a MΦ transfusion of diabetic endothelial nitric oxide synthase-null (eNOS−/−) mice resulted in reduction of glomerulosclerosis and arteriolar hyalinosis. Another recent study demonstrated that M2a MΦ promoted renal tubular cell proliferation and reduced kidney damage in mice with IRI (48). Our group has investigated the relative protective efficacy of M2a and M2c MΦ in AN (6). M2c MΦ displayed a greater protective effect than M2a MΦ against kidney injury, which could relate to the observation that M2c MΦ, but not M2a MΦ, induced Tregs both in culture and in kidney draining lymph nodes. We have investigated mechanisms underlying the protective effect of M2 MΦ in several studies (6, 89, 102). Both M2a MΦ and M2c MΦ can inhibit CD4 T cells, CD8 T cell proliferation, and resident inflammatory MΦ. However, as discussed above, M2c MΦ but not M2a MΦ can induce Tregs. Cytokine-modified MΦ have the potential to provide a relatively easy and safe approach to treat kidney diseases.

FRONTIERS OF KIDNEY MACROPHAGE RESEARCH

Regulatory MΦ. There is compelling evidence that MΦ play an important role in tissue damage in kidney diseases. However, during the last decade, attention has been drawn to the anti-inflammatory and reparative roles of MΦ. Among acute kidney diseases, M2 MΦ appear in kidney during recovery of diseases, including IRI and UUO (12, 48, 64), to play a predominant role in resolution of kidney inflammation and wound healing. Thus it is worth exploring whether M2 MΦ could be not only a mediator but also a marker of remission of kidney injury in acute kidney disease. Kidney M2 MΦ have not been reported convincingly in chronic kidney diseases except lupus nephritis (76, 82). Moreover, in lupus nephritis, M2 MΦ coexist with but are outnumbered by M1 MΦ, perhaps suggesting only a minor role for M2 MΦ. In chronic kidney disease, it is possible that the relative paucity of M2 MΦ may be one factor explaining the progressive nature of kidney injury. Therefore, the appearance of M2 MΦ should be correlated with the severity of CKD, and M2 MΦ number and activity could be manipulated as a means of slowing disease progression.

Tissue-specific MΦ. Kidney MΦ display phenotypic heterogeneity in different types of kidney disease and in different stages of disease, which could relate to kidney microenvironment. However, the phenotypic heterogeneity of kidney MΦ also could be due to their localization in specific kidney compartments, including the glomerulus, periglomerular region, interstitium, cortex, and medulla. MΦ in different kidney compartments could be affected by unique position-specific microenvironments created by tissue factors. As a result, the effects of MΦ in glomeruli, for example, may be different from those of MΦ in interstitium. It is important to define the roles of MΦ in different kidney sites in various kidney diseases.

Signaling pathways and kidney MΦ function. The phenotype and function of kidney MΦ depend in part on the microenviron-ments in which they reside. Kidney MΦ can be stimulated into an M1 phenotype by a wide variety of immunological and stress stimuli via various signaling pathways, including e-Jun amino terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), and activation of nuclear factor-κB (NF-κB). P38 MAPK and JNK have been demonstrated to be pivotal in M1 MΦ-mediated kidney injury in glomerulonephritis (20, 31, 55, 72). Other signaling molecules, including suppressor of cytokine signaling 3 (SOCS3) and IKK, have also been reported to be upregulated in kidney MΦ in nephritis (4, 53). Recently, it was reported that kidney MΦ in UUO highly express mTOR signaling and that rapamycin inhibited inflammatory activity of MΦ via mTOR signaling (10). However, the signaling pathways involved in M2 MΦ function in kidney disease remain unclear. In nonrenal diseases, other signaling pathways have been reported to be important in control of M1 and M2 phenotypes, including, among others, those of C/EBPβ, PPARγ, IRF family, and STAT family, but as yet they have not been studied in kidney diseases (21, 46, 71, 75, 90, 96). Several novel signaling molecules, including, among others, ATF3, Kif4, and HIF-1α, were found by our group to be highly expressed in M1, M2α, or M2c MΦ, respectively (Wang C, Cao Q, Lee VW, Zheng G, Alexander SI, Harris DC, and Wang Y., unpublished observations). Future studies need to focus on the signaling pathways and
transcription factors related to MΦ phenotype and function in kidney disease.

**Therapeutic targeting kidney MΦ.** Specific targeting pathogenic effector M1 MΦ rather than renal MΦ as whole is required for optimal therapeutic outcomes. Treatment to target inflammatory signaling pathways of M1 MΦ, including NF-κB, JNK, and c-fms, has already been developed (1, 20, 24, 55, 56). However, to optimally target kidney injury by depletion of specific subtypes of MΦ, it is necessary to define surface markers and signaling pathways with greater specificity for MΦ phenotypes. Alternatively, transfer of regulatory MΦ (6, 89) or switch of MΦ to an M2 phenotype (5) has been demonstrated to be an effective strategy to reduce kidney inflammation and injury in experimental chronic kidney diseases. Despite a reported in vitro pro-fibrotic effect of wound-healing MΦ, our studies demonstrated that M2a and M2c MΦ reduced kidney fibrosis in mice with Adriamycin nephritis. This paradox needs to be unravelled in greater depth and explored in other disease models, and is likely to have important implications for therapy.

**Subset and source of MΦ determine therapeutic outcome.** Transfer of M2 MΦ has been shown to reduce kidney injury in several models of kidney disease (48, 89, 102). Interestingly, we showed that M2c MΦ are more effective than M2a MΦ in reducing injury in Adriamycin nephropathy, a difference that has not yet been explored in other models. We also found that, unlike M2a MΦ derived from spleen, M2a MΦ derived from bone marrow were not protective, apparently due to continued proliferation and loss of protective phenotype within infiltrated kidney (Cao Q, Zheng D, Wang C, Wang X, Lee VW, Zheng G, Alexander SI, Wang Y, and Harris DC, unpublished observations). These findings suggest that the therapeutic efficacy of M2 MΦ depends on both their subtype and their source. The therapeutic effects of different MΦ subsets and MΦ from different sources should be examined in various kidney diseases.

**Translation of MΦ therapy to human kidney disease.** Regulatory MΦ possess anti-inflammatory and immune suppressive functions, which endow them with great potential for treating human kidney disease. Recently, a regulatory macrophage (Mreg) was generated in vitro by exposure of blood monocytes to M-CSF and IFN-γ applied to patients undergoing kidney transplantation and appeared to be successful in reducing the dose of immunosuppressive drugs (28, 29). Based on this pilot experience, a trial (The One Study; http://www.onestudy.org/) of MΦ therapy in renal transplantation has commenced. This recent progress lays the foundation for the future therapeutic use of M2 MΦ in human CKD (34). It suggests that regulatory MΦ may provide a real and practicable option for treatment of CKD.

In conclusion, based on numerous recent studies, kidney MΦ, by engaging both innate and adaptive immune responses, are believed to play both pathogenic and protective roles in acute and chronic kidney diseases. Careful dissection of the role of MΦ at different stages of various kidney diseases should help to elucidate this complex field and lead to the development of novel diagnostic and therapeutic tools. Increasing evidence suggests an important protective role for regulatory MΦ in dampening kidney injury. Before MΦ can become a therapeutic target or tool for human kidney disease, there needs to be a much greater understanding of the biology of these plastic, phenotypically diverse cells.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

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**REFERENCES**


Macs and macrophage infiltration. and TGF-beta, and increase of chondroitin/dermatan sulfate proteoglycans and matrix metalloproteinase-II expression as a predictor of fibrosis in UUO.

Ramesh G, Reeves WB.


Sica A, Bronte V.

Sung SA, Jo SK, Cho WY, Won NH, Kim HK.


