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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 injury[14] 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 IRI[36] 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