

ISN Forefronts Symposium 2015: The Diverse Function of Macrophages in Renal Disease



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Experimental and human studies indicate that macrophages play a key role within the diseased kidney and represent a target for novel therapies. This brief review outlines the involvement and nature of macrophages in renal disease and highlights the phenotypic plasticity of these cells and their responsiveness to the renal microenvironment.

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Monocytes and macrophages are key components of the mononuclear phagocyte system.¹ Whereas dendritic cells are specialized for immune surveillance and the activation of the adaptive immune system, macrophages are highly phagocytic cells that are involved in tissue development and homeostasis, inflammation, fibrosis, and tissue repair.^{2,3} Difficulties can arise, however, as there is significant overlap between the cell surface markers of macrophages and dendritic cells (e.g., F4/80, CD11b, and CD11c) such that the nomenclature can be confusing and experimental data open to more than one interpretation.^{1,2,4} For example, the majority of resident renal mononuclear phagocytes express CD11c that has often been used as a marker of dendritic cells. However, the analysis of renal F4/80+CD11c+ cells for cell surface markers and function indicates that they express scavenger receptors (CD206 and CD204) and are very phagocytic cells with limited capacity to present antigen—typical features of macrophages.⁵ Additional studies highlight the fact that the kidney contains multiple subpopulations of cells with features of dendritic cells or macrophages.⁶

During disease, the resident macrophage population is increased by the recruitment of monocyte from the circulation driven by chemokines such as CC chemokine ligand 2 and their subsequent differentiation to

macrophages. In addition, renal expression of the monocyte/macrophage growth factor colony stimulating factor-1 (CSF-1) is increased in the inflamed kidney.^{7,8} CSF-1 plays an important role in mediating the survival, proliferation, and differentiation of monocytes and macrophages such that increased CSF-1 expression leads to significant macrophage proliferation that expands the renal macrophage number.^{9–12}

Macrophages encounter myriad stimuli within normal, injured, healing, and fibrotic tissues such as hypoxia, cytokines, chemokines, reactive oxygen species, apoptotic cells, and debris. Macrophages need to integrate these potentially competing signals to adopt a phenotype deemed appropriate to the situation. Experimental *in vitro* and *in vivo* studies have shown that macrophages may adopt a range of diverse phenotypes broadly categorized as the proinflammatory M1 phenotype or the wound healing M2 phenotype.¹³

Exposure to Toll-like receptor ligands such as pathogen-derived endotoxin or damage-associated molecular patterns released during sterile tissue injury¹⁴ and cytokines such as interferon- γ induces M1 macrophage polarization. M1 macrophages upregulate cytotoxic and microbicidal mediators such as tumor necrosis factor- α and inducible nitric oxide synthase (iNOS) and may exhibit increased expression of Ly6C and human leukocyte antigen-antigen D related. Although the M1 phenotype is appropriate for dealing with infective pathogens, it is associated with tissue injury in sterile inflammation.

Transcription factors help regulate the genes involved in macrophage programming. For example, the transcription factor interferon regulatory factor 5 plays a key role in the induction of the proinflammatory M1 phenotype such that small, interfering

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RNA-mediated silencing of interferon regulatory factor 5 can limit M1 macrophage activation and promote M2 macrophage activation *in vivo* with the resultant amelioration of tissue injury in models of cardiac and spinal cord injury.^{15,16}

Exposure to cytokines such as interleukin-10 and interleukin-4, immune complexes, as well as the ingestion of apoptotic cells, induces M2 macrophage polarization. M2 macrophages upregulate arginase activity and typically express increased levels of scavenger receptors such as CD206, CD204, and CD163. Although M2 macrophages are anti-inflammatory and termed wound healing, they are often associated with maladaptive renal fibrosis.

Macrophages may exert immunoregulatory functions and cells termed regulatory macrophages (Mregs) have been implicated in the development of tolerance to allografts.¹⁷ Mregs express few M1 or M2 markers with the production of interleukin-10 being key for their immunosuppressive actions that include the inhibition of CD8+ T-cell responses and induction of regulatory T cells. Recent work, albeit using a murine vascularized cardiac transplant model, suggests that Mreg generation requires the actions of CSF-1 and Toll-like receptor 4 engagement.¹⁸ Mregs expressed the cell surface marker dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (CD209) and were key to the induction of tolerance by costimulatory blockade as the inhibition of these cells abrogated tolerance.¹⁸

Despite the utility of the M1/M2 paradigm, it should be appreciated that the biological reality is much more complex with subtle but important differences between different activation stimuli.^{19–21} As a result, many additional phenotypes will undoubtedly exist including mixed macrophage phenotypes where M1 and M2 markers may coexist.^{22,23}

Insights From Experimental Models of Renal Disease and Macrophage Depletion Studies

In an attempt to mimic human disease, investigators have developed multiple experimental models of renal injury in rodents that can be employed in mice deficient in chemokines (CC chemokine ligand 2) or chemokine receptors (CC chemokine receptor 2 and CX3C chemokine receptor 1) involved in monocyte/macrophage recruitment. This strategy has demonstrated that monocytes/macrophages caused kidney injury in multiple experimental models including nephrotoxic nephritis,²⁴ diabetic nephropathy,²⁵ and renal ischemia-reperfusion injury (IRI).²⁶

Liposomal clodronate is cytotoxic after uptake by cells and has been a useful tool to deplete monocytes/macrophages in various organs as it targets the

phagocytic macrophage. Studies have shown renal protection after clodronate-mediated macrophage depletion in multiple models of kidney injury or disease including cystic renal disease.^{27–31} The development of transgenic mice in which the expression of the human or simian diphtheria toxin receptor (DTR) is under the control of the CD11b promoter has allowed the relative selective depletion of CD11b+ monocytes and macrophages by the administration of DT to mice.³² This system has demonstrated reduced injury or fibrosis after monocyte/macrophage depletion in models of fibrosis,³³ nephrotoxic nephritis,³⁴ and murine transplantation.³⁵ Interestingly, no protection was evident in murine renal IRI³⁶ although the addition of clodronate to DT conferred protection.³⁷

It is important to bear in mind that macrophages are not always injurious or profibrotic as the critical reparative role of the macrophage has been highlighted by studies of macrophage depletion using liposomal clodronate or CD11b/DTR mice in the reparative phase of the renal IRI model. This phase is characterized by the restoration of renal function and tubular repair, and macrophage depletion is highly detrimental as it results in increased mortality, prolonged injury, and failure of tubular repair.^{38–41} During renal repair, macrophages are an important source of mediators such as Wnt7b and IL-22 that promote tubular epithelial proliferation.^{40,42}

Lastly, it should be noted that few studies have attempted to dissect the roles of resident macrophages versus infiltrating monocyte-derived macrophages to determine which macrophage population is key to injury and fibrosis as interventions to deplete macrophages typically exert effects on both populations. To explore this question, Lin *et al.*⁴³ used bone marrow transplantation to generate chimeric CD11b/DTR mice such that the administration of DT would either deplete resident renal macrophages or infiltrating monocyte-derived macrophages. These studies used the model of unilateral ureteric obstruction that exhibits marked interstitial fibrosis with a dramatic macrophage infiltrate. DT-induced depletion of DTR+ infiltrating monocyte-derived macrophages was markedly antifibrotic. In contrast, the targeted depletion of DTR+ resident macrophages did not affect fibrosis despite the fact that they constituted up to 40% of the total macrophage population.

Although the majority of patients with significant renal disease are elderly, the vast majority of experimental rodent studies are undertaken in young animals. It is pertinent that aged mice develop much worse acute kidney injury after renal IRI^{44,45} with the induction of the cytoprotective enzyme hemeoxygenase-1 being less robust compared with young mice. The administration

of the potent hemoxygenase-1 inducer heme arginate strongly protected aged mice from renal IRI with monocyte/macrophage hemoxygenase-1 expression being critical. Other macrophage functions such as the phagocytosis of apoptotic cells have been noted to be abnormal in aging mice⁴⁶ with a defect in both resident and recruited macrophage phagocytosis evident. It is thus likely that the monocytes and macrophages of elderly patients may behave differently to younger individuals.

Although the number and phenotype of endogenous macrophages may be the target of interventions, it is also of interest that the exogenous administration of anti-inflammatory or M2 macrophages can ameliorate both acute and chronic experimental disease.^{47–49}

The Regulation of Macrophage Phenotypes *In Vivo*

It thus appears that macrophages may be cytotoxic (M1), reparative (M2), or profibrotic (M2) within the kidney. An important question that has been addressed recently is whether these differing M1/M2 macrophage phenotypes are directly derived from either resident macrophages or recruited monocytes or whether macrophages can change their phenotype within the kidney as a result of changes in the renal microenvironment. Lee *et al.*³⁹ performed elegant adoptive transfer experiments involving the administration of fluorescently labeled bone marrow-derived macrophages programmed *in vitro* to adopt an M1 phenotype to mice shortly after the induction of renal IRI. The labeled cells were retrieved at later time points and were found to have an M2 phenotype as they exhibited the downregulation of iNOS expression and the upregulation of CD206 expression. This study indicated that macrophage phenotype is dynamic and can evolve during the injury and repair phase of renal injury (Figure 1).

Further work has highlighted the importance of the renal expression of macrophage growth and differentiation factors by tubular epithelial cells in the beneficial reprogramming of proinflammatory M1 macrophages to reparative M2 macrophages with a role for both CSF-1 (also termed macrophage-colony stimulating factor)^{50,51} and granulocyte macrophage-CSF.⁵² The effect of CSF-1 on M1 macrophage reprogramming may be via the induction of microRNA-24⁵³ although renal data are lacking at present.

In the light of the beneficial role of CSF-1 in modulating the phenotype of macrophages, it is intriguing that strategies to inhibit the function of CSF-1 using function blocking antibodies or drugs that target activation of the CSF-1 receptor have been shown to be protective in a wide range of experimental

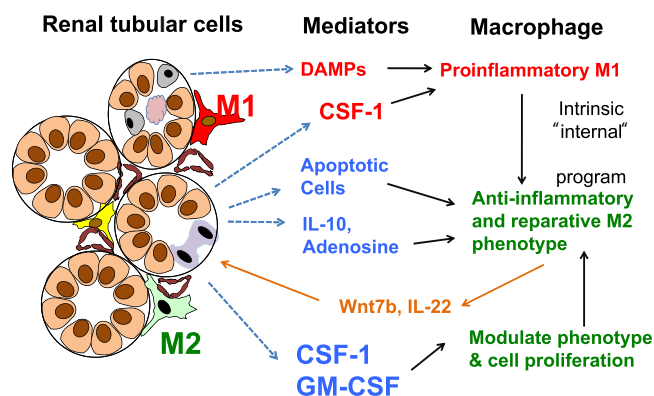


Figure 1. Macrophage phenotype within the diseased kidney. Renal injury and inflammation leads to the release of proinflammatory mediators including chemokines, cytokines, and damage-associated molecular patterns (DAMPs). These and other mediators result in the induction of a proinflammatory cytotoxic M1 macrophage. Upregulated tubular expression of colony stimulating factor-1 (CSF-1) expression induces macrophage proliferation that may promote ongoing renal injury in the presence of persistent inflammation. During repair, various mediators including interleukin (IL)-10, apoptotic cells, and the growth factors CSF-1 and granulocyte macrophage (GM)-CSF promote the phenotypic switch to anti-inflammatory wound healing M2 macrophages that may promote the restoration of tubular integrity by releasing mitogens such as Wnt7b and IL-22.

models.^{54–58} These studies suggest that reducing macrophage proliferation and number is beneficial in situations where there are excessive numbers of macrophages driving injury or fibrosis. In contrast, the exogenous administration of CSF-1 after experimental murine IRI significantly improved renal repair, suggesting that augmenting the population of macrophages involved in renal repair is highly beneficial.⁵⁹

Recent work has suggested an important role for retinoic acid in modulating macrophage phenotype via the direct inhibition of M1 macrophages and the promotion of tubular cell induction of M2 macrophages, indicating that there are multiple pathways to manipulate macrophage phenotype.⁶⁰

Macrophages—Key Players in Human Disease

Macrophages are present in human renal diseases, including diabetes,^{61,62} polycystic kidney disease, kidney allograft rejection,⁶³ chronic allograft nephropathy,^{64,65} and acute kidney injury.^{22,65} Studies have demonstrated a strong association between the extent of macrophage infiltration and functional outcome.⁶⁶ A recent study of pediatric kidney transplant recipients with chronic allograft nephropathy demonstrated CD163+ M2 macrophages in fibrotic areas of the kidney with CD163+ cell number correlating with interstitial fibrosis and renal function.⁶⁴ Interestingly, urine CD163 levels also correlated with fibrosis, suggesting the potential for using urine

markers of macrophage phenotype as a biomarker of renal scarring. Similarly, in a study of 1-year renal transplant biopsies from adult transplant recipients, the numbers of CD206+ macrophages correlated with both fibrosis and renal function at 3 years after transplantation.⁶⁷ Additional recent work highlights the involvement of macrophages in lupus nephritis⁶⁸ with the number of interstitial CD68+ macrophages correlating with renal function and fibrosis. A minority of macrophages were iNOS+ M1 macrophages with the majority being positive for the M206 markers CD206 and CD163. The proportions of iNOS+, CD206, or CD163 varied between glomerular and interstitial compartments and between classes of lupus nephritis. The predominance of M2 macrophages over M1 macrophages may reflect patients undergoing a renal biopsy at a later stage of disease than is usual in experimental models of lupus nephritis as well as the potential effects of drug treatment such as steroids that can induce an M2 phenotype.

Potential Therapeutic Approaches to Target Macrophages

In view of the complexity of macrophage phenotypes and their involvement in multiple aspects of kidney disease (acute kidney injury, renal repair, glomerulonephritis, fibrosis, etc.), the timing of interventions directed to manipulate macrophage phenotypes or numbers will need to be carefully considered. Some therapies currently in use will exert effects on macrophages. For example, glucocorticoids increase the phagocytic capabilities of macrophages and induce an anti-inflammatory phenotype.⁶⁹ Potential strategies to limit macrophage numbers include the inhibition of chemokines involved in the recruitment of monocytes to the kidney, and there are clinical trials in progress that are targeting the CCL2/CCR2 axis in patients with renal disease such as diabetic nephropathy. There is also potential for the inhibition of growth factors such as CSF-1 in situations where macrophages are driving injury and/or fibrosis or the administration of exogenous CSF-1 to bolster a reparative macrophage population. Although macrophage cell therapy for inflammatory renal disease has not been undertaken thus far, the effect of administering donor-derived Mregs generated *in vitro* was examined in 2 patients who underwent live donor kidney transplantation.⁷⁰ Graft function remained stable over 3 years with the patients being maintained on tacrolimus monotherapy. In addition, the peripheral blood gene signature of these patients was similar to that found in tolerant patients. The administration of Mregs is now being tested in the active ONE Study Mreg Trial that aims to increase tolerance in living donor transplant recipients (NCT02085629).

Conclusion

Macrophages are remarkably versatile cells and, although they may assist tissue remodeling, they are often associated with tissue injury and disease progression. A deeper understanding of the cellular and molecular mechanisms that mediate the diverse functions of macrophages in renal disease should allow the development of novel therapies that may have applicability to multiple organs.

DISCLOSURE

All the authors declared no competing interests.

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